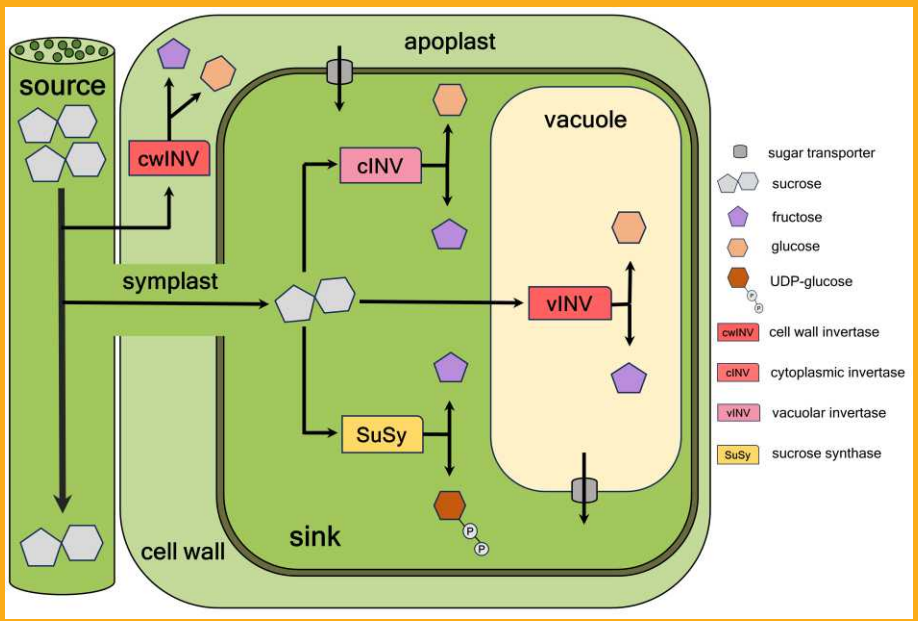
ISSN 0971 - 2976

JOURNAL OF THE BOTANICAL SOCIETY OF BENGAL



Volume 79
Number 1, June 2025

AN
OFFICIAL ORGAN
OF THE
BOTANICAL SOCIETY OF BENGAL
Estd. 1935

BOTANICAL SOCIETY OF BENGAL

(Established in 1935)

Website : www.botansocbengal.org

Aims and Scope

Journal of the Botanical Society of Bengal has a long history of publishing articles since its inception. It is a peer reviewed, biannual journal. The aim of this journal is to encourage publication of scientific knowledge on any branch of plant science, basic and applied. The topics range from cryptogams to phaneragams, morphology-anatomy, plant-microbe interaction, genomics and genetics, physiology and biochemistry, palaeobotany-palynology, plant molecular biology and biotechnology, embryology, systematics and other allied fields of plant sciences. Along with full articles, reviewed articles and short communications are also published.

Copyright and permission

Journal Website

www.botansocbengal.org

Publication Ethics

www.publicationethics.org
https://authorservices.willey.com/ethics-guidelines/index.html

Papers will be accepted only if they abide by the publication ethics and bioethics.

Subscription Information

All communications should be addressed to the Secretary, Botanical Society of Bengal, Department of Botany, University of Calcutta, 35 Ballygunge Circular Road, Kolkata 700019. The subscription price is Rs.2000.00 per volume (two numbers in a year) in India including ordinary Book Post charge. The subscription for foreign countries is US $ 500.00 per volume, including air mail charges.

Office of Publication

Department of Botany, University of Calcutta, 35, Ballygunge Circular Road, Kolkata 700019, West Bengal, India

The figure in the cover page showing : Sucrose catabolism within a sink cell in the article by Mitra *et al.*, *J. Botan. Soc. Bengal,* 79(1): 1-18, 2025

J. Botan. Soc. Bengal 79(1) : i-iv (2025), ISSN 0971-2976

Editorial

BOTANICAL SOCIETY OF BENGAL – 90 YEARS OF LEGACY

Celebrating 90 years of a society is a significant milestone, symbolizing nearly a century old collective effort, growth and legacy. As we mark 90 incredible years of our society, we reflect with pride on a journey built through dedication, unity and common purpose for science and society. From humble beginnings to meaningful strokes,

His excellency Dr. K.N. Katju in a happy mood at the stall of Messrs Talukdar & Co. (Fertilisers) Ltd. in the Botanical & Agri-holticultural Exhibition held by the Botanical Society of Bengal on the 14th April 1951 at 35, Ballygunge Circular Road, Calcutta
(Presented by Talukdar & Co. (Fertilisers) Ltd.)

each generation has contributed to the growth and strength we celebrate today. The anniversary is not just a celebration of the past; it is a tribute to the values that continue to guide us for the bright future. It is a celebration of Heritage and Hope for the future.

The Botanical Society of Bengal, established in the year 1935, is one of the oldest and most vibrant Botanical Societies in the country catering to the botanical needs through its various activities and regular publications of its journals amongst the botanists of the country, particularly in Eastern India. Since its inception, the Botanical

Society of Bengal has been continuously striving to serve the cause of Botany and is forging ahead following its charter of objectives. At present, the Society has about 396 life members. It was after the 22nd session of the Indian Science Congress (Calcutta) in January 1935, the Botanists of Bengal conceived the idea of forming a Society to promote research in Botany as well as to promote a feeling of fellowship among the botanists in Bengal. For this a Committee was formed to ascertain opinions about this from persons in and outside the state. A meeting was organised in the Botany Laboratory, Presidency College, Calcutta, on 11th April 1935, under the Presidentship of Prof. S.P. Agharkar, Head of the Department of Botany, Calcutta University, where it was unanimously resolved that "The Botanical Society of Bengal" be formed which shall be primarily concerned with all matters effecting the study of Botany so far as Bengal is concerned. In the meeting the following resolutions were finally adopted.

1. That the name of the Society should be “Botanical Society of Bengal”.
2. That it shall be primarily concerned with all matters affecting the study of Botany, so far as Bengal is concerned.
3. That under the present financial position, the Society should not have a journal of its own.

A Committee was appointed to draft rules and regulations of the Society which were adopted in a General Meeting held on 25th April 1935 at the Botanical Laboratory, Calcutta University.

1. Dr. S.P. Agharkar, Chairman (Calcutta University); 2. Prof. G.P. Majumdar (Presidency College, Calcutta); 3. Mr. I. Banerji (Calcutta University); 4. Prof. S.N. Banerjee (Ripon College); 5. Prof. M.L. Chakravarty (Bangabasi College); 6. Dr. P.K. Sen Gupta (Calcutta University); 7. Prof. A.K. Ghosh, Secretary (Diocesan College); 8. Dr. Syed Hedayatullah (Calcutta University).

The inaugural meeting of the Society was held at the Botanical Laboratory, University of Calcutta on 13th December 1935. Dr. Shyama Prasad Mookherjee, M.A., B.L., Barrister at Law, M.L.A., the then Vice Chancellor of University of Calcutta presided as President of the meeting. He felt that “this was a move in the right direction. There was a time when it was possible for individuals to carry on research work alone. Specializations had, however, progressed to such a degree that it is now not possible for any individual, however eminent he may be, to do anything very substantial without the cooperation of others." He said "the members of the Society should not confine their activities to the prosecution and publication of scientific researches alone which might bring credit to the individual workers but should also disseminate their knowledge to the general public by arranging for popular lectures, exhibitions, etc." He said that he knew that investigations could not be carried on without money, but he was sure that if proper work or its practical utility was ensured, financial help would be forthcoming, from even the most unexpected quarters. The University was in fact, intended to encourage such activities and the Society can rely on getting necessary help from them, if their work was directed to such objects. He wished the Society a long and successful career of usefulness. This inaugural meeting urged the formation of a society being supported by Sir Prafulla Chandra Ray, Kt., Dr. S.C. Law- the then Sheriff of Calcutta, Mr. C.C. Calder, Director Botanical Survey of India and Superintendent of the Royal Botanic Gardens, Shibpur, Howrah and Professor S.C. Mahalanobis of Presidency College, Calcutta. The celebration of the inaugural meeting was organized by a committee with Prof. S.P. Agharkar, the then Rashbehary Ghose Professor of Botany as its President and since then the Society was christened as Botanical Society of Bengal in 1936 with Late Pofessor G.C. Bose as its first President and Professor Amiya Kumar Ghosh and Professor Ilabanto Banerjee as founder joint Secretaries. His Excellency, the then Governor of Bengal was the Patron of the Society.

Many symbols for the Society were proposed but finally the Great Banyan Tree of the Indian Botanical Garden, Shibpur, Howrah was considered.

Objectives of the Society

To promote the cause of Botany and safeguard the interests of botanists in Bengal.

1. To hold periodical meetings in Calcutta as well as in other parts of Bengal with a view to diffuse botanical knowledge among the public and facilitate mutual intercourse among members.
2. To encourage original investigations.
3. To arrange periodical excursions to different parts of Bengal for the study of its vegetation.
4. To arrange for the preparation of local floras, maps, monographs, etc.
5. To arrange for the publication of its proceedings.
6. To co operate with the Indian Botanical Society and Societies of a similar nature in other parts of India for purposes of common interest, and
7. To do and perform all other acts, matters and things that may assist in, be conducive to or necessary for the fulfilment of the objectives of the Society.

Activities of the Society

The society, holds Ordinary General Meeting, Extra-ordinary General Meeting, Special General Meeting and Annual General Meeting as and when deemed necessary. The Society gives awards to distinguished scientists and students. Medals and cash prizes are also awarded by the Society. The following medals and cash prizes are awarded.

Dr. J. Sen Memorial Medal for students securing First class First rank in U.G. Honours examination in Botany, Calcutta University.

Prof. H. L. Chakroborty Memorial Award for students securing First class First and Second ranks in M.Sc. examination in Botany of Calcutta University.

Dr. Prabir Chatterjee Memorial Award for students securing First class First and Second ranks in UG Honours examination in Botany of Calcutta University.

Prof. Tarachand Nandi Memorial Award for students securing First class First and Second ranks in UG Honours examination in Botany of Calcutta University.

Felicitations to Eminent Senior Botanists by Prof. Subhas Chandra Datta Memorial Committee and by the Society are given every year.

The Executive Council of the Society holds national and international symposia at different intervals and publishes its proceedings. The Society regularly publishes journals, two issues per volume each year. The Society started publishing the Bulletin of the Botanical Society of Bengal since 1947, with Prof P.N. Bhaduri as its first Editor. Presently the bulletin has been designated as the Journal of the Botanical Society of Bengal. Till date Volume 79(1), 2025 has been published. Botanical Society organizes annual botanical excursion-cum picnic to a selected spot every year for the members of the Society.

To provide academic support to the students of undergraduate colleges of Calcutta University and other Universities, the Society publishes Practical Laboratory Note Books in both English and Bengali on U.G. Courses in Botany.

Recent Activities

1st Botanical Congress held during 23rd to 25th March , 2023 at Calcutta University.

2nd Botanical Congress held during 23rd to 24th March , 2024 at University of Burdwan.

3rd Botanical Congress scheduled to be held during 21st to 23 rd August, 2025 at Calcutta University.

Standing at the intersection of history and hope, we are honoured to celebrate 90 incredible years of our society's journey – a legacy built on dedication, resilience and community spirit. This milestone is not just a tribute to the past, but a call to the future. We remember with gratitude the founders whose vision laid our foundation, the members and leaders who carried that vision forward and the generations who kept out purpose alive through changing times. Let us carry this legacy with pride and responsibility. May the values that brought us this far continue to guide us as we look ahead to the century mark and beyond.

For any information please email to: secbsbengal1935@gmail.com

Subir Bera
Ashalata D'Rozario

J. Botan. Soc. Bengal 79(1) : 1-18 (2025), ISSN 0971-2976

REVIEW ARTICLE

An insight into the physiology and biochemistry of flower maturation

Adinpunya Mitra*, Sriraksha A Bapat, Rohit Ghosh, Sinjini Datta

Natural Product Biotechnology Group, Agricultural and Food Engineering Department, Indian Institute of Technology Kharagpur, Kharagpur 721 302, West Bengal, India

Received : 29.05.2025 Accepted : 08.06.2025 Published : 30.06.2025

Floral maturation represents a finely coordinated and tightly regulated developmental process. This phenomenon is characterized by the transition of a bud into a fully open flower, followed by senescence and terminating with the abscission from the mother plant. During maturation, a flower undergoes many changes such as, water potential, redox status, antioxidant activities, and both central and specialized metabolic activities. The entire network of central and specialized metabolism continuously reorganizes to maximally advertise the flowers to ensure successful reproduction. This review begins by discussing various aspects of physiological determinants of flower maturation such as water relations and reactive oxygen species, followed by tracing changes in central metabolism and ending with an overview of specialized metabolic landscape. As flowers occupy a unique ecological niche, a comprehensive understanding of floral maturation will assist researchers, pollination biologists and horticulturists to contribute in crop improvement.

INTRODUCTION

Floral maturation is a complex, tightly regulated and genetically controlled process. The development and maturation of flowers is characterized by cell division, differentiation, growth, enlargement and ultimately cell death (Jhanji *et al.*, 2025). Flowers are unique biological organs due to their modular structure and distinct array of central and specialized metabolites in their constitutive tissues. Generally, the outer whorls of a flower are sites of specialized metabolite accumulation, while the inner whorls of a flower are particularly rich in central metabolites (Borghi, 2025). The reproductive responsibility on top of short-term lifespan, places flowers in a delicate ecological niche in the ecosystem. Therefore, unsurprisingly, various aspects of floral maturationhave been extensively studied by researchers, targeted by plant breeders and relentlessly pursued by horticulturists to make the most out of available time.

Floral maturation is influenced by a combination of internal factors such as water relations, redox status, membrane permeability, phytohormones and changes in central and specialized metabolism (Jhanji *et al.*, 2025).Usually, flowers at their open stage emerge fully expanded, colorful and scented, exuding nectar and pollination-ready (Borghi *et al.*, 2022). The simple sugars resulting from the hydrolysis of polysaccharides contribute to the increased osmotic potential in the developing petal tissues. They are also exported to the apoplast later to be found in the secretion of nectaries (Roy *et al.*, 2017). However, as

**Corresponding author* : adin@iitkgp.ac.in
adinpunya@gmail.com

anthers and pistils are simultaneously developing within the maturing flower, they rely heavily on core energy-producing pathways of glycolysis and tricarboxylic acid cycle intermediates (Prabhakar *et al*., 2010; Chen *et al.*, 2018). Evidently, there exists a pronounced dependency on the mother plant by the developing flowers to fulfil their energy requirements. Therefore, flowers are regarded as powerful sinks.

Historically, flowers have been economic commodities especially for their color and scent (Pichersky, 2023). Ecologically, these compounds are the agents of chemical and visual communication with the potential animal pollinators in most angiosperms (Borghi and Fernie, 2017). Consequently, extensive research has been conducted to decipher the ecological significance, biosynthesis, accumulation and emission patterns of floral specialized metabolites, and subsequent metabolic engineering applications. This review addresses the physiological aspects of flower maturation governed by water potential and importance of reactive oxygen species (ROS) signaling in driving the floral system towards senescence. Further, changes occurring in central metabolism during the process of floral maturation is examined with a special focus on carbohydrate metabolism. Finally, in relation to central metabolism, changes in specialized metabolism have been discussed with an emphasis on floral volatiles and pigments.

Role of water potential in flower maturation

Water potential is the total energy potential of a fluid relative to pure water at rest state (Ali *et al.*, 2022). Higher osmolyte concentration within the cell creates a negative osmotic potential and triggers water influx into the cell. This leads to an increase in cellular volume with simultaneous cell wall stretching and compression of the protoplast. Water movement caused by changes in water potential is crucial for flower opening, which signifies reproductive readiness of a plant. van Doorn and Van Meeteren (2003) reported blockage in water movement at the basal stem impaired opening of cut rose flowers. Petal opening and closing can be modulated by cell expansion or shrinkage mediated by osmoregulation. It was also suggested that the possibility of petal movement in *Gentiana kochiana* and *Kalanchoe blossfeldiana* is through reversible, turgor-driven cell expansion and contraction on the adaxial surface (van Doorn and Van Meeteren, 2003). Additionally, several studies have indicated asymmetric growth of the epidermal cells in the petal tissue brings about the action of unfurling of petals during flower opening. Zhang *et al.* (2024) comprehensively determined that flower opening and the formation of final shape of flowers in *Delphinium anthriscifolium* is due to selective expansion of cells present at different loci of the petal. Usually, the differential growth is characterized by greater increase in cell width of the adaxial epidermal cells than the abaxial side. In *Nelumbo nucifera* flowers, the periodic opening and closing of petals is resulted by periodic longitudinal expansion of epidermal cells present at the petal base (Ishizuna *et al.*, 2024). Young flowers, being the strong sink tissues, contain high quantities of storage polysaccharides such as starch and several other simple sugars. During flower opening, storage polysaccharides break into simple sugars such as sucrose, glucose and fructose which creates a negative osmotic potential inside that triggers influx of water in those cells and in turn results in petal unfurling (Fig. 1A). In Asiatic lily, presence of amylase inhibitor hinders breakdown of starch into simple sugars confirming the role of simple sugars in flower opening (Bieleski, 2000). Apart from flower opening, water potential plays a crucial role in maturation of anthers. During anther maturation especially before dehiscence, sub-epidermal and pollen cells go through a hydration phase where they get inflated by influx of water. The inflation inside the anther results into a lateral tension on the epidermal region – causing a rupture through the weakest point of the epidermal tissue (Fig.1B) (Beauzamy *et al.*, 2014). The pollen tube growth through the style of a gynoecium is an active and turgor driven phenomenon. In growing pollen tubes, SUC1 transporters import sucrose causing reduction of water potential and in turn increase in turgor pressure (Fig.1C). Several other osmolytes such as proline and hydroproline help the pollen tube in turgor driven growth inside the style of a carpel (Beauzamy *et al.*, 2014). During the late maturation stages, flowers lose their turgidity causing the petals to wilt and loss of membrane integrity, which in turn results in leakage of nutrient and ions from cells that may trigger flower senescence (Reid and Chen, 2007).

Role of reactive oxygen species (ROS) in initiation of flower senescence

Senescence is a concerted phenomenon occurring at the terminal developmental phase of a plant.

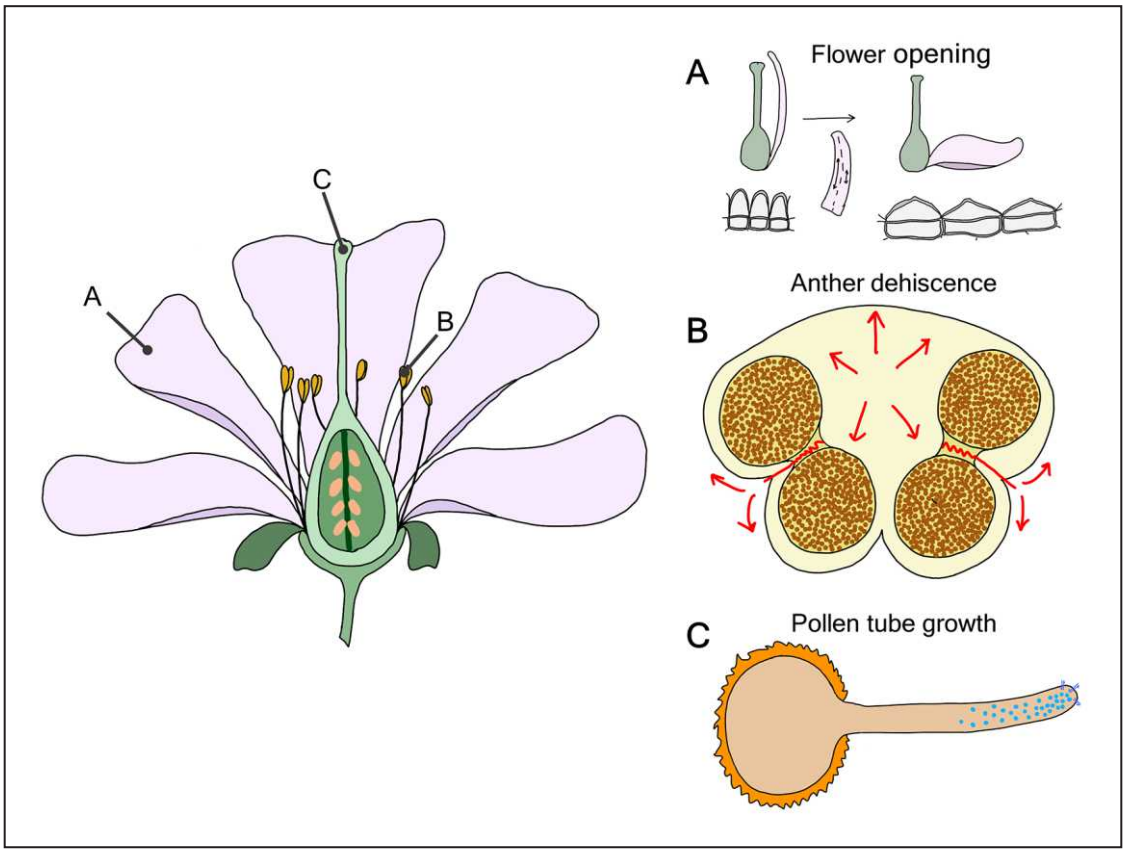


Fig. 1: Illustration showing the role of water in flower maturation. Illustration depicting the role of water in three major events during flower maturation – flower opening **(A)**, anther dehiscence **(B)** and pollen tube growth **(C).**

Senescence in a flower petal is commonly manifested by drooping, wilting, curling and changes in coloration (Rogers and Munné-Bosch, 2016). Several studies have shown reactive oxygen species orchestrate the events by altering the redox balance of the cells (Table 1) (Rogers, 2011, 2012). ROS partake in several metabolic reactions and are key regulators of growth, development and defense pathways in plants. However, ROS are toxic by-products of aerobic reactions and their levels are to be carefully regulated with the help of several metabolites and enzymatic antioxidants to protect the cell from oxidative damages (Rogers, 2011). The enzymatic antioxidant system in vivo comprises of superoxide dismutases (SOD) – located in the mitochondria and cytosol which dismutates O_2^- to H_2O_2. Another enzymatic antioxidant viz. catalases (CAT) that are present in peroxisomes and glyoxysomes converts H_2O_2 into H_2O. Further, the enzymes of Halliwell-Asada pathway, such as ascorbate peroxidase, monodehydroascorbate peroxidase, dehydro-ascorbate reductase and glutathione reductase that are present in the chloroplasts, cytosol, mitochondria and peroxisomes convert the H_2O_2 to water and molecular oxygen (Rogers, 2011). These ROS detoxifying enzymes fine-tune the redox status of cells by controlling the accumulation pattern of ROS during floral maturation, which ultimately translates into flower opening and senescence (Rogers, 2011, 2012; Jhanji *et al.*, 2025). In daylily it was observed that the endogenous H_2O_2 level increases with aging (Panavas and Rubinstein, 1998). Additionally, they reported increased SOD activity and decreased APX and CAT activity with aging. Similarly, in *Murraya paniculata* flowers, SOD activity increased with age whereas, CAT and APX activity exhibited a decreasing trend (Paul *et al.*, 2019). In cut carnations, increased superoxide radical production was observed with aging. The accumulation of superoxide radicals may result from the decrease in APX activity as carnation flowers move towards senescence (Zhang *et al.*,

Table1. Common ROS occurring in biological systems (Murphy *et al.*, 2022)

ROS	Chemical formula	Reactivity
Superoxide radical anion	$O_2^{\bullet-}$	Selectively reactive, does not attack most biological molecules.
Hydrogen peroxide	H_2O_2	Unreactive with most biomolecules. Reacts slowly with most thiols.
Hydroxyl radical	$^{\bullet}OH$	Indiscriminately reactive; reacts with whatever is adjacent to it at near diffusion-controlled rates.
Singlet and triplet oxygen	$^1O_2$, $^3O_2$	Least reactive. Singlet state is more reactive than ground-state triplet O_2.

2006). These indicate that the ROS molecules play a major role in positively controlling the senescence processes in flowers (Fig. 2).

Changes in central metabolism during flower maturation

Metabolically, petals are mixotrophic. Many buds bearing young petals are green and photosynthetically active. With physiological maturity, buds progressively lose their ability to fix carbon and turn completely heterotrophic (Thomas *et al.*, 2003). This transition was also reported by Müller *et al.* (2010) in *Nicotiana tabacum* flowers where the corolla of young *N. tabacum* flowers are photosynthetic as indicated by the presence of chlorophyll and active RuBisCO enzyme. Post anthesis, the photosynthetic ability progressively decreases and petals begin accumulating specialized metabolites such as anthocyanins, carotenoids, flavonols and scent volatiles as required. Additionally, during terminal maturation phase flowers may acquire the sink status as they become site for catabolism after pollination (Rogers and Munné-Bosch, 2016).

Changes in the levels of storage polysaccharides and their breakdown products

Some storage polysaccharides (such as starch) play a crucial role in maintaining biochemical and in turn physiological changes during flower maturation. Starch is a reserve polysaccharide involved in carbohydrate pool in plants. These polysaccharides

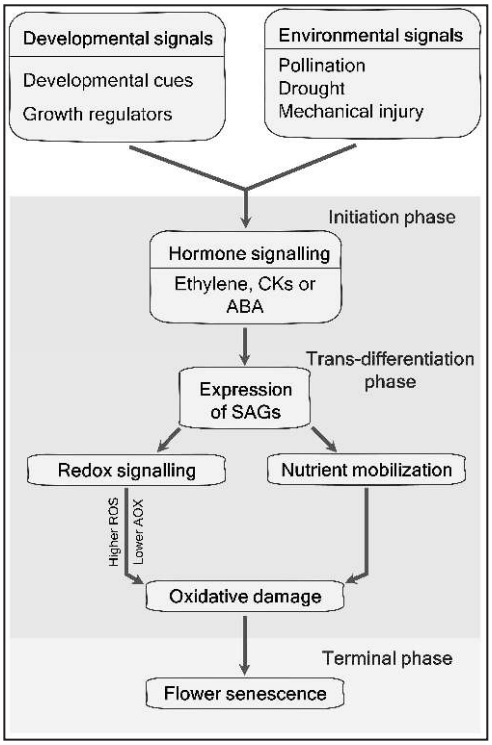


Fig. 2: A schematic model depicting ROS-dependent regulatory processes in floral senescence. Both internal and external stimuli can induce senescence by changing hormonal balance within the cells. Further, transcriptional activation of Senescence Associated Genes (SAGs) triggers redox signaling by altering ROS balance within the cells and nutrient remobilization from the senescing tissues towards other organs. During senescence, several enzymes namely nucleases, proteases, oxidative and cell wall degrading enzymes are produced. Collectively these enzymes push the tissue to the terminal developmental phase and symptoms of senescence become visible. Internally, cells undergo oxidative damage and ultimately enter PCD.

can act as both molecular 'source' and 'sink', depending on the developmental phase of a plant (MacNeill *et al.*, 2017). Plants being energetically conservative accumulate carbon resources which are available before initiating floral transition. The plants will only commit to reproduce if there is enough stored energy available within vegetative tissues for setting viable seeds successfully. In rose buds, substantial increase in starch accumulation observed during initial developing stages and a pattern of gradual starch degradation was observed in the later developmental stages (Kumar *et al.*, 2008). Similarly, a steady decline in the starch content with floral maturation in carnation petals was also observed (Tirosh and Mayak, 1988). Breakdown of the storage polysaccharides during anthesis not only provides necessary osmolytes for petal unfurling, but also necessary for driving the biochemical changes occurring after the onset of petal opening. However, flowers do not rely only on the breakdown products of storage polysaccharides for running its physiological processes; it also depends on the mother plant for continuous supply of carbon pool which they receive in form of sucrose. The sink strength of the floral tissues depends on the activity of sucrose metabolizing enzymes such as sucrose synthase (SuSy) and invertases (Borghi and Fernie, 2017; Borghi *et al.*, 2022). Both of the enzymes are actively taking part in breakdown of sucrose into simpler sugars at the sink tissue, thus keeping a difference in the sucrose gradient at both source and sink ends. The hydrolysis of sucrose is catalyzed by invertases belonging to the β-fructofuranosidase

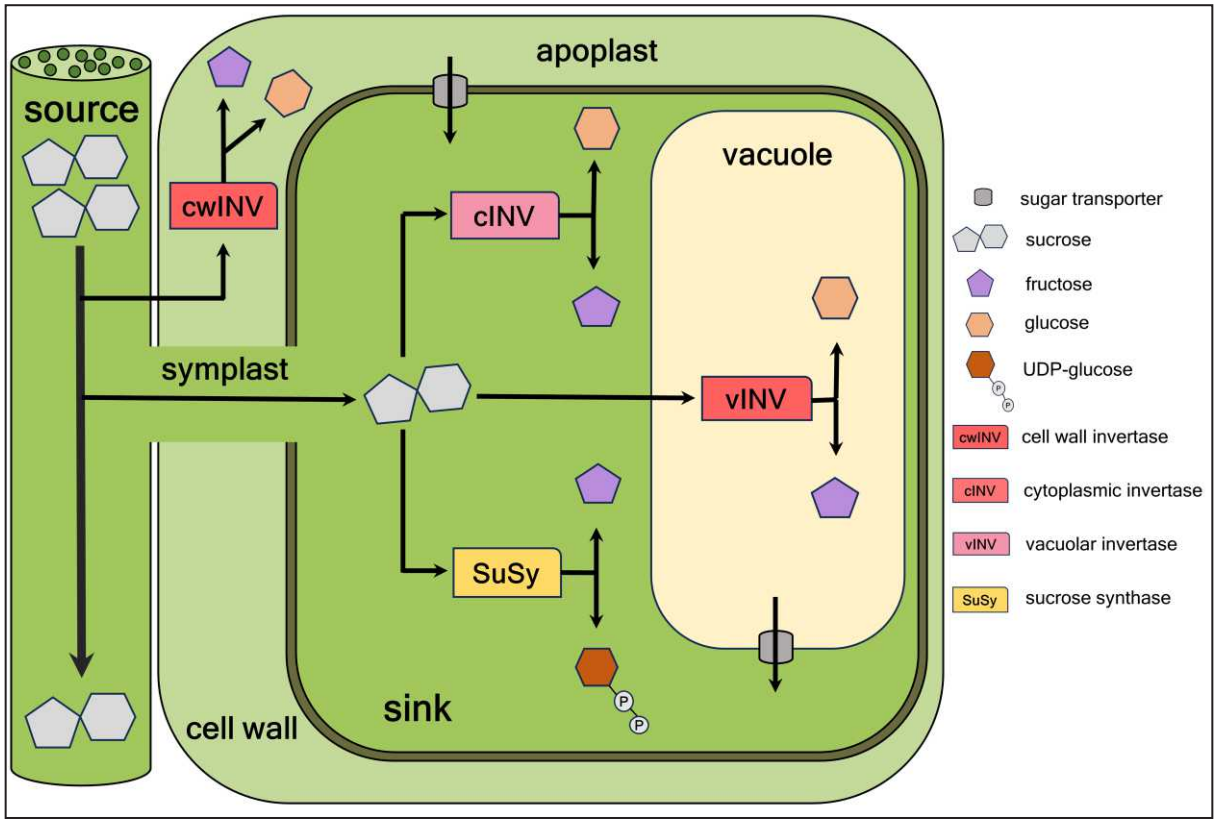


Fig. 3: Sucrose catabolism within a sink cell. Sucrose from the terminal phloem can enter the sink via symplastic (through plasmodesmata) or apoplastic routes. Cell wall invertase (cwINV) in the apoplast hydrolyses sucrose to glucose and fructose. Sugar transporter proteins ferry hexoses into the cytoplasm. In the cytosol, sucrose is hydrolysed by cytosolic invertase (cINV) to glucose and fructose and by sucrose synthase (SuSy) to fructose and UDP-glucose. The sucrose molecules can enter vacuole where they are further hydrolysed by vacuolar invertase (vINV) to glucose and fructose. The sugar transporter proteins carry the hexoses from vacuole to the cytoplasm.

family. They catalyze the hydrolysis of terminal fructose moieties from disaccharides and polysaccharides. Depending on the localization of these enzymes, they are categorized into three classes – cell wall, cytosolic and vacuolar invertases. Another enzyme, SuSy, provide plasticity in sucrose metabolism by creating an alternative route for sucrose metabolism (Rees, 1995). This enzyme is a glycosyltransferase that catalyzes a reversible cleavage of sucrose into fructose and UDP-glucose. Further, the sucrose pool can be modulated by a few sucrose metabolizing enzymes like sucrose phosphate synthase (SPS) and sucrose phosphatase (SPP). Therefore, the apparent redundancy in carbohydrate metabolic pathways can be linked to the developmental stage and energy requirements of a particular plant tissue (Stitt, 2013).

When the transition of flowers occurs from the autotrophic bud stage to the late heterotrophic stages, the floral tissues may get a stronger sink status and hence more nutrients are cargoed from the mother plant. In floral tissue the sugars are unloaded through both – symplastic and apoplastic routes (Fig. 3).

The symplastic transport primarily depend on the functional plasmodesmatal connections, whereas, the apoplastic transport relies on energy driven intermediary transporters. Sucrose loading into the phloem occurs by a coordinated activity of SUGARS WILL EVENTUALLY BE EXPORTED TRANSPORTER (SWEET) and SUCROSE UPTAKE CARRIER (SUC) or SUCROSE UPTAKE TRANSPORTER (SUT) – SUC/SUT carriers. SWEETs are efflux proteins of the plasma membrane, tonoplast and Golgi that transport sucrose and hexoses, and SUC/SUTs are plasma membrane-localized proton-sucrose symporters. Additionally, SUGAR TRANSPORTER PROTEINs (STPs) are localized in the plasma membrane also uptake hexoses from the apoplasmic space (Table 2) (Borghi and Fernie, 2017). Young flower petals possess functional plasmodesmata. Therefore, symplastic mode of sucrose transport can be regarded as the major route of carbon gain. High expression of the proton-monosaccharide symporter SUT13 that transports glucose, fructose and other D-hexoses in the vascular tissues of young petals were reported in Arabidopsis (Nørholm *et al.*, 2006).

Table 2. Sugar transporters expressed in various parts of the flowers of Arabidopsis and substrates transported (Borghi and Fernie, 2017)

Name	Organ or Tissue of Expression	Substrate	References
STP1	Whole flower	Glucose, galactose, mannose, xylose, 3-O-methylglucose, fructose	Sauer *et al.*, (1990)
SWEET2	Sepal, petals, stamen	2-Deoxyglucose	Chen *et al.*, (2015)
SWEET4	Whole flower, stamen	2-Deoxyglucose	Chen *et al.*, (2015)
SWEET1	Pollen grain, pollen tube, petals, sepals	Glucose, galactose	Chen *et al.*, (2010)
STP13	Sepals, vasculature of emerging petals	Glucose, fructose, galactose, mannose	Bi *et al.*, (2005); Nørholm *et al.*, (2006)
SUC8/SUT8	Transmitting tissue	High-affinity sucrose, maltose	Sauer *et al.*, (2004)
SUC4/SUT4	Anthers, pistil, tonoplast	Sucrose (low-affinity transporter)	Weise *et al.*, (2000); Schneider *et al.*, (2011)
SUC3/SUT2	Carpel, pollen grain, and pollen tube	Sucrose, maltose	Meyer *et al.*, (2000, 2004)
SWEET13/RPG2	Tapetum, tetrad	Sucrose	Sun *et al.*, (2013)
SWEET9	Nectary	Sucrose	Ge *et al.*, (2000); Lin *et al.*, (2014)

Changes in the levels of simple sugars

Floral buds are reservoirs of storage carbohydrates. The reserves are steadily accumulated during bud development and rapidly utilized at the time of petal opening to provide the cells a pool of osmolytes which will cause water influx in the petal tissue leading to flower opening (Fig. 4) (Borghi and Fernie, 2017). Androecium and gynoecium are completely heterotrophic and rely on the source tissue for sustenance. Anthers remain symplastically attached to the flower via the filament, which, acts as a channel for the transport of photoassimilates from the source (Mascarenhas, 1989). The obtained carbon is stored as starch granules and soluble sugars (mostly sucrose) in the tapetum of the developing anther (Goldberg *et al.*, 1993). Enzymatic breakdown of these sugars provide energy for the anther maturation. In contrast, mature pollen gains are symplastically isolated from the flowers and rely on apoplastic route for sugar intake. Pollen grains depend on sugars and their specific transporters for development, germination and pollen tube growth. Additionally, the sugars are also involved in the synthesis of pollen cell wall. Pollen grains also store starch in amyloplasts. Once the pollen is ready to be shed, the anther wall needs to be ruptured. This also depends on sugar metabolism leading to increase in osmotic potential of the cells situated between the anther and filament (Borghi and Fernie, 2017). Nectar along with pollen forms a major pollinator attractant. Nectar is a sweet aqueous secretion which plays a vital role in mediating interactions between plants with pollinators and defenders. Nectar is majorly made up of water, sugars and amino acids. It may contain specialized metabolites and antimicrobial proteins. The nectaries possess several sugar metabolizing enzymes such as invertase, sucrose phosphate synthase (SPS) and sucrose synthase which provides the sink status to the nectary tissues. These enzymes in turn catalyze the secretion of sugar rich nectar (Heil, 2011; Roy *et al.*, 2017). As previously mentioned, after pollen grains land on the carpel, they rehydrate, mobilize the sugar resources and pollen tubes emerge out of them (Rounds *et al.*, 2011). Several simple

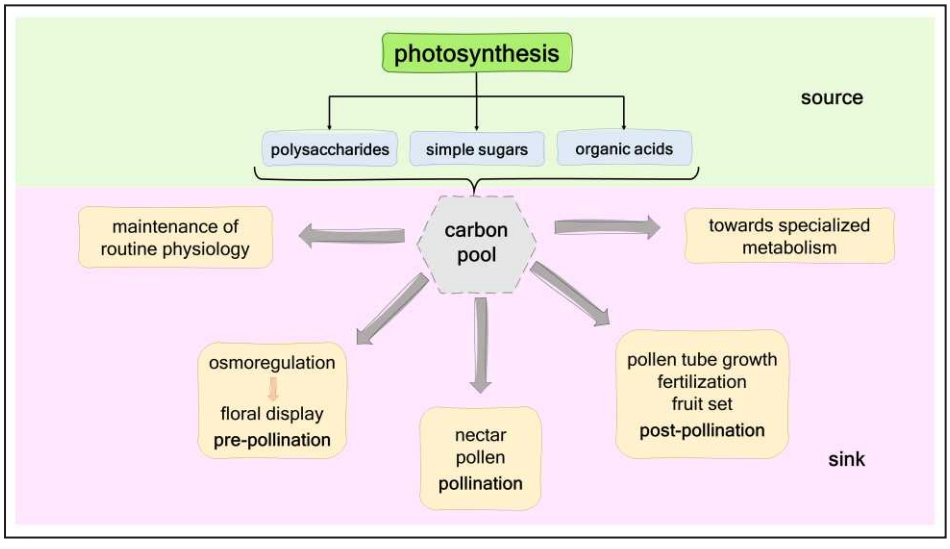


Fig. 4: Schematic representation of diversion of carbon pool in floral tissues. Major end products of photosynthesis such as polysaccharides, simple sugars and organic acids contribute to the carbon pool of a sink tissue. The utilization of the carbon pool depends on the physiological and developmental needs of the sink tissue. In case of flowers, the carbon pool is directed towards various processes such as - maintenance of routine physiology by sustaining cellular respiration and subsequent energy production required for floral organ sustenance; pre-pollination event of floral display mediated by osmoregulation; pollination events like formation and secretion of nectar and pollen; post-fertilization events like pollen tube growth, fertilization and fruit set ensuring successful reproduction; a part of the carbon pool also initiates the synthesis of various specialized metabolites that aid in reproduction, defense and stress tolerance.

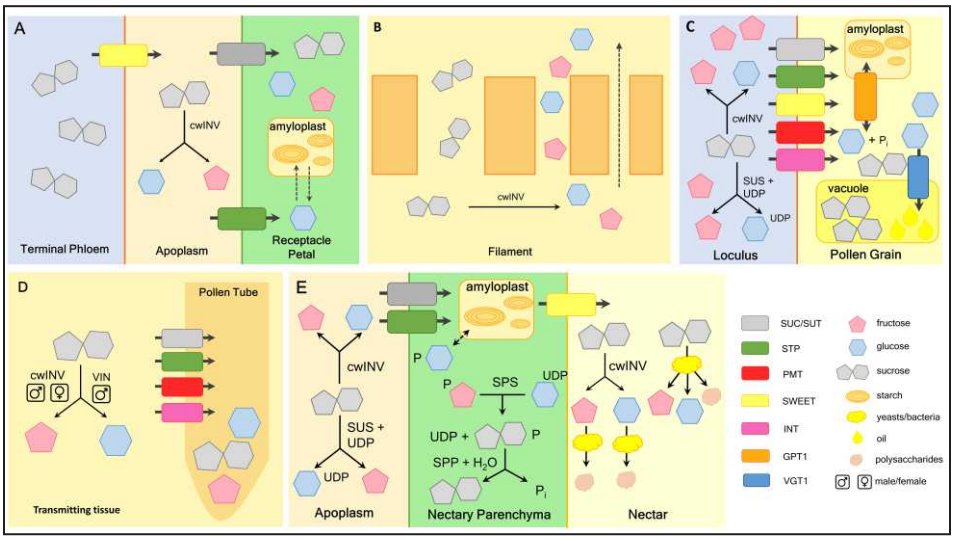


Fig.5: Schematic representation of carbohydrate partitioning among various floral tissues. Sucrose from terminal phloem enters the apoplastic space likely via SWEET protein transporters. Cell wall invertase hydrolyses the sucrose into glucose and fructose. SUCROSE TRANSPORTERS (SUTs) or SUCROSE CARRIERS (SUCs) ferry sucrose and hexoses into the receptacle cells. Receptacle cells store carbohydrates as starch in the amyloplasts (A). Sucrose and hexoses are transported to the anther via plasmodesmata (B). During pollen grain development, glucose-6-phosphate is transported across the membrane of amyloplasts by GLUCOSE PHOSPHATE TRANSPORTER1 (GPT1). Later, glucose produced by the amylolysis is transported to the vacuole by VACUOLAR GLUCOSE TRANSPORTER1 (VGT1) (C). Invertases specific to pollen grain and stigma produce hexoses which aid in pollen tube elongation (D). Amylolytic product sucrose from the nectary parenchyma is hydrolyzed by cell wall invertases to hexoses prior to anthesis leading to the secretion of sugary nectar. Some colonizing microbes in the nectar also add fermented sugars to the nectar (modified from Borghi and Fernie, 2017).

sugars fuel the process by acting as osmolytes which in turn facilitate pollen tube growth and elongation (Fig. 5) (Konar and Linskens, 1966). The combined activity of cell wall invertases of the pollen tube cell wall and vacuolar invertases of the cells of style aid in pollen tube elongation (Borghi and Fernie, 2017).

Similar to anthers, the carpels are also symplastically attached to the flower and receive sucrose from the mother plant and stores it in the form of transitory starch throughout its tissue. The style however, takes up post-phloem sucrose via both symplastic and apoplastic routes. After fertilization, huge amounts of sugars are transported to the endosperm, which subsequently nourish the developing embryo. Nonetheless, the unutilized energy resources present in the different whorls of the flower including nectar, are promptly repurposed by reabsorption and efficient mobilization (Borghi and Fernie, 2017).

Changes in the levels of amino acids

Amino acids are organic forms of nitrogen which fuel different aspects of floral physiology. They are utilized for the synthesis of enzymes, structural proteins and serve as precursors for nitrogen-containing specialized metabolites and signaling molecules. Similar to sugars, the transport of amino acids and some small peptides follow both symplastic and apoplastic route. The vascular tissues ferry them from source to sink (Borghi and Fernie, 2017). However, de novo synthesis of amino acids also can take place in the flowers. Extensive studies on *Petunia hybrida* floral benzenoid volatile synthesis have identified the presence prephenate amino transferase and arogenate dehydratase of phenylalanine biosynthetic pathway to be present in floral tissues. The studies also identified the CATION AMINO ACID PHENYLALANINE TRANSPORTER operating in the plastids. (Maeda *et al.*, 2010; Maeda and Dudareva, 2012; Widhalm *et al.*,

2015). Similar to phenylpropanoid biosynthesis, flowers also possess active asparagine biosynthetic pathway. *ASPAGINE SYNTHASE1 (ASN1)* encodes enzyme that transfers amide nitrogen from glutamine to asparagine. In *Arabidopsis thaliana* flowers, increased levels of *ASN1* transcripts are observed starting from pre-anthesis stage till pollination and even during early embryo development. Asparagine is an efficient nitrogen carrier molecule and storage compound owing to its high nitrogen: carbon ratio. Therefore, continuous supply of asparagine throughout flower maturation ensures a reliable energy reserve (Gaufichon *et al.*, 2017). Additionally, in flowers, proline is found in pollen grains and nectar (Biancucci *et al.*, 2015). This imino acid prevents the desiccation of pollen grains via osmoprotection (Chiang and Dandekar, 1995). Proline may aid in the flight of insect pollinators, in turn ensuring pollination that signifies its unique ecological role (Carter *et al.*, 2006). Conversion of glutamine to pyrroline-5-carboxylate is an essential step in proline biosynthesis in flowers. Pyrroline-5-carboxylate synthase, which catalyses the above conversion, was found to be upregulated in floral tissues (Kishor *et al.*, 2015). Additionally, increased floral proline was attributed to the transcriptional activation of proline transporters (Schwacke *et al.*, 1999).

Volatiles and pigment metabolism in flowers

Floral scent and colour inmost angiosperms collectively act to attract general plant pollinators (Raguso and Weiss, 2015). Both the attributes have unique phenotypic manifestation due to underlying biosynthesis and accumulation patterns. While floral colour provides visual cues of communication, floral scent volatiles act asinvisible cues ultimately mediating plant-environment interaction (Raguso and Weiss, 2015; Kutty *et al.*, 2021). Additionally, plants also reward the pollinators with sugar-rich nectar and nutritious pollen. Overall, the following specialized metabolites - floral scent, colour, nectar and pollen act in unison to ensure the reproductive success of the producer plant. Primary metabolites such as sugars and amino acids act as precursors and provide energy for synthesis of these specialized metabolites (Borghi and Fernie, 2017).

Changes in scent volatiles landscape during floral maturation

Floral volatiles are lipophilic organic compounds of low molecular weight and high vapour pressure. The floral volatiles are mostly classified based on their chemical structures into terpenoids, phenyl-propanoids/benzenoids, and fatty acid derivatives (Pichersky, 2006). Terpenoids constitute a large group of specialized metabolites originating from the five-carbon universal precursor isopentyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) (Tholl *et al.*, 2006). These two molecules are produced by two alternative biochemical pathways- mevalonic acid (MVA) route and methylerythritol phosphate (MEP) route (Dudareva *et al.*, 2004). MVA pathway starts with condensation of two molecules of acetyl-CoA, whereas, one molecule of pyruvate and one molecule of glyceraldehyde-3-phosphate joins enzymatically and begin MEP pathway. Monoterpene, sesquiterpene and diterpene volatiles are produced by these pathways and the catalytic conversion is mediated by a large enzyme family, the terpene synthases (TPSs). TPSs are responsible for producing diverse terpene volatile compounds and these enzymes can use a single substrate to produce multiple terpene volatiles compounds (Degenhardt *et al.*, 2009). Phenylpropanoids/benzenoids are also a diverse and large family of specialized metabolites that not only produce floral volatiles but also specialized metabolites like lignin, polyphenols and flavonoids. Phenylpropanoids are biosynthesized from aromatic amino acid L-phenylalanine (Phe), a product of shikimate pathway operating in cytosol and plastids. The first enzymatic step of phenylpropanoid pathway involves phenylalanine ammonia-lyase enzyme (PAL) that catalyzes conversion of Phe to *trans*-cinnamic acid, the precursor for phenylpropanoid/benzenoid volatile synthesis (Tohge *et al.*, 2013). Fatty acid derivatives that act as volatiles are products of lipoxygenase (LOX) pathway. Unsaturated fatty acids such as linolenic and linoleic acids are converted through this pathway into volatile fatty acid derivatives using either allene oxide synthase (AOS) or hyperoxide lyase (HPL) (Feussner and Wasternack, 2002).

The scent volatile bouquet emitted by flowers can vary based on plant species, stage of development, time of the day or pollination status (Dudareva *et al.*, 2000; Ghissing and Mitra, 2022). Evolutionarily plants have synchronized their floral scent emission in such a way that maximum scent emits when potential pollinators show maximum activity. Thus, the plants that are pollinated by nocturnal insects emit volatiles maximally during the night, whereas for plants pollinated by day-active-insects, diurnal rhythmicity is observed (Schiestl and Ayasse, 2001; Pott *et al.*, 2002). Loughrin *et al.* (1990) showed composition of floral scent bouquet can change based on the time of day, i.e., a plant can emit one set of volatiles during the day and another set is released during the night. Plants have devised this synchroneity to attract different sets of pollinators in a span of 24 h to ensure successful pollination. A circadian, diurnal rhythmicity was reported to exist in the emission of volatile compounds by *Rosa hybrida* cv. Honesty. The rhythmicity was hampered when the flowers were exposed to continuous darkness thereby signifying the incidence of light as the 'Zeitgeber' for the observed circadian rhythmicity (Helsper *et al.*, 1998). Thediversity of the volatile compounds prompts biologists to understand various levels of regulation that might exist at the biosynthesis, storage and release stages. At biosynthesis level, a complex spatio-temporal pattern of enzyme activities involved in scent production in flowers exist. Two such patterns were reported for enzymes involved in the production of benzenoid esters of the fragrant *Clarkia breweri* flowers (Dudareva *et al.*, 1998). Key rate-limiting enzymes of volatile biosynthesis pathways are active during the pre-anthesis phase of the flowers (Datta *et al.*, 2024). This is possibly to ensure the exact timing of floral scent emission. Additionally, the control of rhythmic scent emissions also depends on the level of substrate molecules. In snapdragon flowers, the endogenous substrate pool of benzoic acid exhibited a diurnal fluctuation (Kolosova *et al.*, 2001a). The biosynthesised volatiles are transiently stored in their respective glycosylated forms, subsequently hydrolysed and released at the anthesis (Barman and Mitra, 2018). The volatiles when constantly synthesized can neither stay in the cytoplasm nor in the vacuole due to their lipophilic nature. Therefore, they are stored in specialized lipohilic vacuoles (Hudak and Thompson, 1997). They can also be glycosylated and stored in hydrophilic compartments as observed in *Rosa damascena* (Oka *et al.*, 1999). In *Antirrhinum majus*, *C. breweri* and *Murraya paniculata*, scent volatiles are synthesized in the epidermal layers of petals (Kolosova *et al.*, 2001b; Datta *et al.*, 2024), whereas in tuberose, it was reported to be synthesized in cells surrounding the floral stomata for efficient emission (Maiti and Mitra, 2017).

Changes in accumulation patterns of pigments during floral maturation

Flowers are the most commonly found chromatic elements in the natural world. Three main classes of pigments responsible for flowercolouration are flavonoids/anthocyanins, betalains and carotenoids (Tanaka *et al.*, 2008).

Flavonoids, a group of specialized metabolites belonging to the class of phenylpropanoids, have the widest colour range, from pale yellow to blue. In particular, anthocyanins, a class of flavonoids, are responsible for the orange-to-blue colors found in many flowers, leaves, fruits, seeds and other tissues (Bueno *et al.*, 2012). They exhibit a broad distribution across the plant kingdom, displaying solubility in water and storage within vacuoles. About 19 distinct types of anthocyanidins, which serve as aglycones or chromophores of anthocyanins have been identified. However, among these, only six major anthocyanidins are most abundant - pelargonidin, cyanidin, peonidin, delphinidin, petunidin and malvidin (Mikulic-Petkovsek *et al.*, 2014).The color exhibited by anthocyanin depends on factors such as pH, the presence of colorless co-pigments (typically flavones and flavonols), and the influence of metal ions (Tanaka *et al.*, 2008). Glycosides of these molecules are usually transported to the vacuoles, where the pH of that subcellular compartment determines the colour exhibited by these molecules (Marrs *et al.*, 1995; Kitamura *et al.*, 2004). Cyanidin 3-O-glucoside is responsible for the gradual colour change from white to red in *Combretum indicum* petals (Ghissing *et al.*, 2023).

Carotenoids are isoprenoid compounds primarily consisting of C_{40} backbone with polyene chains that can possess as many as 15 conjugated double bonds (Shumskaya and Wurtzel, 2013). Carotenoids are responsible for yellow or golden color in flowers like *Tagetes*, *Narcissus* and *Calendula*. Carotenoids exhibit distinct characteristics from anthocyanins and betalains, as they fulfil crucial functions in plant physiology. For example, they serve essential roles in photoprotection during photosynthesis and act as substrates for the biosynthesis of abscisic acid (Green and Durnford, 1996; Niyogi, 2000). The pathwayinitiates with the condensation of geranylgeranylpyrophosphate, a precursor derived from the upstream MEP pathway, resulting in the formation of the C_{40} compound namely 15-*cis*-phytoene. This enzymatic step is facilitated by phytoene synthase, an enzyme that acts as a rate-limiting factor in the pathway (Shumskaya and Wurtzel, 2013). The carotenoid biosynthetic pathway enzymes are mostly present inside the plastid associated with the membrane. The C_{40} backboneand conjugated double bondarrangement renders them susceptible to enzymatic or non-enzymatic oxidative cleavage leading to the synthesis of apocarotenoids (Wang *et al.*, 2021). Physiologically, these apocarotenoids can act as pigments, scent volatiles, phytohormones and signaling molecules (Ilg *et al.*, 2014). Crocin, crocetin, β-citraurin and bixin function as apocarotenoid pigments in plants. β-citraurin confers the orange-reddish colour in the peel of citrus fruit (Zheng *et al.*, 2019). *Bixa orellana* seeds contain the red-coloured pigmentbixin (Pereira and Campos, 2019; Pacheco *et al.*, 2019). Crocin and crocetin are the main pigments responsible for the red colourationin saffron stigma (Sánchez and Winterhalter, 2013). Crocin also determines the orange-red colour in *Gardenia jasminoides* fruit and the pale-yellow color in petal tissues (Sommano et al., 2020).

Betalains, a distinct group of compounds, manifest vivid hues in the flowers and fruits of plant species within the Caryophyllales families (Khan and Giridhar, 2015). Betalains are classified into red/crimson betacyanins and yellow betaxanthins. They form immonium conjugates through the linkage of betalamic acid with cyclo-dihydroxyphenylalanine glucoside and amino acid amines, respectively (Strack *et al.*, 2003). Tyrosine functions as the precursor for these pigment molecules and similar to anthocyanins, glycosylation constitutes a crucial biosynthetic step in the production of betalains (Heuer and Strack, 1992; Yamamoto *et al.*, 2001).

Changes in pigment accumulation affect scent emission during floral maturation

Flowers of several plants undergo changes in colouration after opening. Post-anthesis colour change was observed in *Lantana camara* flowers. Mohan Ram and Mathur (1984) had observed that freshly opened flowers were yellow which became orange after 20 h. A further shift in colour to scarlet was noticed in next seven to eight hours followed by magenta after 26 hours. Upon analysis, the flower pigments were identified as delphinidin monoglucoside and β-carotene. In the initial stages of floral maturation, the amount of carotenoid was found to be higher than anthocyanin. A spurt of anthocyanin production occurred after pollination. The shift in colour from orange to scarlet and magenta was possible because of higher accumulation of anthocyanin relative to carotenoids which mask the latter pigments. This work further established that the pollination was the triggering factor for colour change. It was argued that the phenomenon of postpollination colour change could be an advantage in regulating the visitations by pollinators and thus promote seed setting without any disturbance, which in other way help in conserving pollinator energy (Mohan Ram and Mathur, 1984).

Raguso and Weiss (2015) throw further light on concerted changes in floral colour and scent collected from several colour-changing flowers including *L. camara*. They observed that the older, colour-changed flowers emit reduced amounts of linalool and its oxides, along with common aromatic esters and alcohols, whereas abundant sesquiterpene volatiles emitted by floral calyces and other vegetative organs of the plant that did not show any temporal change. While these compounds were not the most abundant floral volatiles, these chemicals responded to

behavioral and electrophysiological–olfactory activities for quite a few species of butterflies (Raguso and Weiss, 2015). Therefore, the dynamic patterns of floral scent emission, which often are overlooked, can identify opportunities for further advancement in these aspects.

Brunfelsia calycina (yesterday–today–tomorrow) is a shrub belonging to the Solanaceae family and is a native to Brazil. The flowers undergo a very rapid colour change: petals unfurl in a dark purple colour, and within three days turn completely white. Because of this rapid colour change, their common name is yesterday–today–tomorrow (Heide, 1963). The decrease in pigment concentrations in *B. calycina* flowers (from dark purple to white) was found to be very rapid, and occurred at a specific and well-defined stage of floral maturation. Active anthocyanin degradation was established as a unique phenomenon occurred in the living flowers of *B. calycina*. It was revealed that colour change in *B. calycina* flowers depends on de novo synthesis of protein (Vaknin *et al.*, 2005). Their study demonstrated that the degradation of anthocyanins was not part of the general senescence process of the flowers but rather a distinctive and specific pathway that involved oxidative enzymatic reactions mediated by peroxidase (Vaknin *et al.*, 2005).

A further study was undertaken by the same group to test whether the biosynthesis of fragrant benzenoids in *B. calycina* flowers depends on the induction of shikimate pathway, or if these volatile benzenoids are formed from the anthocyanin degradation products (Bar-Akiva *et al.*, 2010). If phenolic acids are to be originated from the degraded anthocyanins to serve as precursors for benzenoid and lignin, then these anthocyanin degradation products they would have to be transported out of the vacuole. However, they failed to demonstrate the export of anthocyanin breakdown products, such as the phenolic acids, from the vacuoles. It further remained unclear if the biosynthetic enzymes producing benzenoids in *B. calycina* are expressed in the epidermal cells of the flowers, where anthocyanin pigments usually accumulate (Bar-Akiva *et al.*, 2010).

Osmanthus fragrans Lour. is a member of Oleaceae family highly valued for its bright flower colour and sweet floral scent. Carotenoids are the major pigments determining the floral colour variation in different *O. fragrans* cultivars. Most cultivars of sweet osmanthus bloom in autumn when temperature fluctuates greatly; this may cause changes in floral colour of *O. fragrans* between different years. Therefore, Wang *et al.* (2022) examined if high or low temperature regulates carotenoid accumulation in the flowers of *O. fragrans* needs, resulted in changes of floral colour. They found that high temperature (32°C) changed the floral colour from yellow to yellowish white when compared to the control temperature (19°C), while low temperature (15°C) was shown to turn the floral colour from yellow to pale orange. Further, their study also demonstrated that the total carotenoid content and the content of individual carotenoids (α-carotene, β-carotene, α-cryptoxanthin, β-cryptoxanthin, lutein and zeaxanthin) were inhibited by high temperature, but were enhanced by low temperature. Through gene expression analysis it was confirmed that higher temperature suppressed the floral coloration by repressing the expression of carotenoid biosynthesis genes, and activating the expression of carotenoid degradation genes (Wang *et al.*, 2022). On the contrary, the relative low temperature had opposite role on floral colouration and carotenoid biosynthesis in *O. fragrans*.

Combretum indicum (L.) DeFilipps (syn. *Quisqualis indica* L.) is a well-known perennial liana possesses pendulous racemic inflorescences that bear prominent colour-changing flowers with a gentle fragrance. As a dichogamous and self-incompatible species, attraction of potential pollinators to the *C. indicum* flowers is a requirement for effective seed production. The white (colourless) flowers open in the evening, and change their colour to red through pink initiated on receiving sunlight in the next morning (Yan *et al.*, 2016). Cyanidin 3-*O*-glucoside was identified as the major anthocyanin accumulating in the petals. Acid hydrolysis of anthocyanin extracts further confirmed cyanidin as the major anthocyanidin in floral tissue (Ghissing *et al.*, 2023a). *C. indicum* emits maximal scent in the dark from the colourless white flowers, while minimal emission was observed during the

daytime when the flower gradually changes its colour. A synergistic interaction between temporal scent emission and synchronized developmental changes in colour was clearly observed in the floral tissue (Ghissing *et al*., 2023b).It was further revealed thata dynamic movement of sugars from the source tissue guides the morphological and metabolic transformations via shared biosynthetic pathways. In addition to the advocated painted and scented metabolites, the flower also harbours a considerable amount of nectar in the long green floral tube. A possible pathway for nectar production, transport, and exudation was also mapped by histochemical analysis of the nectariferous tissue (Ghissing *et al*., 2025).

Gardenia carinata Wall. ex Roxb., a member of family Rubiaceae, is one of the popularly cultivated plants well known for its colour changing fragrant flowers. Flowers of *G. carinata* change their colour from white to saffron as they approach senescence. We found the yellow colouration in petals is caused by accumulation of crocins, a carotenoid cleavage product commonly found in saffron stigma. The content of crocins can be more than 10% of the dry mass of the floral tissue (Ghosh and Mitra, unpublished).

Study of emitted volatiles showed the bouquet is mostly dominated by terpenes and phenylpropanoid compounds. Predominantly, emission of these flowers showed a nocturnal pattern. However, the latter yellow stages showed constitutive emission of terpenoid volatiles from the flowers. Carotenoid cleavage volatiles such as cyclo-geraniol and β-cyclocitral was also found in high abundance in the yellow stages. We also observed that the colour changing events in *G. carinata* petals were mostly orchestrated by temperature. Increase in air temperature was shown to accelerates the accumulation of pigments in *G. carinata* floral tissues (Ghosh and Mitra, unpublished). There exists a fine coordination between the synthesis of pigments and scent volatiles which needs to be elucidated.

CONCLUSION

The process of floral maturation encompasses the transition of bud to open flowers and ultimately senescence. Various physiological factors work in co-ordination to achieve successful flower opening and subsequent maturation. Constant variation occurs in the central metabolism to fuel various events encompassing flower maturation. Volatiles and pigments work in a time-bound manner with floral maturation to attract plant pollinators. Anin-depth understanding of the process of floral maturation provides fundamental insights into the fascinating world of floral biology, aid pollination biologistsin selection of floral traits and the horticulturists in prolonging the lifespan of flowers by deferring senescence.

ACKNOWLEDGEMENT

Research on floral scent biology at the authors' laboratory was funded by the Anusandhan National Research Foundation (erstwhile Science and Engineering Research Board), India [CRG/2022/002971, EMR/2015/000247], the Council of Scientific and Industrial Research, India [38(1420)/16/EMR-II, 38(1336)12/EMR-II]and the Department of Science and Technology, Government of India [DST/SSTP/2018/171, INT/RUS/RFBR/P-329].

DISCLAIMER

The author(s) declare no conflict of interest in the work and no funding from any funding agency was used for writing this review.

REFERENCES

Ali, O., Cheddadi, I., Landrein, B. and Long, Y. 2022. Revisiting the relationship between turgor pressure and plant cell growth. *New Phytologist,* **238**: 62-69.

Bar-Akiva, A., Ovadia, R., Rogachev, I., Bar-Or, C., Bar, E., Freiman, Z., Nissim-Levi, A., Gollop, N., Lewinsohn, E., Aharoni, A., Weiss, D., Koltai, H. and Oren-Shamir, M. 2010. Metabolic networking in *Brunfelsia calycina* petals after flower opening. *Journal of Experimental Botany*, **61**: 1393-1403.

Barman, M. and Mitra, A. 2018. Temporal relationship between emitted and endogenous floral scent volatiles in summer - and winter - blooming *Jasminum* species. *Physiologia Plantarum,* **166**: 946-959.

Beauzamy, L., Nakayama, N. and Boudaoud, A. 2014. Flowers under pressure: ins and outs of turgor regulation in development. *Annals of Botany,* **114**: 1517-1533.

Bi, Y., Zhang, Y., Signorelli, T., Zhao, R., Zhu, T. and Rothstein, S. 2005. Genetic analysis of Arabidopsis GATA transcription factor gene family reveals a nitrate - inducible member important for chlorophyll synthesis and glucose sensitivity. *The Plant Journal*, **44**: 680-692.

Biancucci, M., Mattioli, R., Forlani, G., Funck, D., Costantino, P. and Trovato, M. 2015. Role of proline and GABA in sexual reproduction of angiosperms. *Frontiers in Plant Science*,**6**: 680 doi: 10.3389/fpls.2015.00680.

Bieleski, R. 2000. Mechanical aspects of rapid flower opening in Asiatic lily. *Annals of Botany*, **86**: 1175-1183.

Borghi, M. 2025. Roles of sugar metabolism and transport in flower development. *Current Opinion in Plant Biology*, **85**: 102722.

Borghi, M., De Souza, L.P., Tohge, T., Mi, J., Melandri, G., Proost, S., Martins, M.C.M., Al-Babili, S., Bouwmeester, H.J. and Fernie, A.R. 2022. High-energy-level metabolism and transport occur at the transition from closed to open flowers. *Plant Physiology,* **190**: 319-339.

Borghi, M. and Fernie, A.R. 2017. Floral metabolism of sugars and amino acids: implications for pollinators' preferences and seed and fruit set. *Plant Physiology*, **175**: 1510-1524.

Bueno, J.M., Sáez-Plaza, P., Ramos-Escudero, F., Jiménez, A.M., Fett, R. and Asuero, A.G. 2012. Analysis and antioxidant capacity of anthocyanin pigments. Part II: chemical structure, color, and intake of anthocyanins. *Critical Reviews in Analytical Chemistry*, **42**: 126-151.

Carter, C., Shafir, S., Yehonatan, L., Palmer, R.G. and Thornburg, R. 2006. A novel role for proline in plant floral nectars. *The Science of Nature*, **93**: 72-79.

Chen, L., Cheung, L.S., Feng, L., Tanner, W. and Frommer, W.B. 2015. Transport of sugars. *Annual Review of Biochemistry*, **84**: 865-894.

Chen,L., Hou, B., Lalonde,S., Takanaga, H., Hartung, M.L., Qu, X., Guo, W., Kim, J., Underwood, W., Chaudhuri, B., Chermak, D., Antony, G., White, F.F., Somerville, S.C., Mudgett, M.B. and Frommer, W.B. 2010. Sugar transporters for intercellular exchange and nutrition of pathogens. *Nature*, **468**: 527-532.

Chen, W., Gong, P., Guo, J., Li, H., Li, R., Xing, W., Yang, Z. and Guan, Y. 2018. Glycolysis regulates pollen tube polarity via Rho GTPase signaling. *PLOS Genetics*, **14**: e1007373.

Chiang, H. and Dandekar, A.M.1995. Regulation of proline accumulation in *Arabidopsis thaliana* (L.) Heynh during development and in response to desiccation. *Plant, Cell & Environment*, **18**: 1280-1290.

Datta, S., Paul, S., Ballabh, L. and Mitra, A.2024. Histochemical and molecular analyses reveal an insight into the scent volatiles synthesis and emission in ephemeral flowers of *Murraya paniculata* (L.) Jack. *Planta*, **260**: 119 doi.org/10.1007/s00425-024-04552-6.

Degenhardt, J., Köllner, T.G. and Gershenzon, J.2009. Monoterpene and sesquiterpene synthases and the origin of terpene skeletal diversity in plants. *Phytochemistry*, **70**: 1621- 1637.

Dudareva, N., Murfitt, L.M., Mann, C.J., Gorenstein, N., Kolosova, N., Kish, C.M., Bonham, C. and Wood, K. 2000. Developmental regulation of methyl benzoate biosynthesis and emission in snapdragon flowers. *The Plant Cell*, **12**: 949-961.

Dudareva, N., Pichersky, E. and Gershenzon, J. 2004. Biochemistry of plant volatiles. *Plant Physiology*, **135**: 1893-1902.

Dudareva,N., Raguso, R.A., Wang, J., Ross, J.R. and Pichersky, E.1998. Floral scent production in *Clarkia breweri*.*Plant Physiology*, **116**: 599-604.

Feussner, I. and Wasternack, C.2002. The lipoxygenase pathway. *Annual Review of Plant Biology*, **53**: 275-297.

Gaufichon, L., Marmagne, A., Belcram, K., Yoneyama, T., Sakakibara, Y., Hase, T., Grandjean, O., Clément, G., Citerne, S., Boutet - Mercey, S., Masclaux - Daubresse, C., Chardon, F., Soulay, F., Xu, X., Trassaert, M., Shakiebaei, M., Najihi, A. and Suzuki, A. 2017. ASN1 - encoded asparagine synthetase in floral organs contributes to nitrogen filling in Arabidopsis seeds. *The Plant Journal*, **91**: 371-393.

Ge, Y., Angenent, G.C., Wittich, P.E., Peters,J., Franken, J., Busscher, M., Zhang, L., Dahlhaus, E., Kater, M.M., Wullems, G.J. and Creemers-Molenaar, T.

2000. NEC1, a novel gene, highly expressed in nectary tissue of *Petunia hybrida*. *The Plant Journal*, **24**: 725-734.

Ghissing, U. and Mitra, A. 2022. Biology of floral scent volatiles in ornamental plants. *In* Datta SK and Gupta YC (Ed.) Floriculture and Ornamental Plants, Handbooks of Crop Diversity: Conservation and Use of Plant Genetic Resources. Singapore:Springer Nature, 777-817.

Ghissing, U., Goswami, A. and Mitra, A. 2023a. Temporal accumulation of pigments during colour transformation from white to red in *Combretum indicum* (L.) DeFilipps (syn. *Quisqualis indica* L.) flowers. *Natural Product Research*, **37**: 529-533.

Ghissing, U., Kutty, N.N., Bimolata, W., Samanta, T. and Mitra, A.2023b. Comparative transcriptome analysis reveals an insight into the candidate genes involved in anthocyanin and scent volatiles biosynthesis in color changing flowers of *Combretum indicum*. *Plant Biology*, **25**: 85-95.

Ghissing, U., Paul, S., Goswami, A., Ghosh, R. and Mitra A. 2025. Understanding fine coordination among scent emission, anthocyanin accumulation and nectar secretion in colour changing flowers of *Combretum indicum*. *Physiologia Plantarum* [accepted].

Goldberg, R.B., Beals, T.P. and Sanders, P.M. 1993. Anther development: basic principles and practical applications. *The Plant Cell*, **5**: 1217-1229.

Green, B.R. and Durnford, D.G.1996. The chlorophyll-carotenoid proteins of oxygenic photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology*, **47**: 685-714.

Heil,M. 2011. Nectar: generation, regulation and ecological functions. *Trends in Plant Science*,**16**: 191-200.

Heide, O.M. 1963. Effect of temperature and day-length on flower initiation of *Brunfelsiacalycina* (Hook) Benth. *Physiologia Plantarum*, **16**: 104-109.

Helsper, J.P.F.G., Davies, J.A., Bouwmeester, H.J., Krol, A.F. and Van Kampen, M.H.1998. Circadian rhythmicity in emission of volatile compounds by flowers of *Rosa hybrida* L. cv. Honesty. *Planta*, **207**: 88-95.

Heuer, S. and Strack, D. 1992. Synthesis of betanin from betanidin and UDP-glucose by a protein preparation from cell suspension cultures of *Dorotheanthus bellidiformis* (Burm. f.) N. E. Br. *Planta*,**186**: 626-628.

Hudak, K.A. and Thompson, J.E. 1997. Subcellular localization of secondary lipid metabolites including fragrance volatiles in carnation petals. *Plant Physiology*, **114**: 705-713.

Ilg, A., Bruno, M., Beyer, P.and Al-Babili, S. 2014. Tomato carotenoid cleavage dioxygenases 1A and 1B: Relaxed double bond specificity leads to a plenitude of dialdehydes, mono-apocarotenoids and isoprenoid volatiles. *FEBS Open Bio*, **4**: 584-593.

Ishizuna, F., Yo, U., Yoshimura, N., Shirai, A., Aono, T., Tsutsumi, N., Arimura, S. and Takanashi, H. 2024. Structural changes in *Nelumbo* flower petals during opening and closing. *American Journal of Botany*, **111**: e16433.

Jhanji, S., Kaur, G. and Chumber, M. 2025. Deciphering flower senescence physiology: advancements in post-harvest storage and preservation techniques for enhancing longevity. *The Journal of Horticultural Science and Biotechnology*, **100**: 164-32.

Khan, M.I. and Giridhar, P. 2015. Plant betalains: chemistry and biochemistry. *Phytochemistry*, **117**: 267-295.

Kavi Kishor, P.B., Kumari, P.E., Sunita, M.S.L., Sreenivasulu, N. and Sreenivasulu, N. 2015. Role of proline in cell wall synthesis and plant development and its implications in plant ontogeny. *Frontiers in Plant Science*, **6**: 544 doi:10.3389/fpls.2015.00544.

Kitamura, S., Shikazono, N. and Tanaka, A. 2004. TRANSPARENT TESTA 19 is involved in the accumulation of both anthocyanins and proanthocyanidins in Arabidopsis. *The Plant Journal*, **37**: 104-114.

Kolosova, N., Gorenstein, N., Kish, C.M. and Dudareva, N. 2001a. Regulation of circadian methyl benzoate emission in diurnally and nocturnally emitting plants. *The Plant Cell*, **13**: 2333-2347.

Kolosova, N.P., Sherman, D.M., Karlson, D. and Dudareva, N. 2001b. Cellular and subcellular localization of s-adenosyl-l-methionine: benzoic acid carboxyl methyltransferase, the enzyme responsible for biosynthesis of the volatile ester methylbenzoate in snapdragon flowers. *Plant Physiology*, **126**: 956-964.

Konar, R.N. and Linskens, H.F.1966. Physiology and biochemistry of the stigmatic fluid of *Petunia hybrida*. *Planta*, **71**: 372-387.

Kumar, N., Srivastava, G.C. and Dixit, K. 2008. Flower bud opening and senescence in roses (*Rosa hybrida* L.). *Plant Growth Regulation*, **55**: 81-99.

Kutty, N.N., Ghissing, U. and Mitra,A. 2021. Revealing floral metabolite network in tuberose that underpins scent volatiles synthesis, storage and emission. *Plant Molecular Biology*, **106**: 533-554.

Lin, I.W., Sosso, D., Chen, L., Gase, K., Kim, S., Kessler, D., Klinkenberg, P.M., Gorder, M.K., Hou, B., Qu, X., Carter, C.J., Baldwin, I.T. and Frommer, W.B. 2014. Nectar secretion requires sucrose phosphate synthases and the sugar transporter SWEET9. *Nature*, **508**: 546-549.

Loughrin, J.N., Hamilton-Kemp, T.R., Andersen, R.A. and Hildebrand, D.F. 1990. Volatiles from flowers of *Nicotiana sylvestris, N. otophora* and *Malus* × *domestica*: headspace components and day/night changes in their relative concentrations. *Phytochemistry*, **29**: 2473-2477.

MacNeill, G.J., Mehrpouyan, S., Minow, M.A.A., Patterson, J.A., Tetlow, I.J. and Emes, M.J. 2017. Starch as a source, starch as a sink: the bifunctional role of starch in carbon allocation. *Journal of Experimental Botany*, **68**: 4433-4453.

Maeda,H. and Dudareva,N. 2012. The shikimate pathway and aromatic amino acid biosynthesis in plants. *Annual Review of Plant Biology*, **63**: 73-105.

Maeda, H., Shasany, A.K., Schnepp, J., Orlova, I., Taguchi, G., Cooper, B.R., Rhodes, D., Pichersky, E. and Dudareva, N.2010. RNAi suppression of arogenate dehydratase1 reveals that phenylalanine is synthesized predominantly via the arogenate pathway in petunia petals. *The Plant Cell*, **22**: 832-849.

Maiti, S. and Mitra, A.2017. Morphological, physiological and ultrastructural changes in flowers explain the spatio-temporal emission of scent volatiles in *Polianthes tuberosa* L. *Plant & Cell Physiology*, **58**: 2095-2111.

Marrs, K.A., Alfenito, M.R., Lloyd, A.M. and Walbot, V. 1995. A glutathione S-transferase involved in vacuolar transfer encoded by the maize gene *Bronze-2*. *Nature*, **375**: 397-400.

Mascarenhas, J.P.1989. The male gametophyte of flowering plants. *The Plant Cell*, **1**: 657-664.

Meyer, S., Lauterbach, C., Niedermeier, M., Barth, I., Sjolund, R.D. and Sauer, N. 2004. Wounding enhances expression of AtSUC3, a sucrose transporter from Arabidopsis sieve elements and sink tissues. *Plant Physiology*, **134**: 684-693.

Meyer, S., Melzer, M., Truernit, E., Hummer, C., Besenbeck, R., Stadler, R. and Sauer, N.2000. AtSUC3, a gene encoding a new Arabidopsis sucrose transporter, is expressed in cells adjacent to the vascular tissue and in a carpel cell layer. *The Plant Journal*, **24**: 869-882.

Mikulic-Petkovsek, M., Schmitzer, V., Slatnar, A., Todorovic, B., Veberic, R., Stampar, F. and Ivancic, A. 2014. Investigation of anthocyanin profile of four elderberry species and interspecific hybrids. *Journal of Agricultural and Food Chemistry*, **62**: 5573-5580.

Mohan Ram, H.Y. and Mathur, G. 1984. Flower colour changes in *Lantana camara*. *Journal of Experimental Botany*, **35**: 1656-1662.

Müller, G.L., Drincovich, M.F., Andreo, C.S. and Lara, M.V. 2010. Role of photosynthesis and analysis of key enzymes involved in primary metabolism throughout the lifespan of the tobacco flower. *Journal of Experimental Botany*, **61**: 3675-3688.

Murphy, M.P., Bayir, H., Belousov, V., Chang, C.J., Davies, K.J.A., Davies, M.J., Dick, T.P., Finkel, T., Forman, H.J., Janssen-Heininger, Y., Gems, D., Kagan, V.E., Kalyanaraman, B., Larsson, N., Milne, G.L., Nyström, T., Poulsen, H.E., Radi, R., Van Remmen, H. and Halliwell, B. 2022. Guidelines for measuring reactive oxygen species and oxidative damage in cells and in vivo. *Nature Metabolism*, **4**: 651-662.

Niyogi, K.K. 2000. Safety valves for photosynthesis. *Current Opinion in Plant Biology*, **3**: 455-460.

Nørholm, M.H., Nour-Eldin, H.H., Brodersen, P., Mundy, J. and Halkier, B.A. 2006. Expression of the Arabidopsis high - affinity hexose transporter STP13 correlates with programmed cell death. *FEBS Letters*, **580**: 2381-2387.

Oka, N., Ohishi, H., Hatano, T., Hornberger, M., Sakata, K. and Watanabe, N. 1999. Aroma evolution during flower opening in *Rosa damascena* Mill. *Zeitschrift für Naturforschung C*. **54**: 889-895.

Pacheco, S.D.G., Gasparin, A.T., Jesus, C.H.A., Sotomaior, B.B., Ventura, A.C.S.S.B., Redivo, D.D.B., Cabrini, D.A., Gaspari Dias, J.F., Miguel, M.D., Miguel O.G. and da Cunha J.M. 2019. Antinociceptive and anti-inflammatory effects of bixin, a carotenoid extracted from the seeds of *Bixa orellana*. *Planta Medica*, **85**: 1216-1224.

Panavas, T. and Rubinstein, B. 1998. Oxidative events during programmed cell death of daylily (*Hemerocallis* hybrid) petals. *Plant Science*, **133**: 125-138.

Paul, I., Chatterjee, A., Maiti, S., Bhadoria, P. and Mitra, A. 2019. Dynamic trajectories of volatile and non - volatile specialized metabolites in 'overnight' fragrant flowers of *Murraya paniculata*. *Plant Biology*, **21**: 899-910.

Pereira da Costa, D. and Campos Miranda-Filho, K.2019. The use of carotenoid pigments as food additives for aquatic organisms and their functional roles. *Reviews in Aquaculture*, **12**: 1567-1578.

Pichersky, E. 2006. Biosynthesis of plant volatiles: nature's diversity and ingenuity. *Science*, **311**: 808-811.

Pichersky, E. 2023. Biochemistry and genetics of floral scent: a historical perspective. *The Plant Journal*, **115**:18-36.

Pott, M.B., Pichersky, E. and Piechulla, B. 2002. Evening specific oscillations of scent emission, SAMT enzyme activity, and SAMT mRNA in flowers of *Stephanotis floribunda*. *Journal of Plant Physiology*, **159**: 925-934.

Prabhakar, V., Löttgert, T., Geimer, S., Dörmann, P., Krüger, S., Vijayakumar, V., Schreiber, L., Göbel, C., Feussner, K., Feussner, I., Marin, K., Staehr, P., Bell, K., Flügge, U.I. and Häusler, R.E. 2010. Phosphoenolpyruvate provision to plastids is essential for gametophyte and sporophyte development in *Arabidopsis thaliana*. *The Plant Cell*, **22**: 2594-2617.

Raguso, R.A. and Weiss, M.R. 2015. Concerted changes in floral color and scent, and the importance of spatio-temporal variation in floral volatiles. *Journal of the Indian Institute of Science*, **95**: 69-92.

Rees, T.A. 1995. Prospects of manipulating plant metabolism. *Trends in Biotechnology*, **13**: 375-378.

Reid, M.S. and Chen, J. 2007. Flower senescence. *Annual Plant Reviews*, **26**: 256-277.

Rogers, H.J. 2011. Is there an important role for reactive oxygen species and redox regulation during floral senescence? *Plant Cell & Environment*, **35**: 217-233.

Rogers, H.J. 2012. From models to ornamentals: how is flower senescence regulated? *Plant Molecular Biology*, **82**: 563-574.

Rogers, H.J. 2023. How far can omics go in unveiling the mechanisms of floral senescence? *Biochemical Society Transactions*, **51**: 1485-1493.

Rogers, H. and Munné-Bosch, S. 2016. Production and scavenging of reactive oxygen species and redox signaling during leaf and flower senescence: similar but different. *Plant Physiology*, **171**: 1560-1568.

Rounds, C.M., Winship, L.J. and Hepler, P.K.2011. Pollen tube energetics: respiration, fermentation and the race to the ovule. *AoB Plants*, **plr019**: doi:10.1093/aobpla/plr019.

Roy, R., Schmitt, A.J., Thomas, J.B. and Carter, C.J. 2017. Review: nectar biology: from molecules to ecosystems. *Plant Science*, **262**: 148-164.

Sánchez, A. and Winterhalter, P. 2013. Carotenoid cleavage products in saffron (*Crocus sativus* L.). *In* Winterhalter P and Ebeler S (Ed.), *Carotenoid Cleavage Products*, Washington DC, ACS Symposium Series, 45-63.

Sauer, N., Friedländer, K. and Gräml-Wicke, U. 1990. Primary structure, genomic organization and heterologous expression of a glucose transporter from *Arabidopsis thaliana*. *The EMBO Journal*, **9**: 3045-3050.

Sauer, N., Ludwig, A., Knoblauch, A., Rothe, P., Gahrtz, M. and Klebl, F. 2004. AtSUC8 and AtSUC9 encode functional sucrose transporters, but the closely related AtSUC6 and AtSUC7 genes encode aberrant proteins in different *Arabidopsis* ecotypes. *The Plant Journal*, **40**: 120-130.

Schiestl, F.P. and Ayasse, M. 2001. Post-pollination emission of a repellent compound in a sexually deceptive orchid: a new mechanism for maximizing reproductive success? *Oecologia*, **126**: 531-534.

Schneider, S., Hulpke, S., Schulz, A., Yaron, I., Höll, J., Imlau, A., Schmitt, B., Batz, S., Wolf, S., Hedrich, R. and Sauer, N. 2011. Vacuoles release sucrose via tonoplast - localized SUC4 - type transporters. *Plant Biology*, **14**: 325-336.

Schwacke, R., Grallath, S., Breitkreuz, K.E., Stransky, E., Stransky, H., Frommer, W.B. and Rentsch, D. 1999. LeProT1, a transporter for proline, glycine betaine, and Γ-Amino butyric acid in tomato pollen. *The Plant Cell*, **11**: 377-391.

Shumskaya, M. and Wurtzel, E.T. 2013. The carotenoid biosynthetic pathway: thinking in all dimensions. *Plant Science*, **208**: 58-63.

Sommano, S.R., Suppakittpaisarn, P., Sringarm, K., Junmahasathien, T. and Ruksiriwanich, W. 2020. Recovery of Crocins from Floral Tissue of *Gardenia jasminoides* Ellis. *Frontiers in Nutrition*, **7**: 1-11.

Stitt, M. 2013. Progress in understanding and engineering primary plant metabolism. *Current Opinion in Biotechnology*, **24**: 229-238.

Strack, D., Vogt, T. and Schliemann, W. 2003. Recent advances in betalain research. *Phytochemistry*, **62**: 247-269.

Sun, M., Huang, X., Yang, J., Guan, Y. and Yang, Z. 2013. Arabidopsis RPG1 is important for primexine deposition and functions redundantly with RPG2 for plant fertility at the late reproductive stage. *Plant Reproduction*, **26**: 83-91.

Tanaka, Y., Sasaki, N. and Ohmiya, A. 2008. Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids. *The Plant Journal*, **54**: 733-749.

Tholl, D., Boland, W., Hansel, A., Loreto, F., Röse, U.S.R. and Schnitzler, J.P. 2006. Practical approaches to plant volatile analysis. *The Plant Journal*, **45**: 540-560.

Thomas, H., Ougham, H.J., Wagstaff, C. and Stead, A.D. 2003. Defining senescence and death. *Journal of Experimental Botany*, **54**: 1127-1132.

Tirosh, T. and Mayak, S. 1988. Changes in starch content during the development of carnation petals. *Journal of Plant Physiology*, **133**: 361-363.

Tohge, T., Watanabe, M., Hoefgen, R. and Fernie, A.R. 2013. Shikimate and phenylalanine biosynthesis in the green lineage. *Frontiers in Plant Science*, **4**: 1-13.

Vaknin, H., Bar-Akiva, A., Nissim-Levi, R.O.A., Forer, I., Weiss, D. and Oren-Shamir, M. 2005. Active anthocyanin degradation in *Brunfelsia calycina* (yesterday–today–tomorrow) flowers. *Planta*, **222**: 19-26.

van Doorn, W.G. and Van Meeteren, U. 2003. Flower opening and closure: a review. *Journal of Experimental Botany*, **54**: 1801-1812.

Wang, J.Y., Lin, P.Y. and Al-Babili, S. 2021. On the biosynthesis and evolution of apocarotenoid plant growth regulators. *Seminars in Cell & Developmental Biology*, **109**: 3-11.

Wang, Y., Zhang, C., Xu, B., Fu, J., Du, Y., Fang, Q., Dong, B. and Zhao, H. 2022. Temperature regulation of carotenoid accumulation in the petals of sweet osmanthus via modulating expression of carotenoid biosynthesis and degradation genes. *BMC Genomics*, **23**: 418 doi.org/10.1186/s12864-022-08643-0.

Weise, A., Barker, L., Kühn, C., Lalonde, S., Buschmann, H., Frommer, W.B. and Ward, J.M. 2000. A new subfamily of sucrose transporters, SUT4, with low affinity/high capacity localized in enucleate sieve elements of plants. *The Plant Cell*, **12**: 1345-1355.

Widhalm, J.R., Gutensohn, M., Yoo, H., Adebesin, F., Qian, Y., Guo, L., Jaini, R., Lynch, J.H., McCoy, R.M., Shreve, J.T., Thimmapuram, J., Rhodes, D., Morgan, J.A. and Dudareva, N. 2015. Identification of a plastidial phenylalanine exporter that influences flux distribution through the phenylalanine biosynthetic network. *Nature Communications*, **6**: 8142 doi.org/10.1038/ncomms9142.

Yamamoto, K., Kobayashi, N., Yoshitama, K., Teramoto, S. and Komamine, A. 2001. Isolation and purification of tyrosine hydroxylase from callus cultures of *Portulaca grandiflora*. *Plant & Cell Physiology*, **42**: 969-975.

Zhang, H., Xue, F., Guo, L., Cheng, J., Jabbour, F., DuPasquier, P., Xie, Y., Zhang, P., Wu, Y., Duan, X., Kong, H. and Zhang, R. 2024. The mechanism underlying asymmetric bending of lateral petals in *Delphinium* (Ranunculaceae). *Current Biology*, **34**: 755-768.

Zhang, Y., Guo, W., Chen, S., Han, L. and Li, Z. 2006. The role of N-lauroylethanolamine in the regulation of senescence of cut carnations (*Dianthus caryophyllus*). *Journal of Plant Physiology*, **164**: 993-1001.

Zheng, X., Zhu, K., Sun, Q., Zhang, W., Wang, X., Cao, H., Tan, M., Xie, Z., Zeng, Y., Ye, J., Chai, L., Xu, Q., Pan, Z., Xiao, S., Fraser, P.D. and Deng, X. 2019. Natural variation in CCD4 promoter underpins species-specific evolution of red coloration in citrus peel. *Molecular Plant*, **12**: 1294-1307.

J. Botan. Soc. Bengal 79(1) : 19-27 (2025), ISSN 0971-2976

FULL LENGTH ARTICLE

Study of seedling morphology of four species of *Cycas* L. (Cycadaceae) in relation to its taxonomic significance

Sonali Ray
Department of Botany, Surendranath College, 24/2, M. G. Road, Kolkata- 700009

Received : 28.12.2024 Accepted : 05.03.2025 Published : 30.06.2025

Seedling morphology is emerging as an immensely important tool for identification of plants. The present study is on the investigation of the developmental stages of seedling of four species of *Cycas* viz. *C. circinalis* L., C. *revoluta* Thunb., *C. rumphii* Miq. and *C. orixensis* (Haines) R. Singh & J.S. Khuraijam, belonging to the family Cycadaceae, and preparation of an artificial key to identification of the species at the juvenile stage. The morphological study of the seedlings is used to prepare an artificial key for their identification. This study is an attempt to emphasize the significance of seedling study and can be utilized in identifying the taxa at their nascent developmental stage, in natural habitats.

Key-words: Seedling morphology, seedling identification, *Cycas*, cycadaceae.

INTRODUCTION

Seedlings are defined by Jackson (1928) as a plant produced from seeds, in distinction to a plant propagated artificially. Seedling morphological characters are of immense importance for the study of relationships, comparisons, and implied phylogeny of taxa at the level of family, genus and species, as these features are genetically stable. A critical investigation of correlations between the mature and seedling characters has repeatedly proved to have a better understanding of taxonomically difficult taxa (Bokdam,1977). However, vegetative characters can be of primary value in classification but are often ignored or little emphasized (Tomlinson, 1984). Seedlings are rarely preserved as permanent specimens and are not readily available for proper study and identification purpose. Moreover, due to their small size seedlings of many taxa often escape the attention in the natural habitats (Canne, 1983). Paria and his associates reported seedling structures to be key factors in systematics as well as phylogenetic studies of angiosperms (Deb and Paria,1986; Paria, Bhattacharya and Ghosh, 1996; Paria, 2014).

The Cycads of the Gymnosperms are an economical group of plants. They range from being dioecious trees to perennial herbs. They are characterised by having aerial unbranched or dichotomously branched stem or subterranean stem with large, petiolate, coriaceous, spirally arranged, pinnately compound leaves. Cataphylls are prominently present arising alternately with the leaves. They bear specialized coralloid roots which has an association of cyanobacteria that aids in nitrogen fixation. The male cones are large cylindrical

**Corresponding author* : sonyyrr@gmail.com

in shape with spirally arranged microsporophylls containing sori. The female reproductive structure consists of numerous megasporophylls arising from the stem apex, each bearing one or more marginal ovule.

Cycads are a good source of food, fibre, gum and also has an ornamental value. (Kubitzki, 1990, Biswas and Johri, 1997, Arezoo *et al*., 2020). A diverse range of metabolites like flavonoids, phenolic acids, amino acids, fatty acids, lignans, etc have been isolated and chemically analyzed from many species of *Cycas* and these metabolites have been reported for their various pharmacological properties like antibacterial, anti-inflammatory and antipyretic activities (Bhatnagar and Moitra, 1996, Moawad *et al.* 2010*,* Mourya *et al*., 2011, Kalpashree and Raveesha, 2013, Bera *et al.* 2020, Du *et al*., 2023, El-Seadawy *et al.,* 2023). Very fragmentary work has been done on the morphology of seedlings of cycads. Foster (1939) presented a detailed study on the structure and growth of the shoot apex of seedlings and adventitious buds of *Cycas revoluta* Thunb., Morphological study of seeds of *Cycas revoluta, C. media, C normanbyana, C. taiwaniana,* and *C. wadei*was done by Dehgan and Yuen (1983). Reports on albino seedling of *Cycas revoluta* was given by Ruishu and Jian (1997). From the foregoing account, we may conclude that cycads are a significant group of gymnosperms, whose seedling survey will be of potential interest in the light of systematic studies. The threat of endangerment of these species makes it more essential that an artificial key for their identification at the juvenile stage is provided. The present study is an attempt to have a comprehensive knowledge about the seedlings of four important species of *Cycas*, out of which *C. orixensis* is cited as a critically endangered species, *C. circinalis* is cited as endangered and *C. rumphii* is cited as near threatened by IUCN (Khuraijam, 2023, Bösenberg, 2023).

MATERIALS AND METHODS

In the present investigation, seeds and seedlings of the studied taxa of Cycadaceae were collected from different parts of Sikkim, West Bengal and Odisha (Table 1). The seeds of each taxon were observed under binocular stereo microscope (Wild M-3). The descriptions of seeds were prepared in consultation with Martin (1946), Martin and Barkley (1961) and Corner (1976). In case of measurements, an average of at least five to six seeds was considered and for weight of seeds average value was taken by following the methods suggested by Stevens (1932), Datta *et al.* (1980) with slight modifications. The morphological study of the seeds of each species were noted using different magnifications and eventually documentation was done by taking photographs, taken with the help of Nikon Digital Camera (S7000).

The seedling specimens representing different developmental stages were dried and preserved in the form of herbarium sheets according to the procedure as suggested by Farron (1977) and Paria *et al.* (1990). The seedling characters were studied, characterized from fresh specimens immediately after collection and subsequently hand drawn, in different scale of magnification. from the dry specimens. The seedling morphology was described following the scheme of description given by Lubbock (1892), Vogel (1980) and terminology as proposed by Duke (1965), Burger (1972) and Vogel (1980). The parts of the seedlings were observed both in transmitted and incident light by using binocular microscope (Wild M-3 Stereomicroscope) to record the minute characters. With the assortment of the diagnostic characters, an artificial key to the studied specimens was prepared.

RESULTS

1. *Cycas circinalis* L.

Seed Morphology

Seed shape ovoid. *Dimension* 5.6-5.8 × 4.3-4.4 cm. *Apex* rounded. *Base* rounded. *Surface* wrinkled. Colour dull brown. *Seed weight* 310.24 gm / 10 seeds (Fig. 1 A).

Seedling Morphology (Up to 6 leaves stage)

Seedlings hypogeal, cryptocotylar. *Tap root* one to four elongating, tapering downwards, stout, fleshy, branching starts at one foliaceous leaf stage, lateral roots short, stout, thick, fleshy; presence of root nodules on primary as well as lateral roots, giving the appearance of coralloid roots; light brownish in

Table 1. List of investigated taxa of Cycadaceae

List of Studied Taxa	Place of collection
Cycas circinalis L. (Endangered - Bösenberg, J.D. 2023. *Cycas circinalis*. *The IUCN Red List of Threatened Species* 2023: e.T42089A2959396)	Gangtok, Pelling (Sikkim)
Cycas revoluta Thunb.	Gangtok (Sikkim), S-24 Pgns (West Bengal), Angul (Odisha)
Cycas rumphii Miq. (Near Threatened - Bösenberg, J.D. 2023. *Cycas rumphii. The IUCN Red List of Threatened Species* 2023: e.T216470283A69156062.)	Pelling (Sikkim), Satkosia (Odisha)
Cycas orixensis(Haines**)** R.Singh&J.S.Khuraijam (Critically Endangered - Khuraijam, J. 2023. *The IUCN Red List of Threatened Species 2023: e.T103632258A103632295*)	Tikarpara forest,Satkosia (Odisha)

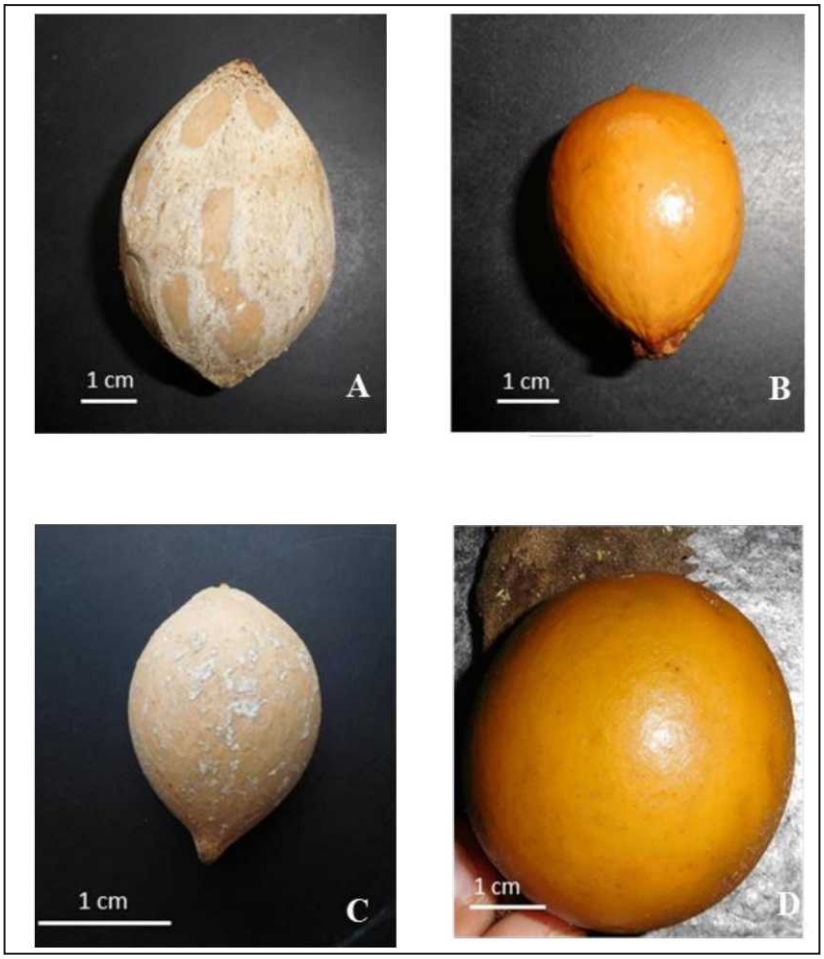


Fig. 1. Morphological diversity of seeds of the investigated taxa.
A. *Cycas circinalis* **B.** *Cycas revoluta*
C. *Cycas rumphii* **D.** *Cycas orixensis*

colour; 4.5-12.0 cm. *Hypocotyl* rudimentary, subterranean, almost continuous with primary taproot without any differentiation. *Cotyledons* two, subterranean, clasping the basal part of the shoot from both sides forming a cotyledonary sheath. *Primary Scale Leaves* one to four, leaves reduced to sessile, large, fleshy scale called cataphylls, adpressed closely on the plumule, triangular in shape, 2.0-4.0 cm, pointed apex, pilose, coriaceous, dark brown in colour. *First foliage leaves* pinnately compound leaves, alternate, showing circinate vernation, exstipulate, petiolate, petiole long, petiole length 3.0-6.5 cm, spinescent through half length, petiole colour light yellow at base becoming dark green gradually upwards; pinnae linear-elliptical, apex acute, base cuneate, margin entire, dark glaucous green in colour, number of pinnae 8-16 on each leaf, length of pinnae on leaf 4.0-12.5 cm × 0.5-1.0 cm, length of leaf 7.0-16.6 cm; presence of a single prominent midrib on each pinnae. *Subsequent foliage leaves* pinnately compound leaves, spirally arranged, pinnae linear-flattened, all other features same as first foliage leaves, except larger in size; size of pinnae 5.5-15.0 cm × 0.5-1.3 cm. (Fig. 2 A-B, Fig 3 A).

2. *Cycas revoluta* Thunb.

Seed Morphology

Seed shape ovoid. *Dimension* 5.8-6.2×2.9-3.1cm. *Apex* truncate with a small point. *Base* slight flattened with a whitish hilum. *Surface* wrinkled on both lateral sides with smooth rounded area. *Colour* orange. Seed weight 105.20 gm /10 seeds (Fig 1 B).

Seedling Morphology (Up to 6 leaves stages)

Seedlings hypogeal, cryptocotylar. *Tap root* one to three, elongating, 7.0-17.0 cm, tapering downwards, stout, fleshy, branching starts at one foliaceous leaf stage, lateral roots short, thick, fleshy; presence of a number of root nodules on primary as well as lateral roots, giving the appearance of coralloid roots; light yellowish in colour. *Hypocotyl* rudimentary, subterranean, almost continuous with primary root without much differentiation. *Cotyledons* two, subterranean, clasping the basal part of the shoot from both sides forming a cotyledonary sheath. *Primary scale leaves* one, two or few more (about five) leaves reduced to sessile, large, fleshy scale called cataphylls, adpressed closely on the plumule, triangular in shape, 2.5-3.5 cm, pointed apex, extremely pilose, coriaceous, dark brown in colour. *First foliage leaves* pinnately compound leaves, alternate, showing circinate vernation, exstipulate, petiolate, petiole long, petiole length 2.5-5.1 cm, pilose entirely, petiole colour light yellow at base becoming bright glaucous green gradually upwards; pinnae linear, apex acuminate, base attenuately subtruncate, margin entire-revolute, dark glaucous green in colour, number of pinnae 10-28 on each leaf, length of pinnae on leaf 1.5-6.0 × 0.3-1.0 cm, length of leaf 5.5 - 13.6 cm; presence of a single prominent midrib on each pinnae. *Subsequent foliage leaves* all characters same as the first foliage leaves except larger in size; size of pinnae 2.8-6.6 × 0.3-0.7 cm (Fig 2 C-D, Fig 3 B).

3. *Cyacs rumphii* Miq.

Seed Morphology

Seed shape ovoid. *Dimension* 5.5-5.6 × 3.6-3.7 cm. *Apex* acuminate. *Base* rounded. *Surface* wrinkled. Colour brown. *Seed weight* 150.43 gm / 10 seeds (Fig 1 C).

Seedling Morphology (Up to 6 leaves stage)

Seedlings hypogeal, cryptocotylar. *Tap root* one to three taproot, elongating, tapering downwards, stout, fleshy, lateral roots short, thick, fleshy; presence of a number of root nodules on primary as well as lateral roots, giving the appearance of coralloid roots; light yellowish in colour; 5.0-12.0 cm. *Hypocotyl* rudimentary, subterranean, almost continuous with primary root without much differentiation. *Cotyledons* two, subterranean, clasping the basal part of the shoot from both sides forming a cotyledonary sheath. *Primary scale leaves* two to three, leaves reduced to sessile, large, fleshy scale called cataphylls, adpressed closely on the plumule, triangular in shape, 1.5-2.5 cm, pointed apex, pilose, coriaceous, dark brown in colour. *First foliage leaves* pinnately compound leaves, alternate, showing circinate vernation, exstipulate, petiolate, petiole long, petiole length 3.0 - 6.0 cm, spinescent almost entirely, petiole colour light

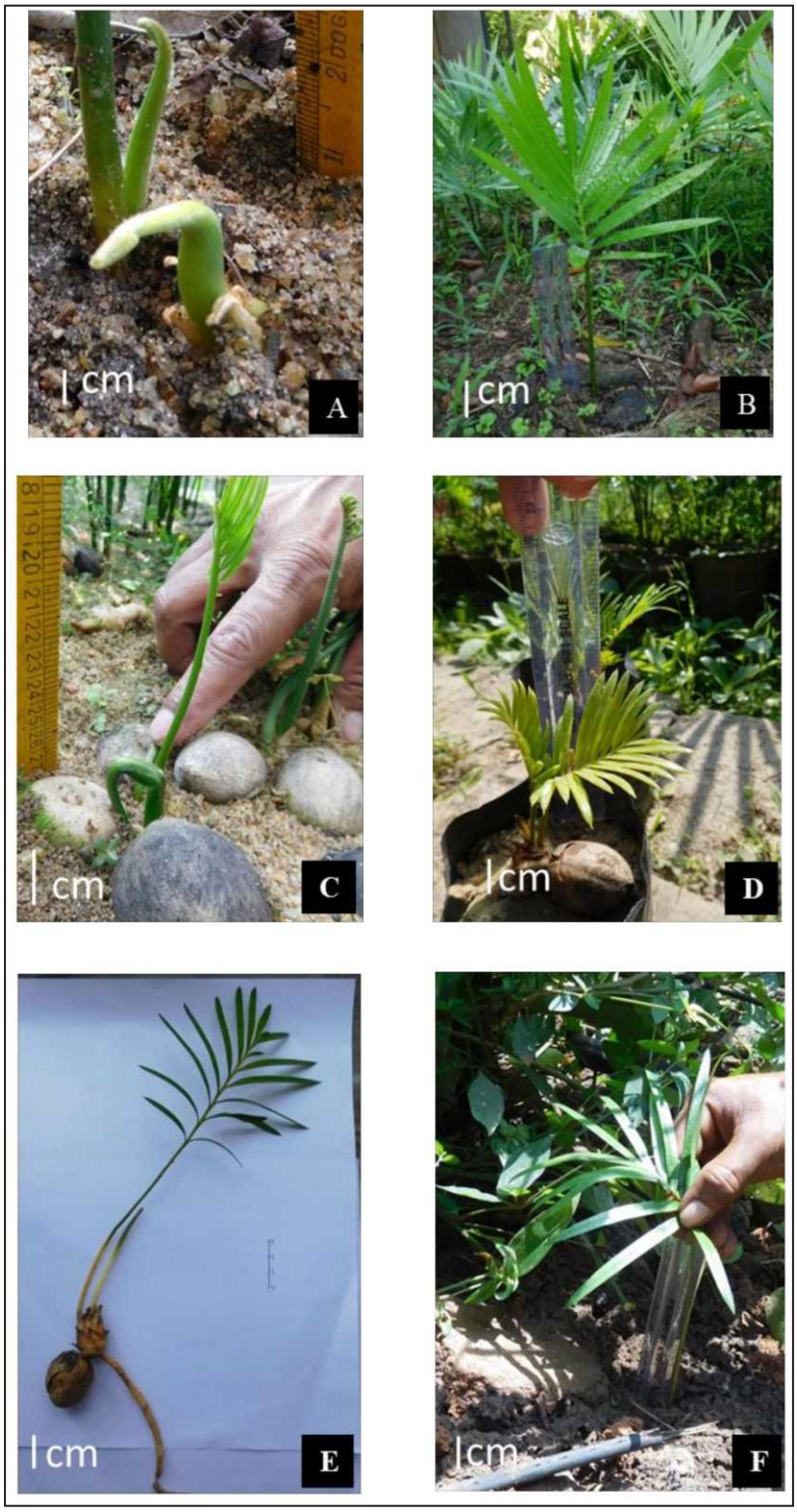


Fig. 2. Morphological diversity of seedling among investigated taxa.
A & **B**. *Cycas circinalis* **C** & **D**. *Cycas revoluta*
E. *Cycas rumphii* **F**. *Cycas orixensis*

yellow at base becoming bright glaucous green gradually upwards; pinnae linear, apex acute, base truncate, margin entire, bright glaucous green in colour, number of pinnae 10-16 on each leaf, length of pinnae on leaf 5.0-13.0 × 0.7-1.2 cm, length of leaf 8.0 -18.0 cm; presence of a single prominent midrib on each pinnae. *Subsequent leaves* pinnately compound leaves, spirally arranged, all other features same as first foliage leaves, except larger in size, size of pinnae 6.0 -15.0 × 0.7-1.4 cm (Fig 2 E, Fig 3 C).

4. *Cycas orixensis*(Haines) R. Singh & J.S. Khuraijam

Seed Morphology

Seed shape ovoid. *Dimension* 3.5-5.0 × 2.7-4.0 cm. *Apex* obtuse. *Base* rounded. *Surface* slightly roughened. Colour dark brown. *Seed weight* 110 gm / 10 seeds (Fig 1 D).

Seedling Morphology (Up to 6 leaves stage)

Seedlings hypogeal, cryptocotylar. *Tap root* one to three taproot, shortly elongating, tapering downwards, stout, fleshy, branching starts at one foliaceous leaf stage, lateral roots short, thick, fleshy; presence of a number of root nodules on primary as well as lateral roots, giving the appearance of coralloid roots; light yellowish in colour; 3.0-5.8 cm. *Hypocotyl* rudimentary, subterranean, almost continuous with primary root without much differentiation. *Cotyledons* two, subterranean, clasping the basal part of the shoot from both sides forming a cotyledonary sheath. *Primary Scale Leaves* one, two or few more (about five) leaves reduced to sessile, large, fleshy scale adpressed closely on the plumule, triangular in shape, 2.5-3.5 cm, pointed apex, pilose, coriaceous, light brown in colour. *First foliage leaves* pinnately compound leaves, alternate, showing circinate vernation, exstipulate, petiolate, petiole long, petiole length 2.5-5.1 cm, moderately spinescent, petiole colour light yellow at base becoming bright glaucous green gradually upwards; pinnae linear-flattened, apex acuminate, sheathing base, margin entire, dark glaucous green in colour, number of pinnae 6 -10 on each leaf, length of pinnae on leaf 0.7-1.2 × 0.3 - 0.4 cm, length of leaf 5.5 -13.6 cm; presence of a single prominent midrib on each pinnae. *Subsequent foliage leaves* pinnately compound leaves, spirally arranged, all other features same as first foliage leaves, except larger in size (Fig 2 F, Fig 3 D).

Artificial key
(Valid for the investigated taxa)

1a. Shape of seed ovoid; leaflets margin revolute ..... ……..... *C. revoluta*
1b. Shape of seed ovoid; leaflets margin flat 2

2a. Petiole extremely spinescent throughout the entire length; leaflets linear, slightly broad at base .... ……..…..…… *C. rumphii*
2b. Petiole spinescent through half the entirelength; leaflets linear …….....3

3a. Apex of seeds rounded; petiole extended; leaflets linear-elliptic ….....……. *C. circinalis*
3b. Apex of seeds obtuse; petiole shorter; leaflets linear flattened ..... *C. orixensis*

DISCUSSION

The seedlings of all the four taxa exhibited cryptocotylar, hypogeal pattern of germination, with subterranean hypocotyls, fleshy cotyledons and pinnately compound first and subsequent leaves. It is observed that *Cycas revoluta* is sharply separated from the other three investigated taxa of *Cycas* in having revolute margins of leaflets of the pinnately compound leaves in the former in contrast to flat margins of leaflets in the other taxa. The shape of the leaflets of *C. circinalis* and *C. orixensis* differ from each other, along with the form of seed apex. The mature leaflets of *C. revoluta* also have revolute margins which is present right from the seedling stage and is an identifying character of the species to differentiate it from the other three. The seedling characters along with the seed morphology presents a summative and diagnostic account of the species that would be aiding in their identification and subsequent protection form deforestation, at the juvenile stage. Since many of the species of *Cycas* are facing threat of survival, the seedling morphological features will play a significant role in the conservation spectrum. The seedling morphological study of these species can be further

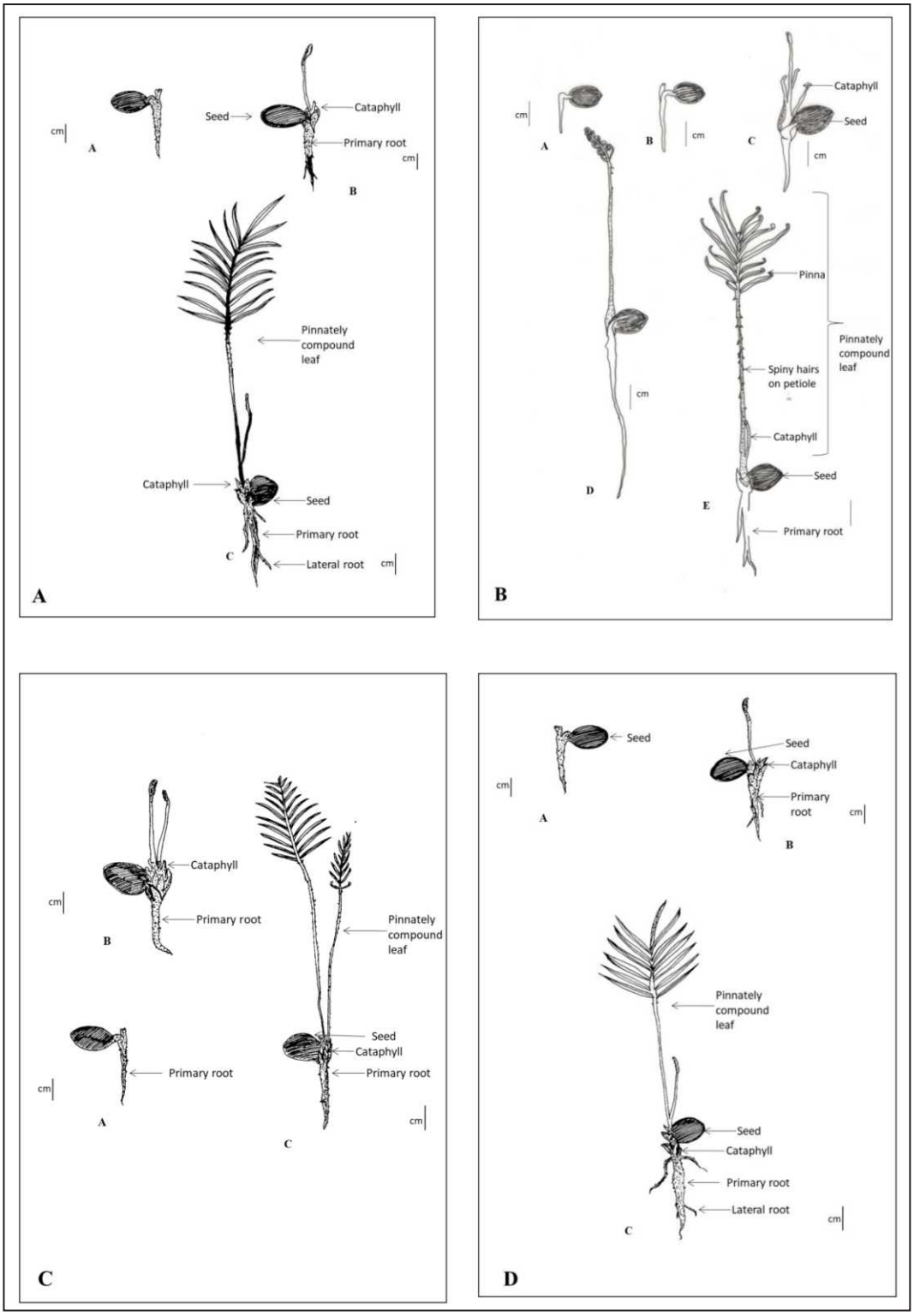


Fig. 3. Illustrations of the developmental stages of seedlings of the investigated taxa.
A. *Cycas circinalis* B. *Cycas revoluta*
C. *Cycas rumphii* D. *Cycas orixensis*

correlated with the other species of cycadaceae as well as species of families under the order Cycadales, and play a key role in understanding phylogenetic interrelationships of taxa.

ACKNOWLEDGEMENT

I would like to express deep gratitude towards Late Prof. N.D. Paria for his valuable guidance and supervision in pursuing this study. I am also grateful to Prof. Subir Bera for his support and guidance.

DISCLAIMER

The authors declared that there has been no conflict of interest while performing the work and preparation of the manuscript.

REFERENCES

Arezoo, E., Mohammadreza, E., Maryam, M. and Abdorreza, M.N. 2020. The synergistic effects of cinnamon essential oil and nano TiO2 on antimicrobial and functional properties of sago starch films. *International Journal of Biological Macromolecules*, **157**: 743-751.

Bera, S., Das, B., De, A., Barua, A., Das, S., De, B. and Samanta, A. 2020. Metabolite profiling and in-vitro colon cancer protective activity of *Cycas revoluta* cone extract. *Natural product research*, **34**(4): 599-603.

Bhatnagar, S.P. and Moitra, A. 1996. *Gymnosperms*. New Age International.

Biswas, C. and Johri, B.M. 1997. *The Gymnosperms*. Springer, Berlin, Heidelberg.

Bokdam, J. 1977. Seedling morphology of some African Sapotaceae and its taxonomical significance. Meded. Landbouwhogeschool. **77**(20): 1-84.

Bösenberg, J.D. 2023. *Cycas rumphii*. *The IUCN Red List of Threatened Species* 2023: e.T216470283A69156062. https://dx.doi.org/10.2305/IUCN.UK.2023-1.RLTS.T216470283A69156062.en.

Bösenberg, J.D. 2023. *Cycas circinalis*. *The IUCN Red List of Threatened Species* 2023: e.T42089A2959396. https://dx.doi.org/10.2305/IUCN.UK.2023-1.RLTS.T42089A2959396.en.

Burger, H.D. 1972. Seedlings of some tropical trees and shrubs, mainly of South-East Asia. Centre for Agricultural Publishing and Documentation (PUDOC), Wageningen.

Canne, J. 1983. The taxonomic significance of seedling morphology in *Agalinis* (Scrophulariaceae). *Canadian Journal of Botany,* **61**(7): 1868-1878.

Corner, E.J.H. 1976. The Seeds of Dicotyledons. Cambridge University Press.

Datta, S.C., Paria, N. and Sahoo, S.K.1980. Reproductive Potential of some Indian Weeds. *Seed Research,* **8**(2):100-107.

Deb, D.K. and Paria, N. 1986. Seedling morphology of some economic trees. *Ind. Agric*., **30**: 133-142.

Dehgan, B and Yuen, C.K.K.H. 1983. Seed morphology in relation to dispersal, evolution and propagation of *Cycas*. *Botanical Gazette*. The University of Chicago Press. **144** (3): 412-418.

Du, Q., Xing, N., Guo, S., Meng, X., Zhang, Y. and Wang, S. 2023. *Cycas revoluta* leaves: As a potential flavonoids source for targeted regulation of immune-related markers in lung adenocarcinoma. *Industrial Crops and Products*, **202**: 116967.

Duke, J. A. 1965. Keys for the identification of seedlings of some prominent woody species in 8 forest types in Puerto Rico. *Annals of Missouri Botanical Garden,* **52**: 314-350.

El-Seadawy, H.M., Abo El-Seoud, K.A., El-Aasr, M. and Ragab, A.E. 2023. Cycadaceae: An Important Source for Biflavonoids and Various Pharmacological Effects of Different *Cycas* Species. Journal of Advanced Medical and Pharmaceutical Research.

Farron, C. 1977. The treatment of seed and seedling collections. *Bauhinia,* **6** (1): 53-60.

Foster, A. 1939. Structure and growth of the shoot apex of *Cycas revoluta*. *American Journal of Botany,* **26**(6): 372-385.

Jackson, B.D. 1928. A Glossary of Botanical terms with their derivation and accent. Duckworth and Co., London. Jacobs, M., 1966. The study of seedlings. Fl. Mal. *Bull*, (21): 1416-1421.

Kalpashree, M.M. and Raveesha, K.A. 2013. Antibacterial activity of *Cycas circinalis* ovules-a naked seeded gymnosperm. *International Journal of Herbal Medicine,* **1**(3): 53-55.

Khuraijam, J. 2023. *Cycas orixensis*. *The IUCN Red List of Threatened Species* 2023: e.T103632258A103632295. https://dx.doi.org/10.2305/IUCN.UK.2023-1.RLTS.T103632258A103632295.en.

Kubitzki, K. ed., 1990. The families and genera of vascular plants (Vol. 1). Berlin: Springer-Verlag.

Lubbock, J. 1892. A contribution to our knowledge of seedlings, Vol. 1 & 2. Kegan Paul, Trench, Trubner and Co., London.

Martin, A. and Barkley, W. 1961. Seed Identification Manual. Berkley, California: University of California Press. 221.

Martin, A.C. 1946. The comparative internal morphology of seeds. *Amer. Midl. Naturalis*, **36**: 513-660.

Moawad, A., Hetta, M., Zjawiony, J.K., Jacob, M.R., Hifnawy, M., Marais, J.P. and Ferreira, D. 2010. Phytochemical investigation of *Cycas circinalis* and *Cycas revoluta* leaflets: moderately active antibacterial biflavonoids. *Planta medica*, 796-802.

Mourya, M.K., Prakash, A., Swami, A., Singh, G.K. and Mathur, A. 2011. Leaves of Cycas revoluta: Potent antimicrobial and antioxidant agent. *World J Sci Tech*, **1**(10): 11-20.

Paria, N.D. 2014. Botanical Research in India in the domain of Seedling Morphology in relation to Taxonomy. *Science and culture*, **80**: 262-270.

Paria, N.D., Bhattacharya, B. and Ghosh, M. 1990. Seedling morphology of some members of Malvales with a note on their cytology. *Journal of the National Botanical Society,* **44**: 1-19.

Ruishu, F. and Jian, L. 1997. The ultrastructure of leaf cells in albino seedlings of *Cycas revoluta*. *Subtropical Plant Research Communications,* **26**(1): 29-31.

Stevens, O.A. 1932. The number and weight of seeds produced by weeds. *American Journal of Botany,* **19**: 784-794.

Tomlinson, P.B. 1984. Vegetative morphology- Some enigmas in relation to plant systematics. *Current Concepts in Plant Taxonomy,* 49-66.

Vogel, E.F. de 1980. Seedling of Dicotyledons: Structure, development, types; Description of 150 woody Malesian taxa. Centre for Agricultural Publishing and Documentation (PUDOC), Wageningen.

J. Botan. Soc. Bengal 79(1) : 28-40 (2025), ISSN 0971-2976

FULL LENGTH ARTICLE

An ethno medicinal survey of some parts of district Nadia (West Bengal) for preservation of traditional knowledge

Smita Ghosh[1a], Tamonna Banerjee[1a], Seemanti Ghosh[a], Smita Ray[a] and Sritama Mukherjee[a]*

[1]Equal contributors

[a]Department of Botany, Bethune College,181, Bidhan Sarani, Kolkata-700006

Received : 30.12.2024 Accepted : 03.03.2025 Published : 30.06.2025

India is a vast repository of flora which has been used for human welfare since the inception of civilization. Plants are used as therapeutics since ancient times and this knowledge was handed down from one generation to another. With the emergence of chemical-based medicines, traditional knowledge is at the verge of extinction. Herbal medicines are beneficial in terms of efficacy, economy, availability and minimal side effects. Thus, ethnomedicine holds immense relevance and its documentation is a necessary aspect before such information is lost forever. The aim of this work is to preserve medicinal plant related Indigenous Traditional Knowledge. The survey was carried out based on interaction with local inhabitants of Bedibhawan and Habibpur villages of the Nadia district of West Bengal. People spanning different age groups and livelihood backgrounds served as the Key Knowledge Holders. Data was analysed in terms of Informant Consensus Factor to evaluate the homogeneity of the information from the participating informants. Forty-seven plant species belonging to 25 families were reported to be used in the treatment of various diseases. Mode of herbal drug administration included fresh consumption, infusion and decoction. Almost all plant parts particularly leaf proved to be the storehouse of the therapeutic active principles. Remedy was obtained for a variety of respiratory, digestive, topical and reproductive diseases. The pharmacological importance of most of the medicinal plants reported and used by the villagers was cited previously in literature. This report provides valuable information for ethnobotany and pharmacology which will pave way for future drug research.

Keywords: Ethnomedicine, Indigenous Traditional Knowledge (ITK), Informant Consensus Factor (ICF), Key Knowledge Holders (KKHs), Nadia district

INTRODUCTION

Ethnobotanical surveys are one of the reliable approaches to discover new drugs and natural products to be used as medicines. Plants supply the main medicinal source for peoples' health care according to the ancient traditional knowledge and modern scientific literature in developing Asian countries (Inglis, 1994; SEPA, 1997; Sheng-Ji, 2001). The indigenous knowledge found in these developing countries of Asia has always contributed to the World's cultural heritage (Sheng-Ji, 2001).The East Indian region consists of the states of Bihar, Jharkhand, Odisha and West Bengal and the union territory of the Andaman and Nicobar Islands. The region inherits its various Eastern Indo-Aryan languages from the historical region of Magadha (https://www.gsi.gov.in/, 2015). Out of 23 districts in

**Corresponding author :* sritama83m@gmail.com

West Bengal, the district of Nadia has astounding historical and geographical richness. The total area of Nadia is 3,927 km² including 3,604.05 km² rural area and 322.95 km² urban area Nadia district has an approximate population of 5,167,600 (https://nadia.gov.in/). The primary occupation of the people is agriculture. Different tribal communities of this area practice indigenous knowledge in their daily lives which leads to the use of the abundant natural resources found here (https://www.westbengal.gov.in/, 2000).

Population growth, loss of biodiversity, resource degradation by uncontrolled overharvesting of medicinal plants, overgrazing by animals, environmental and cultural changes as well as absence of written forms for treatments, have resulted in the loss of indigenous medical knowledge and breakdown of traditional medical system over the last quarter century (Sheng-Ji, 2001;Tripathi 2019).The present work attempts to document the knowledge handed down over generations with respect to the use of herbal medications. The report was drawn upon on site interaction with people spanning different age groups and livelihood backgrounds. Trend in the use of plant-based drugs has been analysed in terms of the diseases addressed, method of administration, parts used and the taxonomic grouping of the frequently used medicinal plants.

METHODOLOGY

MATERIALS AND METHODS

Study Area

The two areas selected for the present survey were Bedibhawan, a local village near Kalyani municipality area (22°58′30″N, 88°26′4″E), about 50 km from Kolkata and Habibpur (23°10′34.96″N, 88°30′58.04″ E), located near Ranaghat municipality area, about 82 km from Kolkata (Fig. 1). These areas experience tropical monsoon type of climate which makes them a rich abode of diverse plant groups including indigenous species. Habibpur, lying to the east of Hooghly River, locally known as Bhagirathi is mainly covered by farming lands and fresh water bodies. The ancestry of the people can be traced back to the ethnic Santhal community.

Data Collection

Indigenous Traditional Knowledge (ITKs) are the beliefs and practices that are developed within an indigenous community and passed down from generation after generations. A survey was carried out for the study, identification, and documentation of several ITKs used by the local villagers for health benefits. Field survey was done in the two villages - Bedibhawan and Habibpur of Nadia District (Fig. 1) of West Bengal during the period December 2020 to November 2021. Twelve respondents were selected as Key Knowledge Holders (KKHs) for each of the traditional practices for the diseases mentioned by them. They included farmers, village chiefs, librarian, housewives and elderly people. The interviews were performed using a structure questionnaire (Jovel, 1996; Buwa-Komoreng, 2019) (Table 1) as well as free-flowing talks to obtain additional information about the methods of treatment. For interacting with the KKHs, Fifteen visits on an average were performed at the location of study. During the field survey, photographs of plants included in ITK were taken, specimens were collected and documentation was done. Photographs of each ITK were digitally captured and used together for confirmation and record. Using the method of enquiry during the study, all necessary features of traditional knowledge about folk practices used in agriculture and usage for each of the traditional practices were obtained.

Data Analysis

Informant Consensus Factor (ICF) was used to evaluate the homogeneity of the ethnobotanical information from the participating informants using the following formula (Heinrich *et al.*, 1998), ICF= (Nur − Nt)/(Nur − 1), where Nur is the number of use reports for each disease category and Nt is the number of species used in that category.

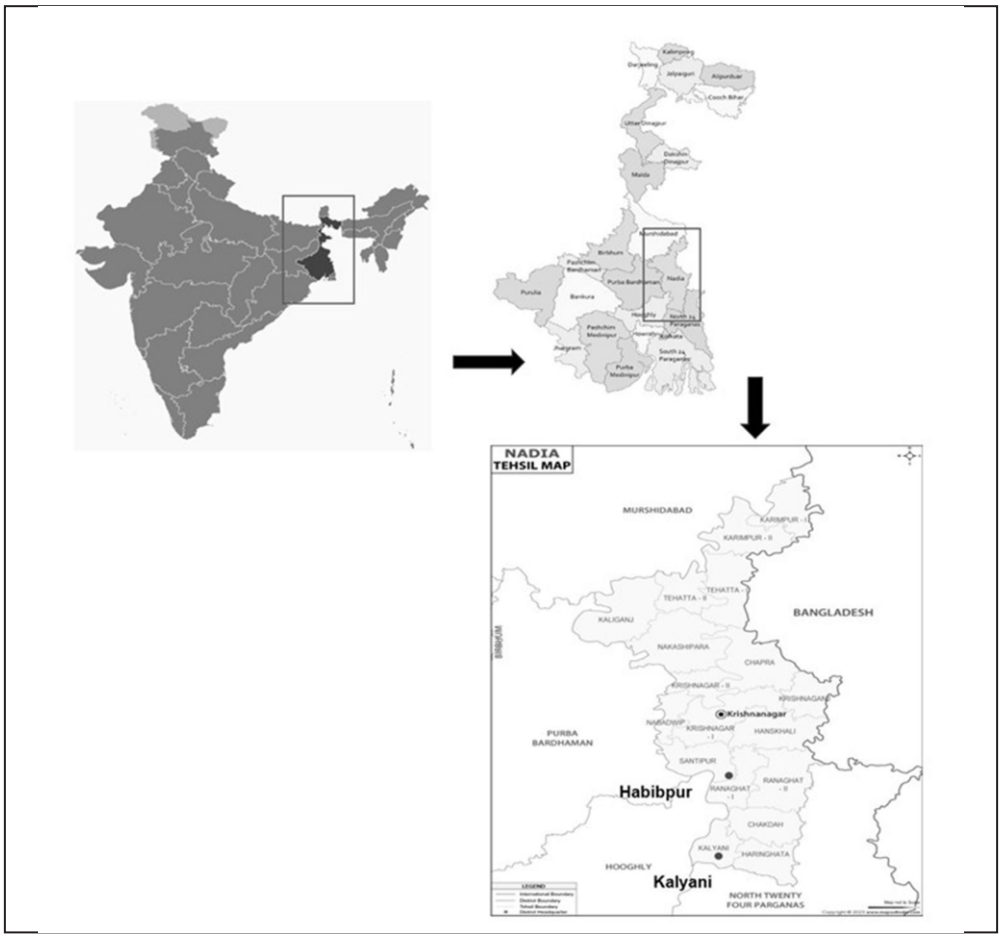


Fig. 1. The map of the study area (Map source: India map and Wikipedia)

Table 1. Questionnaire for ethnobotanical study

Parameter	**Questions**
Informant's Details	Name, Gender, Age, Location/Residence, Occupation
Questions for Ethnobotanical Study	1.Which part or plant product have you used for medicinal purpose?
	2. Which ailment do you use for?
	3. Which part of the plant do you use?
	4. How is it used?

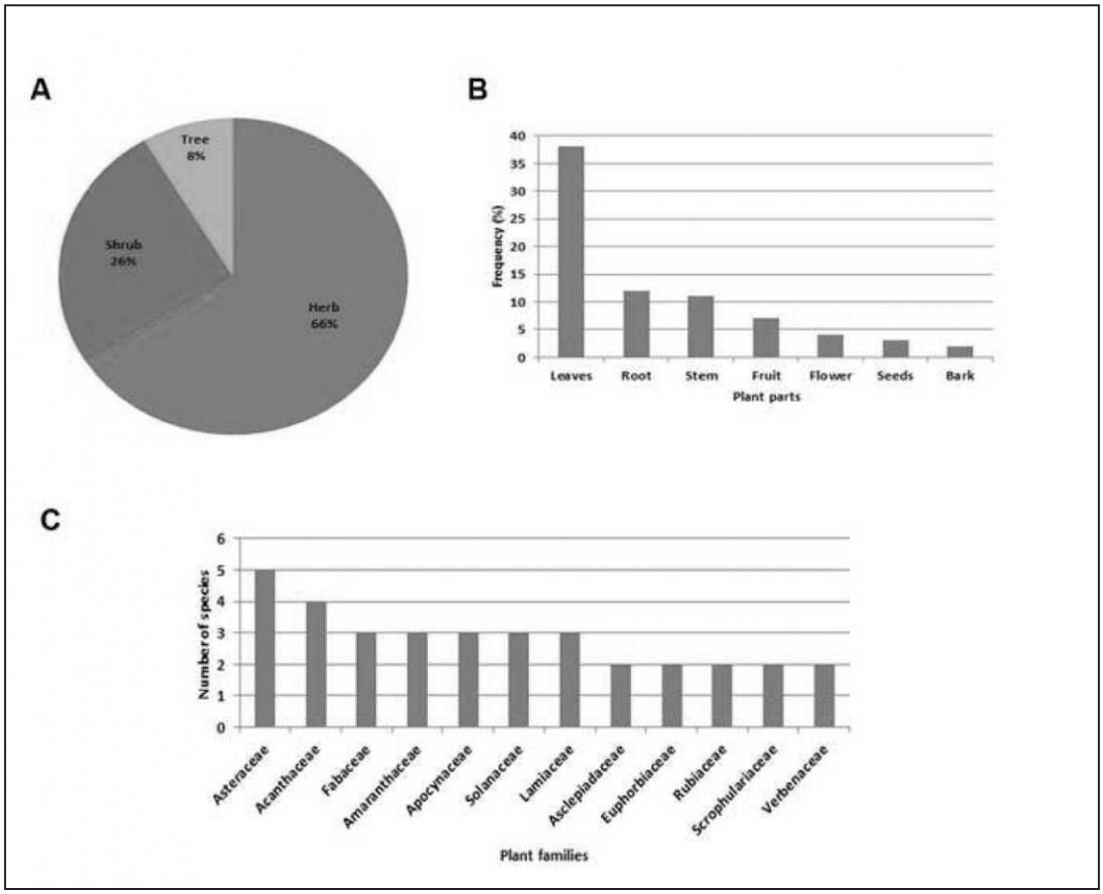


Fig. 2. The growth habit (A), plant parts used (B), and the plant families (C) reported in the present study.

RESULTS

Medicinal plants used in the study area

The present study rendered that the people of Nadia district of West Bengal are still utilizing medicinal plants for sustenance of their daily lives. There are 47 plant species belonging to 25 families which were reported to be used in the treatment of various ailments by the people **(**Table 2). The present study also showed that the most commonly used plants are the members of the family Asteraceae (five species) followed by Acanthaceae (four species), Fabaceae, Amaranthaceae, Apocynaceae, Solanaceae, Lamiaceae(three species each), Asclepiadaceae, Euphorbiaceae, Rubiaceae, Scrophulariaceae, Verbenaceae (two species each) while rest of the 13 families had one species each (Fig. 2).

Plant part used and mode of administration

Seven different medicinal plant parts (Fig. 2) were reported to treat 12 types of ailments in the present study. Leaves (38%) are the most frequently used plant part followed by root (12%), stem (11%), fruit (7%), flower (4%), seeds (3%) and bark (2%). Leaves are the main photosynthetic organs in a plant. Due to their ability to store different photosynthates and their subsequent conversion to secondary metabolites and bioactive compounds they are mostly used to treat (Kumar *et al*., 2021).

During the survey different mode of herbal preparation of the medicinal plant parts were reported. Infusion and decoction preparation from the plant parts were mostly listed during the interview process (Table 2). For cuts, wounds and skin diseases leaf or

Fig. 3. Method of traditional medicine preparation. A-B- Leaf infusion of *Azadirachta indica*; C-D-Direct use of warm *Calotropis* leaf; D-E-Leaf decoction of *Adhatoda vasica;* F- Use of *Moringa oleifera* fruits in vegetable soup; G-H- Leaf infusion of *Ocimum sanctum*; I-Use of *Paederia foetida* in vegetable soup.

Table 2. List of medicinal plants, their uses and methods of preparation and administration.

Family	Scientific Name	Common Name	Uses	Plant part(s) used	Methods of preparation and administration
Acanthaceae	*Andrographis paniculata* (Burm.f.) Nees	Kalmegh	Liver disorders cardiovascular diseases, common cold	Leaves	A cup full of leaf infusion is taken once a day
	Pseudoranthemum carruthersii	Patabahar	Heal wounds, inflammation	Leaves	Leaf paste is directly applied on wounds
	Ruellia humilis Nutt.	Patpati ful	Used as a cooling agent, for urinary problem and high cholesterol	Roots and Leaves	Root and leaf infusion is directly used
	Justicia gendarussa Burm.f.	Bishollokarani	Have beneficial effect in rheumatism, respiratory problem and throat infections	Leaves	Leaf decoction is taken orally
Amaranthaceae	*Alternanthera sessilis* (L.) R.Br. *ex* DC.	Sanche shak	Bronchitis and other lung troubles	Leaves and stem	Leaves are eaten as a vegetable
	Amaranthus cruentus L.	Data shak	Diarrhoea, ulcers, high cholesterol	Leaves, stem and seeds	Seeds and leaves are taken as cereal and vegetable respectively
	Amaranthus spinosus L.	Kanta note	Laxative, purifier, diuretic, for menorrhagia	Leaves, stem and roots	Leaf infusion is used
Apiaceae	*Centella asiatica* (L.) Urban	Thankuni	Stomach disorders, improve mental clarity, treats skin diseases	Leaves	Leaf decoction is taken with black pepper
Apocyanaceae	*Catharanthus roseus* (L.) G.Don.	Nayantara	Diabetes, depression, relieving muscle pain, anticancer	Stem, leaves, roots	Leaf paste is directly used for anthelmintic, root decoction is taken during fever

Contd....

Table 2. (contd.)

Family	Scientific Name	Common Name	Uses	Plant part(s) used	Methods of preparation and administration
Apocyanaceae	*Rauvolfia serpentina* (L.) Benth. ex Kurz	Sarpagandha	Hypertension pressure	Root	Root decoction is taken
	Tabernaemontana divaricata R.Br. ex Roem. & Schult.	Togor	Pharmaceutical products are prepared from flower extract, root is used to treat hypertension	Root, flowers	Flower juice is applied with oil on the skin to treat skin infection
Asclepiadaceae	*Calotropis gigantea* (L.) Dryand.	Akanda	Used as a cooling agent, for urinary problem and high cholesterol	Leaves	Leaves are cleaned, ghee or oil is applied on it and then heated in a hurricane or pan and applied in the areas of pain
	Hemidesmus indicus (L.) R.Br.	Anantamul	Astringent, blood purifier, control rheumatism skin diseases	Root	Root infusion is directly used for urinary tract and skin infections
Asteraceae	*Eupatorium triplinerve* M.Vahl	Ayapan	Control bleeding from open wounds, bloody diarrhoea, bleeding piles, stomach ulcer bleedings	Leaves, stem and root	Decoction is prepared from the whole plant including leaves, stem and root
	Eclipta prostate (L.) L.	Kesuth	Control hair loss, gastrointestinal problems	Leaves	Leaf infusion is directly applied to hairs
	Wedelia trilobata (L.) Hitchc.	Bhringaraj	Control hair loss	Leaves	Leaf infusion is directly applied to hairs
	Synedrella nodiflora (L.) Gaertn.	Relandi/ Tupi ful	Used to treat epilepsy, inflammations treat rheumatism, epilepsy	Leaves	Decoction is prepared from the leaves
	Tridax procumbens L.	Tridhara	Immunomodulatory, hepatoprotective and mosquitocidal properties	Leaves	Leaf decoction is used

Contd....

Table 2. (contd.)

Family	Scientific Name	Common Name	Uses	Plant part(s) used	Methods of preparation and administration
Caricaceae	*Carica papaya* L.	Pempe	Used in the treatment of asthma, aiding in digestion	Ripened fruit	Fresh, ripe fruit are directly taken, unripe fruit is cooked as vegetable
Cucurbitaceae	*Cucurbita maxima* Duchesne	Kumro	Prevention constipation, blood pressure regulation	Fruit and leaves	Cooked as a vegatable
Euphorbiaceae	*Euphorbia milii* Des Moul.	Kanta mukut	Used for the treatment of skin diseases, breathing disorders, gonorrhoea, intestinal parasites	Leaves	Leaf decoction is used
	Euphorbia hirta L.	Boro dudhi	Treats cough, bronchitis, asthma, digestive problems	Leaves	Leaves are cleaned, dried, and crushed, and then mixed with other spices like cumin, salt, turmeric and taken as powder
Fabaceae	*Saraca asoca* (Roxb.) Willd.	Ashok	Astringent, detoxifier, cardiac tonic, reproductive disorders in women	Bark, leaves, flowers, seeds	Flower buds are eaten directly by women, leaf and bark decoction is used
	Cajanus cajan (L.) Millsp.	Arhar	Diabetes, skin irritations, jaundice, measles	Pods	Eaten as dal
	Erythrina vairegata L.	Parijat	Dysentery, menstrual problems, laxative, diuretic	Bark and leaves	Leaf infusion is mixed with honey and engulfed to kill tapeworms and roundworms
Lamiaceae	*Ocimum grastissimum* L.	Ram tulsi	Analgesic, gastroenteritis, cough and cold	Leaves	Decoction is prepared with leaves and honey and is taken as syrup. Fresh leaves are also chewed directly
	Ocimum tenuiflorum L.	Krishna tulsi	Throat infections, respiratory problems	Leaves	

Contd....

Table 2. (contd.)

Family	Scientific Name	Common Name	Uses	Plant part(s) used	Methods of preparation and administration
Lamiaceae	*Ocimum sanctum* L.	Radha tulsi	Common cold, cough, allergic rhinitis	Leaves	
Liliaceae	*Aloe vera* (L.) Burm.f.	Ghritakumari	Used to treat constipation and skin disorders	Leaves	Leaf infusion is used
Meliaceae	*Azadirachta indica* A. Juss.	Neem	Treatment of fungal disease, plaque from teeth, stomach upset, loss of appetite	Leaves and stem	Stems are used as tooth brushes. Decoctions are prepared from leaves. They are also cooked with vegetables
Moringaceae	*Moringa oleifera* Lam.	Sojne	Plant parts act as cardiac and circulatory stimulants, stomach disorders, malnutrition	Entire plant	Flowers, leaves and fruits are taken as vegetales
Nyctaginaceae	*Boerhavia diffusa* L.	Punarnava	Anti-inflammatory and antispasmodic properties	Leaves and stem	Leaves and stem are used as a vegetable
Oxalidaceae	*Oxalis corniculata* L.	Amrul	Rich in vitamin C and used to treat scurvy, ulcers in mouth	Leaves	Leaf infusion is used
Papaveraceae	*Argemone mexicana* L.	Seyal kanta	Anthelmintic, cure skin diseases, purgative and sedative	Root, leaves, seeds	Leaf infusion is used
Polygonaceae	*Rumex crispus* L.	Bon palong	Used to treat gastrointestinal tract disorders, anthelminthic diseases, anti-inflammatory and arthritis	Leaves	Leaf infusion is directly applied to the skin
Portulacaceae	*Portulaca grandiflora* Hook.	Time ful	Used in the treatment of hepatitis, and cirrhosis of the liver	Leaves and stems	Leaf infusion is directly applied to the skin

Contd....

Table 2. (contd.)

Family	Scientific Name	Common Name	Uses	Plant part(s) used	Methods of preparation and administration
Ranunculaceae	*Nigella arvensis* L.	Kalo jira	Seeds are antioxidant and used to treat stomach pain and ulcers, used on cakes as flavoring agent	Seeds	Seeds are used as spices, decoction is prepares with garlic and oil and directly applied to the skin
Rubiaceae	*Paederia foetida* L.	Gandal	Stomach ache, diarrhoea, liver problems	Leaves	Leaves are cleaned and cooked with veggies like potato and carrot
	Ixora coccinea L.	Rangan	Dysentry	Flowers, leaves, fruits	Fusion of leaves and fruits are used directly, flowers are eaten directly or as food supplement
Rutaceae	*Murraya koenigii* (L.) Spreng.	Kari pata	The green leaves are used in treating piles, inflammation, itching, fresh cuts, dysentery	Leaves and roots	Leaves is directly used as flavoring agent in food dishes
Scrophulariaceae	*Scoparia dulcis* L.	Bon dhoney	Leaves are used for diabetes, digestive problems, dysentery, diabetes, insect bite	Leaves	Leaves and stem are used as a vegetable
	Mazus pumilus (Burm.f.) Steenis	Tutra	Leaves and stem are used to treat diabetes and typhoid	Leaves and stem	Leaf infusion is used
Solanaceae	*Capsicum annum* L.	Lanka	Relieve the pain of post-therapeutic neuralgia and other pain syndromes, anticarcinogenic	Fruits, seeds	Fruits are eaten both raw and cooked
	Datura metel L.	Dhutro	Prevent stomach and intestinal problems, toothache, anti-hairfall	Leaves, root and fruit	Roasted leaves are used
	Physalis angulata L.	Bontepari	Analgesic and tranquilizing activities	Fruits	Fruits are eaten raw or cooked
Verbenaceae	*Phyla nodiflora* (L.) Greene	Bhuni okra	Diarrhoea, joint pain, indigestion, asthma, bronchitis, piles	Leaves and roots	Leaf and root infusion are used
	Lippia alba (Mill.) N.E.Br. ex Britton & P. Wilson	Sada matmatia	Respiratory illnesses such as asthma and influenza as well as headache, depression, anxiety	Leaves	Leaf infusion is used once a day

Table 3. Ailment category and ICF (Informant Consensus Factor) Values

Ailment Category	Nur	Nt	ICF
Respiratory diseases	21	9	0.6
Reproductive system diseases	6	3	0.6
Cardiovascular diseases	17	9	0.5
Musculoskeletal and neural disorders	7	4	0.5
Dental problems	3	2	0.5
Bacterial and viral diseases	9	5	0.5
Cancer	8	5	0.42
Fever	8	5	0.42
Vitamin deficiency	10	7	0.33
Diabetes	7	5	0.33
Wound, ulcers, sores and skin diseases	20	14	0.31
Digestive system diseases	19	16	0.16

Respiratory diseases: Asthma, cough, and colds; shortness of breath and bronchitis; Reproductive system diseases: Menorrhagia, urinary tract infection and women infertility; Cardiovascular diseases: Hemorrhage, high and low blood pressure, high cholesterol, hypertension; Musculoskeletal and neural disorders: Headaches, chest pain, arthritis, rheumatism, epilepsy and body pain; Dental problems: toothache, Bacterial and viral diseases: Influenza, typhoid, hepatitis, measles, gonorrhoea; Cancer: Cancer; Fever: Fever; Vitamin deficiency: Scurvy, malnutrition; Diabetes: Diabetes; Wound, ulcer, sores and skin diseases: Ulcers, wound, insect bite, mosquito bite itching, sore, warts and hair loss Digestive system diseases: Stomach disorders, infections by parasites, jaundice, constipation, vomiting, diarrhea and dysentery; Nur: Number of reports; Nt: Number of taxa; ICF

root infusions are directly applied. Often the leaves are ground to prepare fine powders and are directly applied to the cut site or skin infection. For respiratory diseases mostly decoctions are prepared with the plant part and honey. Using the fresh plant material without preparation is another method of administration for different health issues like orthopaedic, urinary, digestive and respiratory problems. Sometimes roasted leaves are also taken for digestive problems. Seeds, leaves, fruits are often taken as raw vegetable or in cooked form to treat digestive problems, fever and to develop immunity (Fig. 3).

Ailment category and determination of ICF

The medicinal plants which are frequently mentioned by the informants were: *Datura metel*, *Scoparia dulcis*, *Murraya koenigii*, *Paederia foetida, Oxalis corniculata, Boerhavia diffusa, Moringa oleifera, Azadirachta indica, Aloe vera, Ocimum sanctum, Cajanus cajan, Saraca asoca, Euphorbia milii, Carica papaya, Eclipta prostata, Hemidesmus indicus, Catharanthus roseus, Centella asiatica, Justicia gendarussa, Andrographis paniculata*. There were at least 14 plants: *Moringa oleifera, Azadirachta indica, Aloe vera, Ocimum sanctum, Murraya koenigii, Paederia foetida, Oxalis corniculata, Boerhavia diffusa, Centella asiatica, Ocimum tenuiflorum, Ocimum gratissimum, Eclipta prostata, Catharanthus roseus, Centella asiatica* commonly used in the two villages of Nadia district (Bedibhavan and Habibpur).

The 47 plants reported from the present survey were used to treat 12 types of ailments that includes respiratory diseases, reproductive system diseases, cardiovascular diseases, musculoskeletal and neural disorders; dental problems, infectious diseases caused by bacteria and viruses, cancer, fever, vitamin deficiency, diabetes, wounds, ulcers and skin diseases and digestive system diseases (Table 3). The highest

ICF value (0.6) was observed for respiratory and reproductive system diseases due to the ability of the informants to diagnose followed by easy treatment the diseases. The other diseases with high ICF values (0.5) are cardiovascular, musculoskeletal and neural disorders, dental problems and infectious diseases caused by bacteria and viruses. As reported by the villagers, there were nine plant species viz. *Justicia gendarussa, Alternanthera sessilis Euphorbia milii, Euphorbia hirta, Ocimum grastissimum, Ocimum tenuiflorum, Ocimum sanctum, Phyla nodiflora* and *Lippia alba* reported to cure respiratory problems like asthma, cough, and colds; shortness of breath and bronchitis. Menorrhagia, urinary tract infection and women infertility were reported under the reproductive system diseases category and *Ruellia humilis, Amaranthus spinosus, Saraca asoca* were the plants for their treatments (Tables 2 and 3). Sometimes single plant species like *Eupatorium triplinerve, Justicia gendarussa, Ocimum sanctum, Moringa oleifera, Rumex crispus,* were documented to have medicinal importance against wide array of diseases.

DISCUSSION

The documentation of 47 medicinal plant species belonging to 25 families from the two villages in Nadia district of West Bengal speaks about its rich ethnomedicinal heritage that passes on from one generation to another. The plants were reported to treat a myriad of ailments like respiratory diseases, reproductive system diseases, cardiovascular diseases, musculoskeletal and neural disorders etc. Families such as Asteraceae, Acanthaceae, Fabaceae, Amaranthaceae, Apocynaceae, Solanaceae and Lamiaceae were represented with high number of species having ethnomedicinal potentialities. It is interesting to note that some of the plant species were used to treat a specific disease whereas others were active against a wide array of diseases. As per previous reports Lamiaceae, Asteraceae, Fabaceae are the most preferred families with ethnomedicinal importance (Wanda *et al*., 2015; Raj *et al*., 2018; Gras *et al*., 2021; Cordero *et al*., 2022). Majority of the plant species had more than one important plant part in use out of which leaves (38%) were mostly used (Cordero *et al.*, 2022) followed by root (12%) stem (11%) and fruit (7%).

The pharmacological importance of most of the medicinal plants reported and used by the villagers- *Azadirachta indica, Eclipta prostrata*, *Paederia foetida, Ocimum* sp., *Eupatorium triplinerve* were cited previously in literature. The ethno-pharmacological importance Indian Sarsaparilla (*Hemidesmus indicus*) and their recent progress in phytochemical research are cited in the literature (Nandy *et al*., 2020). The ethnopharmacological importance of plants encountered in the current survey supports previous studies conducted from different parts of India involving neem. The ethnomedicinal importance of Neem in India (Moin *et al*., 2021); wide use of *Eclipta prostrata* as traditional medicine in different countries including India for skin, liver and stomach problems (Timalsina and Devkota, 2021); the ethnomedicinal tribal plant of northeastern India, *Paederia foetida* (Chanda *et al.*, 2013); the ethnomedicinal importance of the queen of herbs, 'tulsi' (Joshi *et al.*, 2017); ethnomedicinal importance of *Eupatorium triplinerve* (Cheriyan *et al.*, 2019) were some more evidences of ethnopharmacological importance of the medicinal plants.

CONCLUSION

Developed from experience which is being gained over centuries and applying the knowledge in day-to-day medicinal practice according to the locality and environment; indigenous traditional knowledge is culturally and scientifically accepted over ages. It plays a crucial role in protecting the environment and provides valuable resources for the development of new drugs. The present study revealed important information on medicinal plants to treat different ailments by the villagers and herbalists of the Nadia district of West Bengal. The efficacy of the therapeutic claims of these medicinal plants can be pharmacologically investigated and validated. The indigenous traditional knowledge of Nadia district is passed on from generation to generation verbally and documentation is urgently needed before it is forgotten due to social and environmental challenges. Hence, the present study provides valuable information in the field of ethnobotany and pharmacology that might be helpful in future drug research as well as for generating responsibility towards conservation of biodiversity.

ACKNOWLEDGEMENT

The authors acknowledge the local inhabitants of Nadia district for sharing their valuable traditional knowledge). The authors are further thankful to DBT Star College Scheme for financial assistance.

DISCLAIMER

The authors declared that there has been no conflict of interest while performing the work and preparation of the manuscript.

AUTHOR(S) CONTRIBUTION

SM and SG[a] conceptualized the work. SG[1a] and TB had equal contribution in performing the survey, data recording and analysis of results. All authors drafted the manuscript.

REFERENCE

Buwa-Komoreng, L.V., Mayekiso, B., Mhinana, Z. and Adeniran, A.L. 2019. An Ethnobotanical and Ethnomedicinal Survey of Traditionally Used Medicinal Plants in Seymour, South Africa: An Attempttoward Digitization and Preservation of Ethnic Knowledge, *Pharmacognosy Mag,* **14**: 115.

Chanda, S., Sarethy, I., De, B. and Singh, K. 2013. *Paederiafoetida* – a promising ethno-medicinal tribal plant of northeastern India. *J Forestry Res,* **24**: 801-808.

Cheriyan, B.V., Scarlet, S., Joshi, P.S. and Mohamed, S.S. 2019. *Eupatorium triplinerve* (Vahl): An Ethnobotanical Review, *Asian J Pharm. Res,* **9(3)**: 200-202.

Cordero, C.S., Meve, U. and Alejandro, G.J.D. 2022. Medicinal plants used by the indigenous Panay Bukidnon in Lambunao, Iloilo, Philippines, *Frontiers in Pharmacol,* **12**: 790567.

Gras, A., Hidalgo, O, D'Ambrosio U., Parada, M., Garnatje, T. and Vallès, J. 2021.The role of botanical families in medicinal ethnobotany: a phylogenetic perspective. *Plants* (Basel), **10**(1): 163.

Heinrich, M., Ankli, A., Frei, B., Weimann, C. and Sticher, O. 1998. Medicinal Plants in Mexico: Healers' Consensus and Cultural Importance. *Soc Sci Med,* **47**: 1859-1871.

Inglis, J. 1994. Introduction. *Nature & Resources,* **30**: 3-4.

Joshi, R.K., Setzer, W.N. and da Silva, J.K. 2017. Phytoconstituents, traditional medicinal uses and bioactivities of Tulsi (*Ocimum sanctum* Linn.): A review, *American J Essential Oils and Nat Pro,* **5**(1): 18-21.

Jovel, E.M., Cabanillas, J. and Towers, G.H. 1996. An ethnobotanical study of the traditional medicine of the Mestizo people of Suni Mirano, Loreto, Peru. *J Ethnopharmacol*, **53**:149-56.

Kumar, M., Rawat, S., Nagar, B., Kumar, A., Pala, N.A., Bhat, J.A., Bussmann, R.W., Cabral-Pinto, M. and Kunwar, R. 2021. Implementation of the use of ethnomedicinal plants for curing diseases in the Indian Himalayas and its role in sustainability of livelihoods and socioeconomic development. *Int J Environ Res Public Health,* **18**(4): 1509.

Moin, M.S., Siddiqui, J.I., Alam, M.A., Khatoon, F., Khan, S. and Minhajuddin, A. 2021. Ethnomedicinal potential of widely used plant *Azadirachta indica* A. Juss: A comprehensive review. *The J Phytopharmacol,* **10**(6): 456-467.

Nandy, S., Mukherjee, A., Pandey, D., Ray, P. and Dey, A. 2020. Indian Sarsaparilla (*Hemidesmus indicus*): Recent progress in research on ethnobotany, phytochemistry and pharmacology. *J Ethnopharmacol,* **254**:112609.

Raj, A.J., Biswakarma, S., Pala, N.A. *et al*. 2018. Indigenous uses of ethnomedicinal plants among forest-dependent communities of Northern Bengal, India. *J Ethnobiol Ethnomed*, **14 (8)**:1-28.

SEPA, National status report on biodiversity of China, States Environment Protection Agency (SEPA). 1997. China Environment Press. Beijing.

Sheng-Ji, P. 2001. Ethnobotanical Approaches of Traditional Medicine Studies: Some Experiences from Asia, *Pharmaceutical Biol,* **39**:74-79.

Timalsina, D. and Devkota, H.P. 2021. *Eclipta prostrata* (L.) L. (Asteraceae): Ethnomedicinal uses, chemical constituents, and biological activities. *Biomolecules,* **11**(11): 1738.

Tripathi, S. 2019. Ethnomedicine and future challenges. *Glob J Archaeol Anthropol*, **10**(5): 87-90.

Wanda, J.M., Gamo, F. and Njamen, D. 2015. Medicinal plants of the family of Fabaceae used to treat various ailments. In: Classification, Nutrient Composition and Health Benefits Series edited by W. Garza (NY: Nova Science Publishers, Inc.).

J. Botan. Soc. Bengal 79(1) : 41-46 (2025), ISSN 0971-2976

University of Calcutta, Kolkata 700 019, India

FULL LENGTH ARTICLE

Moullava digyna (Rottler) Gagnon & G.P. Lewis (Leguminosae: Caesalpinioideae), A new distributional record for the flora of Telangana

A. Appaiah[1], J. Swamy[2], G. Ravi[1], Nagendra Singh Chauhan[3] and P. Shivakumar Singh[4]*

[1]Bharatiya Engineering Science and Technology Innovation University, Anantapur, Andhra Pradesh-515731

[2]Botanical Survey of India, Acharya Jagadish Chandra Bose Indian Botanic Garden, Howrah, West Bengal-711103

[3]Drugs Testing Laboratory, Avam Anusandhan Kendra, Raipur 492010, Chhattisgarh, India

[4]Department of Botany, Palamuru University, Mahabubnagar, Telangana-509002

Received : 24.01.2025 Accepted : 02.04.2025 Published : 30.06.2025

Moullava digyna (Rottler) Gagnon & G.P. Lewis (Fabaceae: Caesalpinoideae) is a both medicinal and economically important plant and also has high potential of ornamental value. The species is presented here as a new record to Telangana State based on its recent collection from Bhadradri Kothagudem District of the State. A detailed description of it along with a photographs and taxonomic note is provided to facilitate its easy identification.

Keywords: *Moullava digyna*, Fabaceae, Telangana.

INTRODUCTION

The genus *Moullava* Adans.is represented by four species, of which three species are distributed in tropical and subtropical part of Southern Asia and one species namely, *Moullava welwitschiana* (Oliv.) Gagnon & G.P. Lewis is distributed in tropical Africa (Gagnon *et al*., 2016). In India, the genus is represented by three species namely, *Moullava digyna* (Rottler) Gagnon & G.P. Lewis, *M. spicata* (Dalzell) Nicolson and *M. tortuosa* (Roxb.) Gagnon & G.P. Lewis, of which *M. spicata* is endemic to Goa, Karnataka, Kerala and Maharashtra (Sanjappa, 2010 and 2020). It is already stated by Baker in FBI, that this species is distributed in "Eastern Himalaya, Eastren and Western Penusular and Ceylon", also in Malay islands. So it is expected that these plants is present in Telangana state but not been collected as a recorded and documented species, as become it has already been reported from Odissa and Tamil Nadu as well as Andhra Pradesh (as stated by Sanjappa, 2010). Mostly in flower lower sepal arching over the upper sepal as a character of ceasalpina are recorded.

In a field exploration tour to Bhadradri-Kothagudem District, Telangana, a collection of *Moullava* species was made which on critical examination was identified as *Moullava digyna* (Rottler) Gagnon & G.P. Lewis. So far, it has not been reported from Telangana (Pullaiah, 2015; Reddy and Reddy, 2016) of come a distributional data for a is available from the either literature in estern Himalaya as, Andhra Pradesh, Assam, Bihar, Orissa (Odissa), Tripura,

**Corresponding author* : drpsksingh1@gmail.com

Tamil Nadu, West Bengal (Sanjappa, 2010) and thus the present collection forms the first generic report of this species Telangana state.A detailed description along with a photographs and taxonomic note is provided to facilitate easy identification.

TAXONOMIC TREATMENT

Moullava digyna (Rottler) E. Gagnon & G. P. Lewis in Phytokeys 71: 67.2106. *Caesalpinia digyna* Rottler in Ges. Naturf. Freude Berlin NeueSchriften 4: 200.1803; Baker in Hook.f., Fl. Brit. India 2: 256.1878; Chaudary, Assam Flora, (Present status of Vascular plants), Prain, Plaugail, Pl: 323, 1903, (1963 Reb.) 2010. Gamble, Fl. Press, Madras 1: 394 (279Reb). 1919; Sanjappa, Legumes India 11.2010. Pal in Pal *et al*. (edu.)., Fl. West Bengal. 2: 8: 2015; Sanjappa in A.A. Mao & S.S. Dash, Fl. Pl. India Annot. Checkl. Dicotyledon 1: 310.2020.*Caesalpinia oleosperma* Roxb., Horst benz. 32. 1834. Fl. Ind. 2:359.1832.

Large scrambling, prickly shrub, usually to 5 m high. Stem bark dark brown stem with strong recurved prickles; young parts densely rusty brown hairy; branchelets shining, prickly and prickles 4-5 mm long; lower prickles straight and sharp. Leaves alternate, bipinnate, 10-20 cm long pinnae; 8-13 pairs., of pinnae; stipules subulate, reaches 3 mm long, slightly hairy, caduceus rachis with prickles at each pair of pinnae 4-5 cm long, unarmed; leaflets opposite, 7-12 pairs per pinna, sub-sessile, membranous, oblong or ovate-oblong, 5-11 × 2.5-4.5 mm, oblique-truncate at base, entire along margin, rounded, obtuse, sub-truncate at apex, glabrous on upper surface, appressed short-hairy beneath, glabrescent with age, lateral veins obscure. Inflorescences axillary, supra-axillary and terminal as 8-30 cm long racemes, predenele glabrous or hairy; floral bracts somewhat navicular, c. 4 × 0.4 mm, hairy; pedicels, slender, 1.5-3 cm long, glabrous or with a few hairs, articulated at the base. Flowers yellow, 1.5-1.8 cm across., calyx tube very short, oblique, persistent,0.5-0.8 mm long; lobes 5, oblong, 4-8 × 2-4 mm, hooded, rounded at the top, imbricate in the bud, the upper nearly to 8 mm long, arching over the lower 4-5 mm long sepals and falling after quartered petels 5, inserted on the nines of the calyx tube, orbicular, obovate or oblong, rounded, imbricate the bud, emarginated at apex, shortly up to 2 mm long clawed at base. 5-10 × 3-8 mm stamens 10, slightly excreted, free; filaments 10-12 mm long, dilated woolly at the base; anthers 1.2-1.5 mm long, glabrous. Ovary 3-4 mm long, glabrous or silky hairy, 2-4-ovuled; style 6-8 mm long, glabrous; stigma nearly 0.3 mm diam., short hairy along the margin. Pods shortly stipulate with persistent hypanthium, sub-torulose, 2.5-5 × 1.5-2 cm, oblong, elliptic-oblong, rounded at base, acute or short-beaked at apex, with thickened sutures, fleshy, indehiscent, 1 to 3 seeded, seeds sub-globose, 2 cm diam., dark brown.

Flowering time: August to October.
Fruiting time: January-March.
Habitat & Ecology: Plant grows along the streams in mixed dry deciduous forests.
Distribution: India: Andhra Pradesh, Arunachal Pradesh, Assam, Bihar, Chhattisgarh, Maharashtra, Odisha, Sikkim, Tripura, Tamil Nadu and West Bengal (Sanjappa, 2020) and Telangana. Bangladesh, Bhutan, Burma, Cambodia, China, Laos, Malesia, Nepal, Srilanka, Thailand, and Vietnam.

Specimen examined: India, **Telangana**, Bhadradri-Kothagudem district, Palwancha Forest Division, Aswaraopeta Range, Katkur RF block, Anantharam Section, Panduvarigudem beat, 30.09.2022, *A. Appaiah* & *J. Swamy* 628.

Note: *Moullava digyna* is allied to *Mezoneuron enneaphyllum* (Roxb.) Wight & Arn.ex Voigt and *Biancaea decapetala* (Roth) O.Deg. by its In this case habit and colour can be considered as important character. Actually fleshy fruit beaked, only 1-4 seeded condition and the characters are the important for this species. Thus cannot be compared with species of Mezenogone or even Bianaceae, it can be compare with the species of Moullava or of Ceasalpinia but it can be easily recognised by its fleshy, indehiscent, sub-torulose, wingless pods and sub-globular seeds whereas in later two species, the pods are dehiscent, not sub-torulose, winged like extension seeds found as compressed nature.

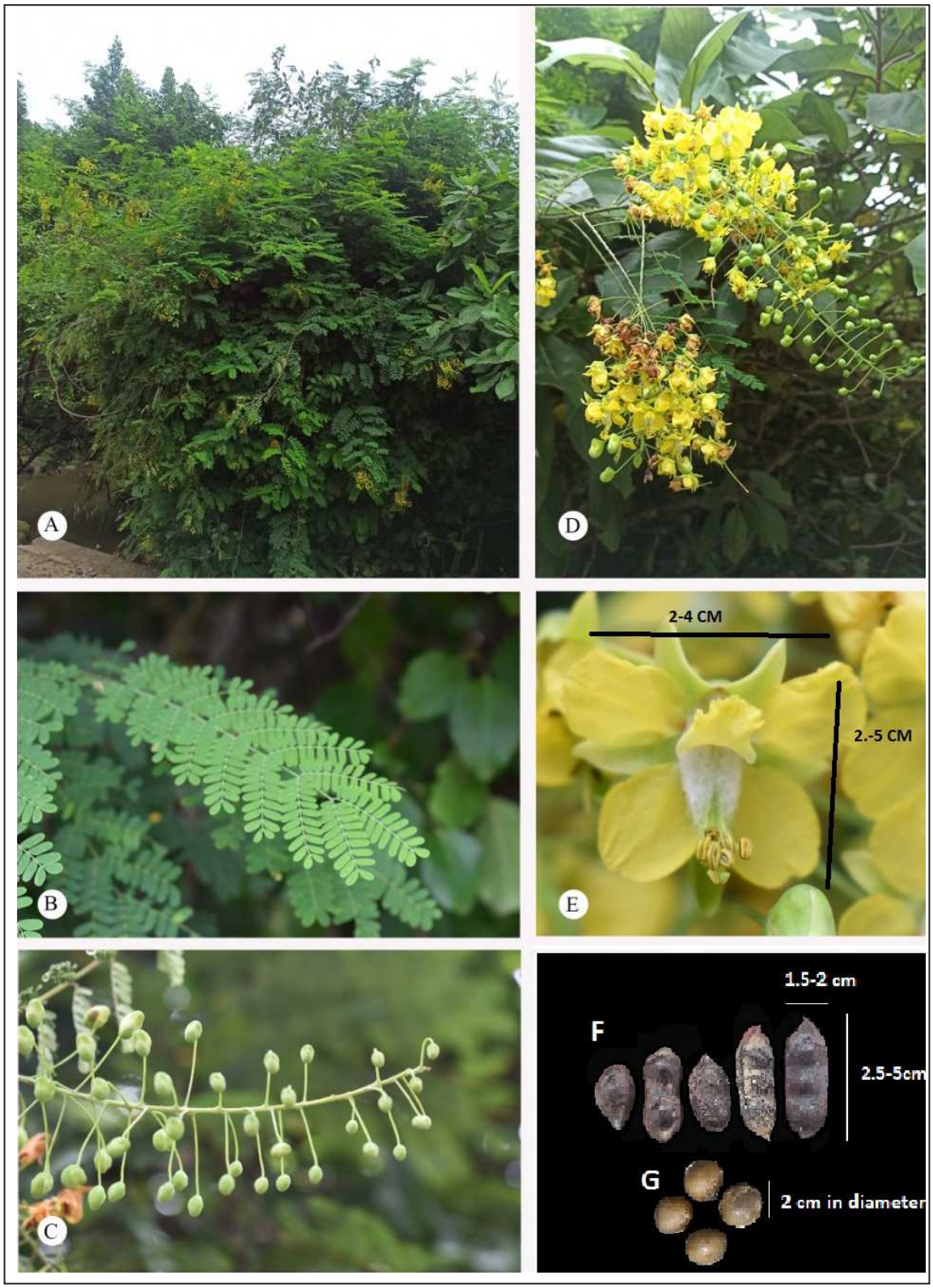


Fig. 1. Moullava digyna (Rottler) Gagnon & G.P. Lewis:
A. Habit; **B.** Leaf; **C-D.** Inflorescence; **E.** Flower; **F.** Pods; **G.** Seeds

ACKNOWLEDGEMENTS

Authors are grateful to the Director, Botanical Survey of India (BSI), Kolkata, Scientist In-charge of BSI, Deccan Regional Centre, Hyderabad and AJC Bose Indian Botanic Garden, Howrah for facilities and encouragements and to the officials of Telangana State Forest Department, for forest permission and logistic support.

DISCLAIMER

The author(s) declare no conflict of interest in the work.

REFERENCE

Gagnon, E., Bruneau, A., Hughes, C.E., De Queiroz, L.P. and Lewis, G.P. 2016. A new generic system for the pantropical Caesalpinia group (Leguminosae). *PhytoKeys* **7**1: 1-160. doi: 10.3897/phytokeys.71.9203.

Pullaiah, T. 2015. *Flora of Telangana, the 29th State of India*. vol. 1.Page no: 232, Regency Publications, New Delhi.

Reddy, K.N. and Reddy, C.S. 2016. *Flora of Telangana State, India*. M/s Bishen Singh Mahendra Pal Singh, Dehra Dun, India,

Sanjappa, M. 2020. Fabaceae. In A.A. Mao and S.S. Dash (eds.), *Flowering Plants of India: An Annotated Checklist (Dicotyledons)* Vol. **1**. Botanical Survey of India, Kolkata. pp. 310-311.

Sanjappa, M. 2010, Legume of India. M/s Bishen Singh Mahendra Pal Singh , Dehardun, P. no 11

J. Botan. Soc. Bengal 79(1) : 45-57 (2025), ISSN 0971-2976

FULL LENGTH ARTICLE

Effects of whole plant extract of *Artemisia vulgaris* L. on insect larvicidal activity and brine shrimp toxicity

Arghya Ghosh[1] and Prantik Hazra[2]*

[1]Department of Botany, Jhargram Raj College, Jhargram

[2]Department of Zoology, Darjeeling Government College, Darjeeling

Received : 27.01.2025 Accepted : 17.06.2025 Published : 30.06.2025

Over the past few decades, there has been a growing interest in alternative insecticides, especially those made from plants and referred to as botanical insecticides, due to the negative effects that synthetic pesticides have on non-target creatures and the environment. Nevertheless, the supply of commercially accessible botanical pesticides is restricted. The basic insecticidal properties of *Artemisia vulgaris* L. in methanol, acetone, and benzene crude extracts of entire plant bodies are presented in this research paper. The test organisms utilised in this instance were the larvae of *Culex* sp., *Chironomus* sp., *Anopheles* sp., and brine shrimps. The acetone and benzene extracts of the total plant body exhibited the most larvicidal and brine shrimp killing properties among the three. The acute toxicity of *Artemisia vulgaris* L. on aquatic macroinvertebrates was demonstrated by a study conducted with insect larvae and brine shrimps, and this phenomenon may be used to manage vector-borne disease and lower environmental dangers.

Keywords: *Artemisia vulgaris* L., Toxicity Effects, Whole Plant Extracts, Insect Larvae, Brine Shrimp.

INTRODUCTION

The most popular method of preventing agricultural pest insects and disease-carrying insects is to use synthetic insecticides and larvicide (Brain, 1979; Mkenda *et al*., 2015; Ghosh, 2018; Hazra and Ghosh, 2024). It is widely acknowledged that applying pesticides shields crops from insect damage and increases agricultural yield. According to Dougan *et al*. (1979), there would be a 35–45% reduction in global food output if pesticides were not used, and that view was also supported by Zhang *et al*. (2022) and Anand and Rai (2024). However, since the release of Silent Spring (Janardhanan and Lakshmanan, 1985), the use of artificial pesticides and larvicides has grown to be one of the most contentious issues and is still a top regulatory concern in many nations.

Because larvicides and insecticides are toxic, their careless application has resulted in pollution of the water, soil, crops, and air; it has also caused insect pests and vectors to reappear and become more resistant; and it has caused toxicity to creatures that are not intended targets (Huizing *et al*., 1985; Oerke, 2006; Guedes *et al*., 2016). Botanical insecticides are an alternative to synthetic pesticides. Insect pests can be controlled by botanical pesticides, which are made of compounds extracted from plants, dried and pulverized plant materials, or crude plant extracts

**Corresponding author :* prantikhazra@gmail.com

(Carson, 1962; Joseph *et al*., 2004; Brisibe*et al*., 2011; Bernardes *et al*., 2015; Alanazi, 2018; Dubale *et al*., 2023). Additionally, plant-based products are starting to show promise as a substitute for artificial pesticides. According to Senthil-Nathan (2020), phytochemicals from several chemical classes, including as steroids, alkaloids, terpenes, and phenolic components, have been investigated earlier for their potential as pest control agents and have proven to be effective.

The protective effect against insects is typically attributed to secondary metabolites found in plants, such as terpenoids, alkaloids, non-protein amino acids, steroids, phenols, flavonoids, glycosids, glucosinolates, quinones, and tannins (Carson, 1962; Bernardes *et al*., 2015; Alanazi, 2018; Balasubramani *et al*., 2018; Dubale *et al*., 2023). Additionally, there is less environmental damage because botanical pesticides are naturally occurring, work swiftly, and decompose soon. Selectivity, low toxicity to animals and natural enemies, and, with a few notable exceptions, low toxicity to mammals are characteristics of botanical insecticides (Cycon *et al*., 2017; Campos *et al*., 2019). Since local farmers grow plants for local use rather than using synthetic ones, botanical insecticides especially those plant-derived extracts from them are more affordable to prepare and easier to apply (Cycon*et al*., 2017; Campos *et al*., 2019).

In many countries, including China, the USA, Hungary, Bulgaria, Romania, Turkey, Argentina, Italy, France, Spain, and India, the medicinal herbal plant *Artemisia* spp. (Asterales: Asteraceae) grows naturally between 1000 and 1500 m above sea level. It is typically a weedy herb with a single stem that can reach a height of 2 to 3 m (Mittal and Subbarao, 2003; Brisibe *et al*., 2011; Alanazi, 2018). This herbaceous plant has long been utilized for a variety of reasons, such as medicinal and pest management. While the same plant materials were employed to eradicate mosquitoes in China without having any harmful impacts on the environment, they were also used to cure the major flea plague in the United Kingdom in the 18th century (Mittal and Subbarao, 2003).

The essential oil extracts of *Artemisia vulgaris* L. leaves and flowers have a larvicidal impact on *Aedes aegypti* larvae (Mya *et al*., 2016; Balasubramani *et al*., 2018). Numerous empirical research have demonstrated how different *Artemisia* spp. may control different species of mosquitoes. For example, hexane, methanol, and other extracts of *Artemisia annua* and *Artemisia absinthium* were discovered to have a greater toxicity against *Aedes aegypti* and *Anopheles sinensis* by Shri Ram (2011) and Sofi *et al*. (2022).

Our study's objective was to assess the methanolic, acetone, and benzene extracts from *Artemisia vulgaris* L. whole plant body for larvicidal and brine shrimp toxicity tests. Meyer *et al*. (1982) employed the brine shrimp lethality test (BST) to forecast the existence of bioactive chemicals in the extract. The larvae of *Anopheles* sp., *Culex* sp., and *Chironomus* sp. were used to test the larvicidal properties. In contrast, Chironomus is a harmless dipteran fly whose larval stages can be found in almost any aquatic or semiaquatic habitat, including tree holes, bromeliads, rotting vegetation, soil, sewage, and artificial containers. *Anopheles* and *Culex* mosquitoes are well known vectors of numerous diseases, including Malaria, Filariasis, Japanese Encephalitis, etc.

They make up a sizable portion of the macro zoo-benthos in the majority of freshwater environments. Because certain species have evolved to live in nearly anoxic environments and are prominent in contaminated waters, they are frequently linked to degraded or low-biodiversity ecosystems.

MATERIALS AND METHODS

Gathering of vegetation

In the Department of Botany at Jhargram Raj College, Jhargram, the entire *Artemisia vulgaris* L. plant was identified after it was taken in March 2024 from ShelpuKhasmahal, Kurseong, Darjeeling District, West Bengal, India.

Extract and sample solution preparations

The entire plant body of *Artemisia vulgaris* L.

weighing 5 kg and shade dried was ground into a fine powder and extracted three times in three litres of 90% aqueous methanol, chloroform, and benzene, each at room temperature and for seven days in the dark (Ghosh, 2018; Hazra and Ghosh, 2024; Ghosh and Hazra, 2024). Dark brownish residue was obtained by filtering and concentrating each of the several extracts under reduced pressure, up to a volume of 100 ml. Diverse bioactive evaluation techniques were then used on the diverse residual masses that were obtained, after which they underwent additional phytochemical screening.

The dark brownish residue was dissolved in a few drops of propylene glycol to create the test solution, which was then diluted with sterile water at a concentration of 125 mg ml^{-1} to 1 mg ml^{-1} (Ghosh *et al*., 2013). As a control, a small amount of sterile water was added to a drop of propylene glycol. A membrane filter with a pore size of 0.02 µm was used to filter all of the dilutions in order to sterilise them. The extract was also further screened using different biochemical experiments to examine the presence of various phyto-constituents. The following phytochemical screens were conducted independently using extracts of ethanol, acetone, and benzene.

Investigation for the presence of different Phytochemicals

Ketone and aldehyde screening

To one millilitre of the material, a saturated solution of 2, 7-diamino fluorene was added. To make the solution acidic by capillary, was added with two to three drops of glacial acetic acid (Donno *et al*., 2015). If there is an aldehyde or ketone present, a yellow to crimson tint develops.

Testing for Alkaloids

For a few minutes, 2.6 g of bismuth carbonate and 7 g of sodium iodide were heated in 25 ml of glacial acetic acid. The precipitated sodium acetate was filtered out after 12 hours. Then it was added with 0.5 ml of water after combining 20 ml of the red-brown filtrate with 80 ml of ethyl acetate. The stock solution was kept in a dark bottle for storage.

100 ml of glacial acetic acid, 2.4 ml of ethyl acetate, and 1 ml of the sample were combined with 1 ml of the stock solution (Matijevic, 1969; Auwal *et al*., 2014; Abodunrin *et al*., 2015).

Checking for Amines and Alkaloids

Ten millilitres of ethanol and five drops of 65% nitric acid were added to one millilitre of the sample. The combination was examined under a UV light source following an extended heating period of 120°C (Matijevic, 1969; Auwal *et al*., 2014; Abodunrin *et al*., 2015).

Assessing for Corticosteroids

Before usage, 2 millilitres of 50% sulfuric acid and 2 millilitres of a 2% methanolic solution of 4-hydroxybenzaldehyde were newly combined. The material was then put in 1 ml and heated to 105°C for 3 to 4 minutes (Matijevic, 1969; Shaikh and Patil, 2020).

Verification of Ergot alkaloids

After dissolving 0.125 g of 4-dimethyl amino benzaldehyde in 65 ml of 97% sulfuric acid and 5 ml of water, 0.05 ml of 5% aqueous iron (III) chloride solution was added. The mixture was allowed to cool (Abodunrin *et al*., 2015; Shaikh and Patil, 2020; Dubale*et al*., 2023).Then, 1 ml of the sample solution was added to this combination.

Looking for Essential oils

Diphenyl picryl hydrazyl (0.06 g) was dissolved in 10 millilitres of chloroform. One millilitre of the sample was added to this mixture. For five to ten minutes, the experimental set was heated to 110°C (Matijevic, 1969; Butnariu and Ioan, 2018).

Searching for Flavonoids

One millilitre of the material was combined with a 25% aqueous solution of basic lead acetate. It is examined under a long wavelength UV light after being heated for two minutes at 65 degrees Celsius (Matijevic, 1969; Shaikh and Patil, 2020; Dubale *et al*., 2023).

Examining Glycolipids

Two millilitres of 10% ethanolic diphenylamine solution, ten millilitres of 37% hydrochloric acid, and eight millilitres of glacial acetic acid were combined. One millilitre of the sample was added to that mixture. It was then heated to 100°C for five to ten minutes (Matijevic, 1969; Shaikh and Patil, 2020).

Glycoside screening

Five millilitres of 0.5% methanolic magnesium acetate solution were added to one millilitre of the sample. According to Matijevic (1969), Auwal *et al*. (2014), and Dubale *et al*. (2023), the mixture was then heated to 90°C for five minutes.

Reviewing for Ketoses

5 ml of acetic acid, 3 ml of 85% phosphoric acid, 1 ml of water, and 0.3 g of anthrone were added to the mixture. One millilitre of the sample was added to that reaction mixture, and it was heated to 110°C for five to six minutes (Matijevic, 1969; Auwal *et al*., 2014).

Pointing for Lipids

A 0.01% ethanolic fluorescein solution was combined with 1 millilitre of the sample solution. The mixture was then allowed to sit for ten minutes. Warm air was used to dry the mixture following incubation (Matijevic, 1969; Butnariu and Ioan, 2018).

Confirming for Organic phosphate esters

Acetone was mixed with 1% anhydrous cobalt (II) chloride solution. One millilitre of the sample solution was then added to the mixture. For five minutes, the reaction mixture was incubated at 40 to 50°C (Matijevic, 1969).

Screening for Peroxides

The mixture of 128 ml methanol, 25 ml water, and 1 ml glacial acetic acid was used to dissolve 1.5 g of N, N-dimethyl-1.4-diphenylene di ammonium dichloride. One millilitre of the sample solution was added to that mixture (Matijevic, 1969).

Looking for Persulfates

A solution of 0.05 g benzidine was prepared in 100 ml of 1 N acetic acid. One millilitre of the sample solution was added to that mixture (Matijevic, 1969).

Confirming for Phenols

10 cc of 97% sulfuric acid were used to dissolve 0.1 g of vanillin. One millilitre of the sample was added to the reaction mixture, and it was heated to 120°C until the colour's intensity changed (Matijevic, 1969; Shaikh and Patil, 2020; Dubale *et al*., 2023).

Examining for Phosphoric acid for keto sugars

5 ml of ethanol were used to dissolve 0.3 g of 5, 5-dimethyl cyclohexane-1,3-dione (dimedone), and 2 ml of 85% ortho phosphoric acid were added. 1 millilitre of the sample was added to this solution, and it was then incubated at 110°C for 15 to 20 minutes (Matijevic, 1969; Shaikh and Patil, 2020).

Evaluating for Polyalcohol

Mixture II: Add 50 ml water, 20 ml acetone, and 10 ml 0.2 N hydrochloric acid to a solution of 1.8 g of benzidine in 50 ml ethanol. Mixture I: 0.5% aqueous sodium meta-periodatesolution. Now, 1 ml of Mixture I was added to 1 ml of the sample. After that, it was incubated at ambient temperature for five minutes. Addition of Mixture II (1 ml) was then made (Matijevic, 1969).

Analysing for Polyphenyls

100 cc of 65% nitric acid was used to dissolve 0.3 g of cerium (IV) sulfate. A millilitre of the sample was added to that reaction mixture, and it was heated to 120°C for 15 to 20 minutes (Matijevic, 1969).

Studying for Polysaccharides

5 ml of acetic acid, 3 ml of 85% phosphoric acid, 1 ml of water, and 0.3 g of anthrone were added to the mixture. One millilitre of the sample was added to that reaction mixture, and it was heated to 110°C for five to six minutes (Matijevic, 1969; Auwal *et al*., 2014).

Accessing for Sugars

After dissolving 0.5 g of carbazole in 9.5 ml of ethanol, 0.5 ml of 97% sulfuric acid was added. One millilitre of the sample was added to the reaction mixture, and it was heated to 120°C for ten minutes (Matijevic, 1969; Shaikh and Patil, 2020).

Going over for Sugar alcohols

After adding 1 ml of saturated aqueous silver nitrate solution and swirling it into 2 ml of acetone, water was added drop by drop until the silver nitrate completely dissolved. One millilitre of the sample was added to the reaction mixture, and it was heated for one hour at 80°C in a lightprotected chamber filled with ammonia vapours until the sample turned light brown. The surplus silver nitrate was then extracted using a solution of 10% sodium thiosulfate (Matijevic, 1969).

Understanding for Sugar phosphates

After dissolving 0.5 g of ammonium molybdate in 2 ml of water, 0.5 ml of 25% hydrochloric acid and 0.5 ml of 70% perchloric acid were added. It was filled with five millilitres of acetone once it had cooled to room temperature. Before used, the solution was left to stand for at least a day. One millilitre of the sample solution was added to that combination. Following sample addition, the set was heated to 110°C for 5 to 10 minutes and exposed to an infrared lamp for 2 minutes at a distance of 30 cm (Matijevic, 1969).

Auditing for Reducing sugars

10 ml of acetone was used to dissolve 4 g of diphenylamine, 4 ml of aniline, and 5 ml of 85% phosphoric acid. After adding one millilitre of the sample solution, the temperature was raised to 85°C for ten minutes (Matijevic, 1969; Auwal *et al*., 2014; Shaikh and Patil, 2020).

Addressing for Steroid sapogenins

After dissolving 30 g of zinc chloride in 100 ml of methanol, the insoluble material was filtered off. Currently, 1 ml of the sample was added to 1 ml of the reaction mixture mentioned above. After heating the entire set to 105°C for an hour, the layer was promptly covered with a glass plate to prevent moisture impact (Matijevic, 1969; Shaikh and Patil, 2020).

The study of Steroids and triterpene glycoside

Before using, thoroughly mix and allow to cool completely 1 millilitre each of acetic anhydride, 97% sulfuric acid, and the sample solution. Two millilitres of ethanol were added to the mixture and chilled. A 10-minute incubation period at 110°C was used for the entire set (Matijevic, 1969; Shaikh and Patil, 2020; Dubale *et al*., 2023).

Look at for Sterols

One millilitre of acetic anhydride, one millilitre of 97% sulfuric acid, one millilitre of the sample solution, and the mixture were thoroughly mixed and freshly cooled before being added to three millilitres of ethanol. Following treatment, all of the pieces were incubated at 110°C for 10 minutes (Matijevic, 1969; Shaikh and Patil, 2020).

Inspectionof Steroids

One millilitre of a 20% ethanolic tungsto-phosphoric acid (T.P.A.) solution was added to one millilitre of the sample solution. A temperature of 120°C was applied to the combination (Matijevic, 1969; Shaikh and Patil, 2020).

Investigation of Straight-chain lipids

Two milliliters of α-cyclodextrin 30% ethanolic solution were mixed with one milliliter of the sample solution. The entire set was placed in an iodine vapour-filled chamber and incubated for ten minutes at room temperature (Matijevic, 1969).

Analysis for brine shrimp toxicity and larvicidal activity

The Brine Shrimp Cytotoxicity Assay employed condensed preparations of the entire plant body of *Artemisia vulgaris* L., namely separate extracts of benzene, methanol, and acetone (Mkenda *et al*., 2015; Meyer *et al*., 1982; Ghosh, 2018; Hazra and Ghosh,

2024; Ghosh and Hazra, 2024). Brine shrimp eggs were incubated in a shallow 22 by 32 by 12 centimetres rectangular container, with one-third filled with saline water. An aluminium divider with several 2 mm holes was fastened into the dish to produce uneven divisions. The 50 mg of eggs were sprinkled into the larger, darker compartment while the smaller section remained lit. The phototropic nauplii were collected by pipette from the lighter side of the set, which was maintained at a temperature of between 30ºC and 32°C for 48 hours, after being separated from their shells by the divider.

Live brine shrimp show rapid cell division like tumor cells, as telomerase remains active there. After 24 hours of hatching, control over cell division occurs (Meyer *et al*., 1982). So, we have taken a 24-hour treatment period against our sample to study the cytotoxic activity. As we know that each cytotoxic biological sample has some potent side effects on the cell (Chudzik *et al*., 2015; Matsuo *et al*., 2022), we made a serial dilution of the sample to test the minimum concentration of cytotoxic activity against brine shrimp and screed at 3 hours of intervals.

Twenty brine shrimps were added to each sample(s) vial using a 23 cm disposable pipette, and the volume was then reduced to 10 ml by adding saline water. The quantity of nauplii in the pipette stem may be counted against a light background. A drop of dry yeast suspension (3 mg in 5 ml of saline water) was added to each vial. Twenty prawns and salt water were the only ingredients in the negative control. The vials were stored under light and at room temperature. To determine the percentage of death for each dose and control, the remaining prawns were counted every three hours for a maximum of twenty-four hours.

The larvae in their second instar were sourced from the natural habitats and breeding grounds of *Chironomus* sp., a small shallow ditch. The eggs of *Anopheles* and *Culex* mosquitoes were taken from outdoor concrete tanks and placed in five-litre metal containers for incubation. They were allowed to grow to the second instar stage before the bioassay test. Twenty larvae of each species were individually added to the vial containing the sample(s) using a 23 cm disposable pipette. After that, water from their breeding grounds was added to get the level down to 10 ml. The larvae in the pipette stem may be counted against a light background. Twenty larvae of each species, independently collected, were contained in each vial. The only material used in the negative control that was extracted from their breeding was water.

Death (%) = Test Control × 100 / Control

Three copies of each dose level were created, and during a 24-hour observation period, the LC_{50} values were determined.

Analytical statistics

The data was analysed using Paleontological Statistics, Version 4.3 (PAST). The observed data were expressed using the mean ± standard error. To statistically evaluate all of the experiment values, multiple sample repeated measurements using ANOVA were employed.

RESULTS

Phytochemical screening

In the case of the methanolic extract, the MIC (Minimum Inhibitory Concentration) starts at 100 µg/ml of the sample concentration, whereas in the case of the Acetone and Benzene Extracts, the MIC starts at 25 µg/ml of the sample concentration, showing higher efficacy than the methanolic extract. Some larvae require a 3-hour incubation period, while others require up to 24 hours to reach the MIC number (the number at which half of the initially taken larvae die), also called $Lc_{50.}$

Methanolic Extract

The methanolic extract contained dissolved peroxides, flavonoids, aldehydes, and ketones (Table 1).

Acetone Extract

Glycolipids and ketones were positive (Table 1).

Table 1. Table of Phytochemical Screening of *Artemisia vulgaris* L., whole plant extracts in different solvents.

Sl. No.	Extraction Solvent Used	Nature of Extracted Phytochemical	Present/Absent (Denoted as +/- respectively)	Polarity
1	Methanol	Aldehyde and ketone	+	Polar
2	Methanol	Flavonoids	+	Polar
3	Methanol	Dissolved Peroxide	+	Polar
4	Methanol	Persulfates	-	Polar
5	Methanol	Sugar Alcohols	-	Polar
6	Acetone	Alkaloids	-	Polar / Non-Polar
7	Acetone	Amines and Alkaloids	-	Polar
8	Acetone	Ergot Alkaloids	-	Polar
9	Acetone	Glycolipids	-	Polar
10	Acetone	Glycosides	+	Polar
11	Acetone	Ketones	+	Polar
12	Acetone	Polyalcohols	-	Polar
13	Acetone	Dissolved Polysaccharides	-	Non-Polar but Water
14	Acetone	Sugars	-	Soluble due to ‘H’-bonding
15	Acetone	Phosphate in Sugars	-	Non-Polar but Water
16	Benzene	Steroids	+	Soluble due to ‘H’-bonding
17	Benzene	Essential Oils	+	Polar
18	Benzene	Lipids	-	Non-Polar
19	Benzene	Organic Phosphate Esters	+	Non-Polar
20	Benzene	Dissolved Phenols	+	Non-Polar
21	Methanol	Phosphoric Acid for Keto Sugars	-	Polar
22	Benzene	Polyphenols	+	Non-Polar
23	Benzene	Reduced Sugar Levels	-	Non-Polar
24	Benzene	Steroid Sapogenins	-	Non-Polar
25	Benzene	Triterpene Glycosides and Steroids	+	Non-Polar
26	Benzene	Sterols	+	Non-Polar
27	Benzene	Steroids	+	Non-Polar
28	Benzene	Lipids with a Straight Chain	-	Non-Polar

Benzene Extract

Steroids, essential oils, polyphenols, dissolved phenols, organic phosphate esters, triterpene glycosides, and phenols were all present in positive amounts in the extract (Table 1).

Effect of Methanolic Extract

Brine Shrimps: This sample's LC_{50} value at 24 hours was 125 µg/ml (Table 2, Figs. 1 and 2). The concentration of crude extract increases with the death rate of shrimp.

Anopheles larvae: According to Table 2, Figs. 1 and 2, the sample's LC_{50} value at 21 hours was 100 µg/ml. As crude extract concentration rises, the rate at which larvae die increases.

Culex larvae: According to Table 2, Figs. 1 and 2, the sample's LC_{50} value at 24 hours was 125 µg/ml. As crude extract concentration rises, the rate at which larvae die increases.

Chironomus larvae: Table 2, Figs. 1 and 2, show that the sample's LC_{50} value at 21 hours was 125 µg/ml. As crude extract concentration rises, the rate at which larvae die increases.

Effect of Acetone Extract

Brine Shrimps: At 24 hours, the sample's LC_{50} value was 100 µg/ml (Table 2, Figs. 1 and 2). Shrimp death rates rise as crude extract concentration rises.

Anopheles larvae: Table 2, Figs. 1 and 2, show that the sample's LC_{50} value was 25 µg/ml after 12 hours. The rate at which larvae die rises as crude extract concentration rises.

Culex larvae: According to Table 2, Figs.1 and 2, this sample's LC_{50} value was 50 µg/ml at 15 hours. The rate at which larvae die rises as crude extract concentration rises.

Chironomus larvae: Table 2, Figs. 1 and 2, shows that the sample's LC_{50} value was 50 µg/ml at 15 hours. The

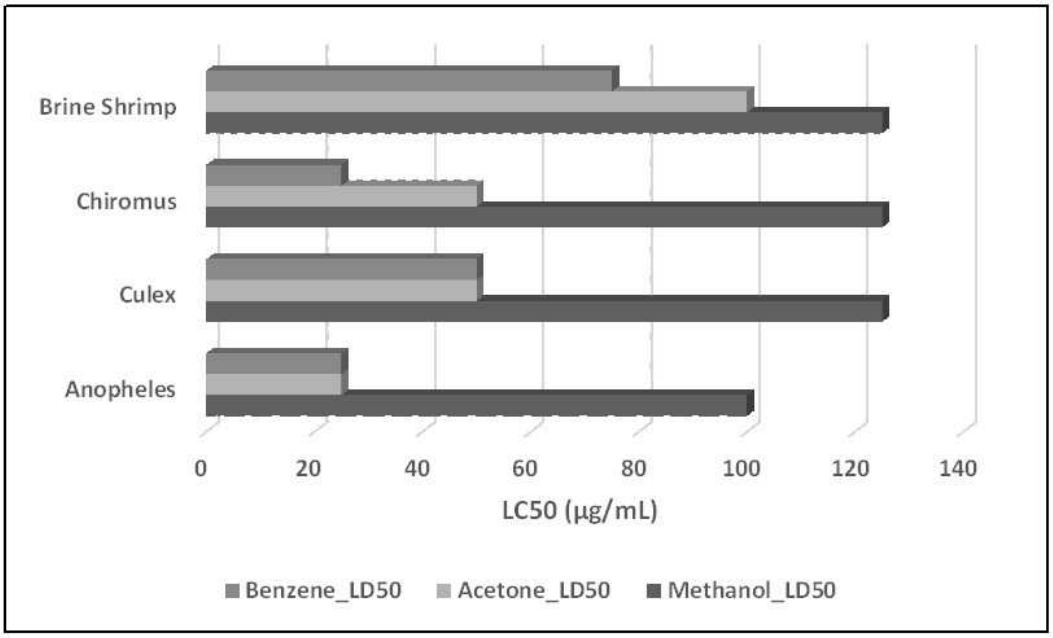


Fig.1. Variations of LC 50 values of the whole plant extract of *Artemisia vulgaris* L., against different test organisms at various concentrations (µg/mL).

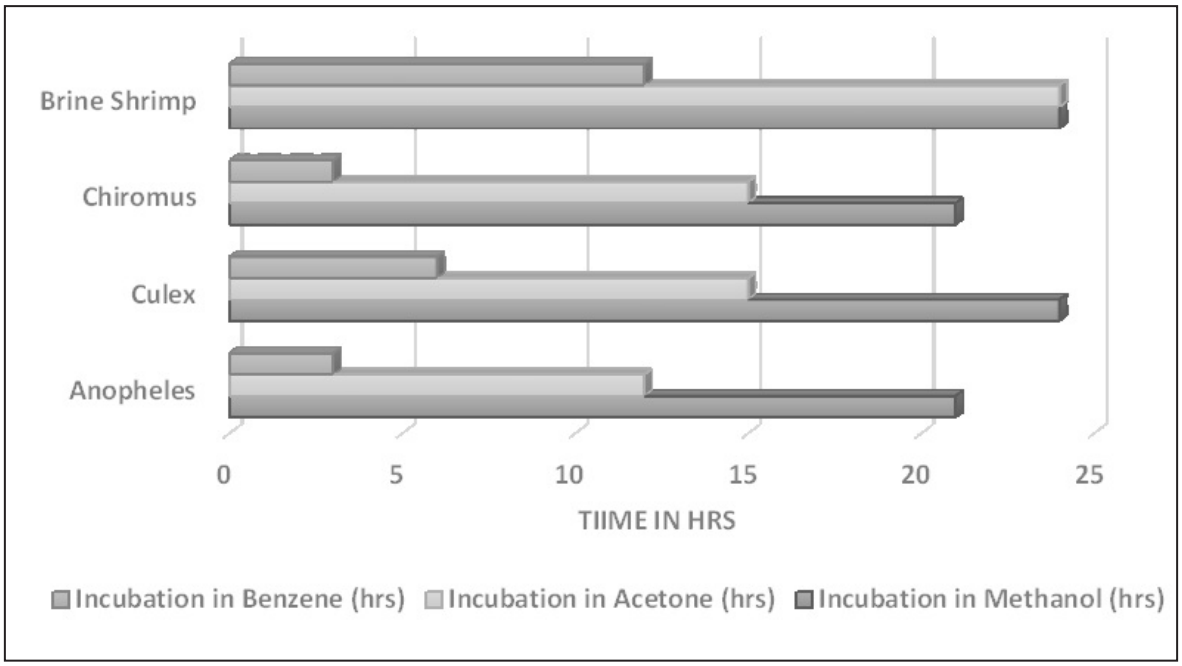


Fig. 2. Variations of LC_{50} values of the whole plant extract of *Artemisia vulgaris* L., against different test organisms at various incubation in periods (hours).

Table 2. LC_{50} values at different concentrations (µg/ml) of *Artemisia vulgaris* L., whole plant extracts in various solvents, were given below:

	Methanol Extract		**Acetone Extract**		**Benzene Extract**	
	Conc.	Hrs.	Conc.	Hrs.	Conc.	Hrs.
Anopheles	100	21	25	12	25	3
Culex	125	24	50	15	50	6
Chironomus	125	21	50	15	25	3
Brine Shrimp	125	24	100	24	75	12

rate at which larvae die rises as crude extract concentration rises.

Effect of Benzene Extract

Brine Shrimps: At 12 hours, the sample's LC_{50} value was 75 µg/ml (Table 2, Figs. 1 and 2). Shrimp death rates rise as crude extract concentration rises.

Anopheles larvae: Table 2, Figs. 1 and 2, show that the sample's LC_{50} value at 3 hours was 25 µg/ml. The rate at which larvae die rises as crude extract concentration rises.

Culex larvae: According to Table 2, Figs. 1 and 2, this sample's LC_{50} value at 6 hours was 50 µg/ml. The rate at which larvae die rises as crude extract concentration rises.

Chironomus larvae: Table 2, Figs. 1 and 2, shows that the sample's LC_{50} value at 3 hours was 25 µg/ml. The rate at which larvae die rises as crude extract concentration rises.

The efficacy of the three distinct solvent extracts on the shrimp and insect larvae varies significantly (F= 31, df=2, P< 0.001) (Table 3). There is also a substantial variation in the three extracts' duration of action (hours) (F= 55.8, df=2 P< 0.001). Shrimp and insect larvae responses exhibit notable differences in the three extracts' concentrations (F= 6.25, df=3 P= 0.028) and action time (F= 7.8, df=3 P= 0.018) (Table 3).

DISCUSSION

Many transmissible illnesses, including Filariasis, Dengue, Malaria, and Chikungunya, which are endemic in Asia and Africa and have a serious negative impact on human health, are mostly spread by mosquitoes (Syed *et al*., 2013). Moreover, organic products made from plants are preferable for controlling insect vectors of human diseases because chemical pesticides have detrimental effects on both the environment and human health. According to Chore *et al*. (2014), Jawale (2014), Joseph *et al*. (2004), Azmathullah *et al*. (2011), Hazra and Ghosh 2024, Ghosh and Hazra 2024, the effectiveness of secondary metabolites such as alkaloids, isoflavonoids, flavonoids, saponine, and steroids have the ability to cause mosquito larvicides. In this study the presence of flavonoids, dissolved peroxides, glycolipids, ketones, aldehydes, polyphenols etc. in whole plant extracts of *Artemisia vulgaris* L. also supports those previous findings.

Among the different phytochemicals present in the extracts (Table 1), Phenols, Triterpene Glycosides, and Sterols have potent cytotoxic activities (Chudzik *et al*., 2015; Cunha *et al*., 2020; Matsuo *et al*., 2022).

Table 3. Mean, Standard Errors and Several samples repeated measures ANOVA of LC_{50} values of *Artemisia vulgaris* L., whole plant extracts in three different solvents on brine shrimps and insect larvae are summarised below.

	Variance in concentrations			Variance in Hrs of Treatment		
Variables	**Mean ± SE**	**F***	**P**	**Mean ± SE**	**F***	**P**
Methanol	118.75 ± 6.25			22.5 ± 0.86		
Acetone	56.25 ± 15.73	31^{2}	< 0.001	16.5 ± 2.59	55.8^{2}	< 0.001
Benzene	43.75 ± 11.97			06 ± 2.12		
	Variance in concentrations			Variance in Hrs of Treatment		
Variables	**Mean ± SE**	**F***	**P**	**Mean ± SE**	**F***	**P**
Anopheles	50 ± 25.00			12 ± 5.20		
Culex	75 ± 25.00	6.25^{3}	0.028	15 ± 5.19	7.6^{3}	0.018
Chironomus	66.67 ± 30.05			13 ± 5.30		
Brine Shrimp	100 ± 14.43			20 ± 4.0		

F* : 2 = d.f. 2; 3 = d.f. 3.

There are several reports (e.g. Azmathullah *et al*., 2011; Brisibe *et al*., 2011; Chore *et al*., 2014; Alanazi, 2018; Campos *et al*., 2019) of the efficiency of these metabolites against brine shrimps as well as insect larvae. Especially triterpene and sesquiterpene, both produced from isoprenoids, have cytotoxic effects (Tabopda *et al*. 2007) and potential brine shrimp (Ngassapa *et al*. 2022) and insect larvicidal activity (Kabir *et al*. 2013).

The LC_{50} values for benzene, acetone, and methanol extracts for *Chironomus* larvae are 25 µg/ml, 50 µg/ml, and 125 µg/ml, respectively. Accordingly, the extracts of acetone and benzene had the greatest harmful effects in this instance. Previous research on toxicity has also indicated that, despite being sensitive to crude oil (David *et al*., 1989), *Chironomids* are comparatively resistant of toxicants (Nondo *et al*., 2011).

The methanolic extracts of *Artemisia vulgaris* L. demonstrated nontoxic brine shrimp toxicity with an LC_{50} value of 125 µg/ml, and effect at that level of concentration can be considered as nontoxic (Monson, 1939). Conversely, extracts of the entire plant body containing acetone and benzene had somewhat higher harmful effects (LC_{50} values of 50 µg/ml and 75 µg/ml, respectively) (Table 2). This may be explained by the fact that the acetone and benzene extracts contain greater concentrations of the hazardous bioactive chemicals. The notion is further supported by the significant differences in treatment hours and the concentrations of three distinct extracts (Table 3).

The results of this study, which used mosquito, *Chironomus*, and brine shrimp larvae as test subjects, show that the entire plant extract of Artemisia vulgaris has an acutely toxic effect on freshwater macroinvertebrates. These kinds of research are unquestionably crucial for creating vector control and environmental risk reduction techniques.

CONCLUSION

Many secondary metabolite components, found in *Artemisia vulgaris* L., whole plant extracts have larvicidal activities. That findings also corroborate with different works by other authors. Therefore, it has high potentiality to use as ecofriendly pest or vector control management in future and thereby reducing harmful chemical pesticide use. So, further research is required to evaluate larvicidal and other bio-toxic activities of *Artemisia vulgaris* L. to create a future path of sustainable use.

ACKNOWLEDGEMENT

We sincerely thank the West Bengal Government's Department of Higher Education.

DISCLAIMER

The authors declared that there has been no conflict of interest while performing the work and preparation of the manuscript.

REFERENCE

Abodunrin, T., Uhuegbu, C. and Olugbuyiro, J. 2015. Phytochemical Analysis of Leaf-Extracts from Eight Tropical Trees: Prospects for Environmentally-Friendly Dye Compounds for Smart Windows. *International Journal of Scientific & Engineering Research,* **6**(3): 682-698.

Alanazi, N.A. 2018. Larvicidal effect of *Artemisia annua* (Asterales: asteraceae) against the dengue fever mosquito vector *Aedes aegypti* (Diptera: Culicidae). *International Journal of Mosquito Research,* **5**(2): 35-38.

Anand, U. and Rai, N.R. 2024. Pesticide Reduction through Organic Farming for Promoting Public Health Management and Food Security. *South Eastern European Journal of Public Health*, Posted: 14-06-2024. p. 246-250. DOI:10.70135/seejph.vi.783

Auwal, M.S., Saka, S., Mairiga, I.A., Sanda, K.A., Shuaibu, A. *et al*. 2014. Preliminary phytochemical and elemental analysis of aqueous and fractionated pod extracts of *Acacia nilotica* (Thorn mimosa). *Veterinary Research Forum*, **5**(2): 95-100.

Azmathullah, N.M., Asrar, S.M. and Sultan, M.A.K. 2011. Phytochemical Screening of Calotropis procera Flower Extracts and Their Bio-Control Potential on *Culex* sp. Mosquito Larvae and Pupae. *Int. J. Pharma. Bio. Arch.*, **6**: 718-21.

Balasubramani, S., Sabapathi, G., Moola, A.K., Solomon, R.V., Venuvanalingam, P. and Diana, R.K.B. 2018. Evaluation of the Leaf Essential Oil from *Artemisia*

vulgaris and Its Larvicidal and Repellent Activity against Dengue Fever Vector *Aedes aegypti* - An Experimental and Molecular Docking Investigation. *ACS omega,* **3**(11): 15657-15665.

Bernardes, M.F.F., Pazin, M., Pereira, L.C. and Dorta, D.J. 2015. Impact of pesticides on environmental and human health. In Toxicology Studies: Cells, Drugs and Environment; Andreazza, A.C., Scola, G., Eds.; In Tech: Rijeka, Croatia. pp. 195-233.

Brain, K.R. 1979. Accumulation of L-Dopa in cultures from Mucuna pruriens. *Plant Sci. Lett.*, **7**: 157-161.

Brisibe, E.A., Adugbo, S.E., Ekanem, U., Brisibe. F. and Figureueira, G.M. 2011. Controlling bruchid pests of stored cowpea seeds with dried leaves of *Artemisia annua* and two other common botanicals. *Afri. J Biotechnol*., **10**(47): 9586-9592.

Butnariu, M. and Ioan, S. 2018. Essential Oils from Plants. *Journal of Biotechnology and Biomedical Science,* **1**(4): 35-43. DOI:10.14302/issn.2576-6694.jbbs-18-2489.

Campos, E.V.R., Proença, P.L.F., Oliveira, J.L., Bakshi, M., Abhilash, P. and Fraceto, L.F. 2019.Use of botanical insecticides for sustainable agriculture: Future perspectives. *Ecol. Indic.*, **105**: 483-495.

Carson, R. 1962. Silent Spring. Anniversary Edition; Houghton Miffin Co.: New York, NY, USA.

Chore, J.K., Obonyo, M., Wachira, F.N. and Mireji, P.O. 2014. Larvicidal Activity of Selected Aloe Species Against Aedes aegypti (Diptera: Culiciade). *J. Insect Sci.,* **14**(202):1-3. doi: 10.1093/jisesa/ieu064.

Chudzik, M., Korzonek-Szlacheta, I., Król, W. 2015. Triterpenes as Potentially Cytotoxic Compounds. *Molecules,* **20**(1):1610-1625.

Cunha, G.O.S., da Silva, D.M., dos Santos, M.L., de Moraes Filho, M.O., Pessoa, C.D., Guimaraes, C. de J., Silva, M.F.S. and Menezes, A.C.S. 2020. Chemical constituents and cytotoxic activity of *Miconia burchellii* Triana (*Melastomataceae*) leaves. *South African Journal of Botany,* **137**: 345-350. https://doi.org/10.1016/j.sajb.2020.11.008.

Cycon, Mrozik, A. and Piotrowska-Seget, Z. 2017. Bioaugmentation as a strategy for the remediation of pesticide-polluted soil: A review. *Chemosphere*, **172**: 52-71.

David, P., Kendall, A.W. and David, W.J.D. 1989. Chronic toxicity of cadmium to Chrironmousripariusmeigen-effects upon larval development and adult emergence. *Hydrobiologia,* **175**:109-115.

Donno, D., Beccaro, G.L., Cerutti, A.K., Mellano, M.G. and Bounous, G. 2015. Bud extracts as new phytochemical source for herbal preparations: Quality control and standardization by analytical fingerprint. In Phytochemicals-Isolation, Characterisation and Role in Human Health; Rao, V., Rao, L., Eds.; Tech: Rijeka, Croazia, pp. 187-218.

Dougan, D., Wade, D. and Mearric, K.P. 1975. Effects of L-DOPA metabolites at a dopamine receptor suggest a basis for 'on-off' in Parkinson's disease. *Nature*, **254**: 71-72.

Dubale, S., Kebebe, D., Zeynudin, A., Abdissa, N. and Suleman, S. 2023. Phytochemical Screening and Antimicrobial Activity Evaluation of Selected Medicinal Plants in Ethiopia. *Journal of Experimental Pharmacology*, **15**: 51-62. doi: 10.2147/JEP.S379805.

Ghosh, A., Ghosh, P.D. and Chatterjee, P. 2013. Isolation, purification and characterisation of 2, 7, (14), 10 bisabolatriene-1,9,12 triol, a bisabolene type sesquiterpene isolated from *Curcuma longa* L. *Natural Products: An Indian Journal,* **9**(3): 117-122.

Ghosh, A. 2018. Bioformulation of antifungal herbal extract from *Curcuma caesia* Roxb. and *Ixora coccinea* L. against Botrytis cinerea Pers. *The Journal of Phytopharmacology,* **7**(1): 56-59.

Ghosh, A. and Hazra, P. 2024. Assessing the brine shrimp toxicity and larvicidal potentiality of *Tephrosia purpurea* L. *J. Botan. Soc. Bengal,* **78**(1): 71-82.

Guedes, R.N.C., Smagghe, G., Stark, J.D. and Desneux, N. 2016. Pesticide-induced stress in arthropod pests for optimized integrated pest management programs. *Annu. Rev. Entomol*, **61**: 43-62.

Hazra, P. and Ghosh, A. 2024. Evaluation of Cytotoxic Activities of Methanolic Extracts of *Calotropis procera* (Aiton) W.T. Aiton. *Jour. Pl. Sci. Res.*, **40**(1): 190-196.

Huizing, H.J., Wijnsma, R., Batterman, S., Malingre, T.H. M. and Wichers, H.J. 1985. Production of L-DOPA by cell suspension cultures of Mucuna pruriens. *Plant Cell Tiss. Org. Cult.,* **4**: 61-73.

Janardhanan, K. and Lakshmanan, K.K. 1985. Studies on the pulse, Mucuna utilis. Chemical composition & anti-nutritional factors. *J. Food Sci. Tech.,* **22**: 369-37.

Jawale, C.S. 2014. Larvicidal activity of some saponin containing plants against the dengue vector Aedes aegypti. *Trends in Biotechnology Research*, **3**:1-11.

Joseph, C.C., Ndoile, M.M., Malima, R.C. and Nkunya, M.H. 2004. Larvicidal and mosquitocidal extracts, a coumarin, isoflavonoids and pterocarpans from Neorautanenia mitis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **9**(8):451-5. doi: 10.1016/j.trstmh.2003.10.008.

Kabir, K.E., Choudhary, M.I., Ahmed, S. and Tariq, R.M. 2013. Growth-disrupting, larvicidal and neurobehavioral toxicity effects of seed extract of *Seselidiffusum* against *Aedes aegypti* (L.) (Diptera: Culicidae). *Ecotoxicology and Environmental Safety,* **90**: 52-60.

Matsuo, Y., Tsuchihashi, H., Takatori, K. and Fukaya, H. 2022. Cytotoxic triterpene and steroidal glycosides from the seeds of Digitalis purpurea and the synergistic cytotoxicity of steroidal glycosides and etoposide in SBC-3 cells. *Bioorganic Chemistry*, **122**: 105697. https://doi.org/10.1016/j.bioorg.2022.105697.

Meyer, B.N., Ferrign, R.N., Putnam, J.E., Jacobson, L.B., Nicholas, D.E. and McLaughlin, J.L. 1982. Brine Shrimp:A Convenient General Bioassay for Active Plant Constituents. *Planta Medica*, **45**: 31-34.

Mittal, P.K. and Subbarao, S.K. 2003. Prospect of using herbal product in the control of mosquito vectors. *ICMR Bulletin*, **33**(1): 0377-4910.

Mkenda, P., Mwanauta, R., Stevenson, P.C., Ndakidemi, P., Mtei, K. and Belmain, S.R. 2015. Extracts from field margin weeds provide economically viable and environmentally benign pest control compared to synthetic pesticides. *PLoS ONE*, **10**: e0143530.

Monson, D. 1939. The Action of Certain Assamese Plants as Larvicides. *J. Malaria Inst. India*, **2**: 35.

Mya, M.M., Oo, N.N., Ha, T., Oo, A.W., Htay, T.M., New, C.T., Thaung, S. and Maung, Y.N. 2016. Larvicidal effect of *Artemisia vulgaris* leaves, flower and leaves essential oil extracts against *Aedes aegypti* larvae. *Journal of Biological Engineering Research and Review*, **3**(2): 25-34.

Ngassapa, O.D., Runyoro, Deborah K.B.S., Maregesi, M., Sangeda, R.Z., Msemo, S., Mwakalukwa, R., Kidukuli, A.W. and Marandu, E. 2022. Antimicrobial activity and brine shrimp toxicity of propolis collected from various regions of Tanzania. *The Journal of Phytopharmacology,* **11**(4): 275-280.

Nondo, R.S.O., Mbwambo, Z.H., Kidukuli, A.W., Innocent, E.M., Mihale, M.J., *et al.* 2011. Larvicidal, antimicrobial and brine shrimp activities of extracts from *Cissampelos mucronate* and *Tephrosia villosa* from coast region, Tanzania. *Complementary and Alternative Medicine*, **11**: 33. doi: 10.1186/1472-6882-11-33.

Oerke, E.C. 2006. Crop losses to pests. *J. Agric. Sci.*, **144**: 31-43.

Shaikh, J.R. and Patil, M.K. 2020. Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies*, **8**(2): 603-608. DOI:10.22271/chemi.2020.v8.i2i.8834.

Senthil-Nathan, S. 2020. A review of resistance mechanisms of synthetic insecticides and botanicals, phytochemicals, and essential oils as alternative larvicidal agents against mosquitoes. *Front. Physiol.,* **10**: 1591.

Shri Ram. 2011. Research output on Artemisia (*Artemisia annua*): a bibliometric study. *Ann. Libr. Inf. Stud.*, **58**: 237-248.

Sofi, M.A., Nanda, A., Sofi, M.A., Maduraiveeran, R., Nazir, S., Siddiqui, N., Nadeem, A., Shah, Z.A., Rehman, M.U. 2022. Larvicidal activity of *Artemisia absinthium* extracts with special reference to inhibition of detoxifying enzymes in larvae of *Aedes aegypti* L. *Journal of King Saud University – Science*, **34**. https://doi.org/10.1016/j.jksus.2022.102248.

Syed, A.M.Y., Ravikumar, S. and Beula, J.M. 2013. Mosquito larvicidal activity of seaweeds extracts against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. *Asian Pac. J. Trop. Dis.,* **3**(3): 196-201. DOI:10.1016/S2222-1808(13)60040-7.

Tabopda, T.K., Liu, J., Ngadjui, B.T., Luu, B. 2007. Cytotoxic triterpene and sesquiterpene lactones from Elephantopusmollis and induction of apoptosis in neuroblastoma cells. *Planta Med.,* **73**(4): 376-80. doi: 10.1055/s-2007-967132. Epub 2007 Mar 15.

Zhanga, Y.W., McCarl, B.A., Cao, Z., Li, Q., Yanga. S. and Liu, H. 2022. Operating pesticide use reduction within the boundary of food security in peri-urban settings. *Fundamental Research,* **2**(4): 635-647.

J. Botan. Soc. Bengal 79(1) : 58-70 (2025), ISSN 0971-2976

FULL LENGTH ARTICLE

Microbiological evaluation of probiotics from milk and products thereof

Snehali Kundu, Sushovan Bera, Ujjayinee Ray* and Subhasree Bhattacharjee*
Post Graduate Department of Botany, Jogamaya Devi College, University of Calcutta

Received : 30.01.2025 Accepted : 31.05.2025 Published : 30.06.2025

Probiotics are live microorganisms which confer health benefits after consumption by the host. Probiotic microbes should be safe for food and clinical use, can colonize human intestine, antagonize pathogenic bacteria, possess validated health effects, and stable during processing and storage. The aim of this study is to analyze different probiotics from raw milk and milk products (curd, paneer, Yakult). Strains grown on de Man Rogosa Sharpe agar (MRSA)under microaerophilic conditions showed either milky white or creamy white, mucoid, small or medium-sized colonies. All were gram-positive, Methyl Red-Voges-Proskauer (MR-VP) negative, and the majority showed dextrose and lactose fermentation. Both catalase-positive and negative strains were obtained. Carbohydrate fermentation with various sugars revealed mixed results among the strains. Interestingly, all the strains were able to grow at optimal pH (pH 5.5) and NaCl concentration (2.5%). The majority showed tolerance towards 2.5-8.5% NaCl and pH 2-6. Results suggested the presence of *Saccharomyces sp., Lactobacillus sp*., and *Enterococcus sp*. in the samples. Bile tolerance, antagonism against pathogenic bacteria, phylogeny, etc. remain to be evaluated. Analysing these strains and identifying the potential probiotic strains will affirm their use in the development of functional foods for the health benefits.

Key words: Probiotics, dairy products, MRS agar, lactic acid bacteria, carbohydrate fermentation.

INTRODUCTION

Probiotics are the live microorganisms that provide benefits to human health when they are included in the diet in an adequate amount. Probiotics are often termed as "good bacteria" and are also found in the human gut. There are various dietary sources for these probiotics, such as beverages, ice cream, yogurt, bread, and many others by the food industry, and they also show anti-inflammatory, anti-cancerous, immunity-enhancing properties. Fermented foods are examined, and it shows that they contain some active microbes that have similar genetic properties as are found in probiotic strains that are used. Fermented food transforms substrates and produces bioactive and bioavailable end-products and thereby improving the functional and nutritional aspects (Marco *et al*., 2017). It has been revealed in a study that an approximate consumption of 10^9 colony-forming units (CFU)/day acts as an effective dose(Hill *et al*., 2014).

Milk and milk products have long been valued for their nutritional content, offering essential vitamins, minerals, and proteins crucial for human health. Curd is known to be one of the richest sources of probiotics. Curd is a natural food with healing properties because

**Corresponding author :* ujjayineeray3@gmail.com
subhasreebhattacharjee70@gmail.com

of the probiotics that are present in it, which prevent numerous enteric diseases. It contains lactic acid bacteria and boosts the immunity of the host (Das *et al.*, 2019). Presently, dairy companies are active producers of the probiotic-rich yogurts, buttermilks, tofu, and probiotic drinks like Yakult. These products are artificially supplemented with probiotics and are easily available options for consumers.

In recent years, there has been growing interest in probiotics in dairy products, studied due to the increasing awareness of the gut microbiota's role in maintaining overall health and well-being. Studies have demonstrated the potential of probiotics to modulate the gut microbiota composition, enhance digestive health, boost immune function, and even exert systemic effects beyond the gastrointestinal tract (Latif *et al*., 2023).Probiotic microorganisms are thought to have a wide range of health benefits and exert antimicrobial activity against enteric pathogenic bacteria; stimulate mucosal and systemic immune responses of the host; provide a remedy for colon cancer, with anti-cholesterol and anti-inflammatory effects; and improve nutritional status. These also reduce symptoms of diarrhoea, lactose intolerance, and allergic reactions (Taye *et al*., 2021).

It has been observed that the main mechanisms that probiotics mainly use to exhibit their beneficial properties on host are (1) competitive exclusion of pathogens, (2) improvement in intestinal barrier functions, (3) immunomodulation in the host's body, (4) and production of neurotransmitters. Probiotics compete with pathogens for nutrients and receptor-binding sites, making the survival of the pathogen difficult in the gut (Plaza-Diaz *et al*., 2019). Probiotics produce substances such as short chain fatty acids (SCFA), organic acids, hydrogen peroxide which act as antimicrobial agent (Ahire *et al*., 2021). Probiotics also stimulate the mucin production in the host body and thereby improve the intestinal barrier function (Chang *et al*., 2021). They also regulate the expression of tight junction proteins, including occludin and claudin 1which in turn regulates the immune response in the gut (Bu *et al*., 2022).

A study was conducted to determine the presence of antimicrobial activities among the probiotics incorporated into these different food products against common microbial pathogens (Chuayana *et al*., 2003). Another study aimed to characterize the probiotic properties of lactic acid bacteria (LAB) strains isolated from cheddar cheese, yoghurt, and cow milk (Vanniyasingam *et al*., 2019). Furthermore, understanding the impact of probiotics on milk and milk products holds significant implications for the dairy industry, offering opportunities to develop innovative functional foods with enhanced nutritional value and health benefits.

Therefore, an attempt has been made to study the probiotics present in milk and milk products collected from different places in Kolkata (Sahanagar and Parnasree). The bacterial colonies were isolated from the samples using specific culture techniques and characterized biochemically. Through rigorous analysis and experimentation, this research endeavors to elucidate the different strains of probiotics found in milk and milk products, paving the way for the development of novel and impactful dairy products with tangible health benefits.

MATERIALS AND METHODS

Sample Collection

Four different samples were collected from different places. listed in Table 1.

Table 1. Samples and collection sites

Sample	Place	Latitude/Longitude/ coordinates
Milk	Sahanagar, Kol 26	22.5145416/ 88.3421391
Curd	Parnasree Flying Club, Kol 60	22.57687/ 88.35047
Paneer	Parnasree Flying Club, Kol 60	22.57687/ 88.35047
Yakult	Parnasree, 60Kol	22.57687/ 88.35047

Raw milk was collected from a local cow shelter in Sahanagar, Kol 25 and the curd, paneer and Yakult were collected from local shops of Parnasree Flying Club locality.

Serial dilution and culturing of strains

The four samples (Milk, Paneer, Curd, Yakult) were first subjected to serial dilution done from 10^{-1} to 10^{-3} using 0.9% saline water. MRSA media (agar 15g/l) (Table 2) was used for plating. Spread plating was done from 10^{-1} and 10^{-3} solutions for each sample. Then plates were covered, labelled and kept for incubation at 37ºC for 24h in an inverted manner under microaerophilic conditions.

Media composition of de Man Rogosa Sharpe (MRS) media (pH 5.5) that has been used as the basal media for the growth of the bacterial colonies is noted in Table 2.

Determination of colony morphology

From the mother plates all the colonies were observed and their morphology was noted. After that 10 strains were selected from the colonies of the mother plate for further confirmatory tests based on their morphology (shape, size, texture, colour, margin) and were sub-cultured by quadrant-streaked method in a zig-zag manner. For sub-culturing, MRS Agar plates were used. The strains were named as SK 1, SK 2, SK 3, SK 4, SK 6, SK 9, SK 12, SK 14, SK 15, and SK 16.

Table 2. Media composition of de Man Rogosa Sharpe (MRS) media (pH 5.5).

Components	Gram/Litre
Peptone	10
Yeast Extract	5
Beef Extract	10
Glucose	20
Polysorbate 80	1ml/l
Sodium Acetate	5
Magnesium Sulphate	0.1
Manganese Sulphate	0.05
Disodium Phosphate	2

Gram Staining

Gram staining was performed for all the selected strains following the standard method (Mannan *et al*., 2017), and the result is noted.

Catalase Test

A single colony was added to one drop of sterile water on a sterilised slide and freshly prepared 15% H_2O_2 was added on the slide. Effervescence was observed and time of effervescence was also observed (Reiner, 2010). For each strain, the process was repeated on 3 different slides.

Kligler'sIron Test

Kligler iron agar medium (pH 7.4 ± 0.2) is used for this test (Table 3). Kligler Iron Agar is a differential medium that is used for the identification of enteric bacteria based on the fermentation of dextrose, lactose and H_2S production. Slants were prepared and were inoculated by streaking on the slant and stabbing deep into the centre of the solid culture into the tube within 3-5mm from the bottom. Triplicates were used for each strain. The tubes were then kept for incubation at 37ºC and the tubes were studied for acid production of the slant/ butt, gas, and hydrogen sulphide reaction after 24 and 48 h (Mannan *et al*., 2017).

MR-VP Test

For MR-VP test the MR-VP broth (pH 6.9 ± 0.2) was used (Table 4).

- **Methyl red solution**: 0.1 g of methyl red was completely dissolved in 300 ml of ethanol (95%). Add 200 ml of deionized water was added to it to make 500 ml of a 0.05% (wt/vol) solution in 60% (vol/vol) ethanol. The solution was freshly prepared.

- **Voges-Proskauer reagents Barritt's reagent A:** 5% (wt/vol) a-naphthol was dissolved in absolute ethanol.

- **Barritt's reagent B:** 40% (wt/vol) KOH was dissolved in deionized water (this might be replaced by a 40% (wt/vol) NaOH solution).

All the Reagents were freshly prepared. **Voges-Proskauer reagents Barritt's reagent A** and **Barritt's reagent B** are also referred to as VP-1 and VP-2 or VP-A and VP-B, respectively (McDevitt, 2009).

All the tubes were inoculated with the selected strains, except the blank, and were kept for 24 hrs of incubation at 37ºC. After the incubation the tubes for MR tests were taken and methyl red solution were added to it and colour change was observed if any. In case of VP test, at first the VP-A solution was added, and after that solution VP-B was added. Then colour change was observed, if any. For each strain, 'n' no. of tubes was used (n=3).

Table 3. Media composition for Kligler's Iron test (pH 7.4 ± 0.2).

Components	Gram/Litre
Peptone	15
Beef Extract	3
Yeast Extract	3
Proteose peptone/ Casein hydrolysate	5
Lactose	10
Dextrose	1
Ferrous Sulphate	0.2
Sodium Chloride	5

Table 4. Composition of the buffered peptone-glucose (MR-VP) broth.

Components	Gram/Litre
Buffered peptone	7
Dipotassium Phosphate	5
Dextrose	5

Casein Hydrolysis Test

For this test, Skimmed Milk Agar (pH 7) (Table 5) was used. The casein hydrolysis test is a biochemical test that is used to determine the ability of bacteria to synthesize the caseinase enzyme. Some bacteria produce caseinase, a proteolytic enzyme capable of hydrolysing casein into smaller amino acids and peptides. This proteolysis will result in the formation of a clear transparent zone in the agar plate where casein has been hydrolysed, indicating a positive result (Khushboo *et al*., 2023).

Plates were inoculated with the selected strains in a quadrant-streaking manner and were kept for incubation at 37ºC for 96 h in an inverted manner. Readings were noted after 24, 48, 72, and 96 h. Each

Table 5. Media composition of Skimmed Milk Agar.

Components	Gram/Litre
Skim Milk Powder	28
Yeast	2.5
Tryptone	5
Glucose	1
Agar	15

strain was streaked 3 times in a different quadrant.

Growth Curve at Optimal pH 5.5

MRS broth at pH 5.5 is made. Bacterial cultures were inoculated into MRS broth in test tubes and were kept for incubation at 37ºC. Each strain was inoculated 3 times in 3 different tubes. OD was noted at 600nm for each strain from 0 min to 50 h at a time interval of 2 h. In between the readings, the tubes were kept in an incubator under shaking conditions at 37ºC.

pH Tolerance at pH 2, 4, 6

MRS broth at pH 2, 4, and 6 is made. Bacterial cultures were inoculated into MRS broth in test tubes and were kept for incubation at 37ºC.OD was noted at 600nm for each strain from 20 h. till 50 h at a time interval of 2 h. In between the readings, the tubes were kept in an incubator under shaking conditions at 37ºC. 3 replicates were used for each strain.

Growth Curve at Optimal NaCl concentration (2.5%)

MRS broth with a 2.5% NaCl concentration is made. Bacterial cultures were inoculated into MRS broth in test tubes and were kept for incubation at 37ºC. Each strain was inoculated 3 times in 3 different tubes. OD was noted at 600nm for each strain from 18 to 50 h at a time interval of 2 h. In between the readings, the tubes were kept in an incubator under shaking conditions at 37ºC.

Salinity tolerance at different NaCl concentrations (4.5%, 6.5%, 8.5%)

For salinity tolerance, MRS media with different NaCl concentrations. (4.5%, 6.5%. 8.5%) was used. Bacterial cultures were inoculated into MRS broth in test tubes and were kept for incubation at 37ºC. Triplicates were used for each strain. OD was noted at 600nm for each strain from 18 h to 50 h at a time interval of 2 h. In between the readings, the tubes were kept in an incubator under shaking conditions at 37ºC.

Carbohydrate fermentation

For carbohydrate fermentation, MRS broth was used with different sugars at a concentration of 2%. Phenol red was also added to the media at a concentration of 0.05%. The sugars that were used for the tests were Dextrose, Fructose, Lactose, Arabinose, and Sucrose. 5 ml of media was poured into each tube for the 10 selected strains, and a Durham's tube was added to it after ensuring that no bubbles had entered the tube. Then the tubes were inoculated with the respective strains, except for the blank. Then the tubes were kept in an incubator at 37ºC for 48 h. Colour change and bubble formation were observed if any after 24 and 48 h. 3 replicas were used for each strain.

RESULTS

Four different samples of milk and milk products were collected from different places around Kolkata, as mentioned previously. The samples were serially diluted up to $10^{-3}$and a spread plate was made using MRS media with the 10^{-1} and 10^{-3}plates. After colony formation, colony morphology and CFU/ml of each sample were noted.

Most of the colonies were round-oval in shape, creamy white to milky white in colour, and mucoid. The size of the colonies varied from large to very small. The margin of the colonies also varied. Some had smooth margins, whereas the others had wavy margins. The colony from milk has the highest CFU/ml of 5.92×10^{6}. The colonies from Curd have the highest CFU/ml count of 1.92×10^{6}. One of the colonies isolated from Paneer were characterized by irregular shape, chalky texture, and creamy white in colour. These colonies were spread out in bunches. The colony isolated from Yakult, which has the highest CFU/ml, has a value of 0.076×10^{6}(Fig. 1A).

10 strains were selected from these plates based on their morphologies (Table 6).

Gram staining

The selected strains were then tested for Gram character (Fig. 1B). All of them tested positive. SK 2

Table 6. Morphology of selected strains.

Strain	Morphology	Sample	CFU/ml x 10^6
SK 1	Large; White; Elevated at Centre; Mucoid; Smooth Margin	CURD	0.08
SK 2	Oval; Large; Milky White; Elevated at Centre; Smooth Margin	CURD	0.028
SK 3	Medium; Milky White; Elevated at Centre; Mucoid; Smooth Margin	CURD	1.92
SK 4	Irregular Shape; White; Mucoid; Wavy Margin	YAKULT	0.004
SK 6	Round; Small; White; Mucoid; Smooth Margin	YAKULT	0.02
SK 9	Oval; Wavy Margin; Creamy White; Mucoid	PANEER	0.064
SK 12	Irregular; Creamy White; Chalky Texture	PANEER	--
SK 14	Round; Creamy White; Medium; Mucoid; Smooth Margin	MILK	0.116
SK 15	Oval; Creamy White; Medium; Mucoid; Wavy Margin	MILK	0.012
SK 16	Round; Creamy White; Very Small; Mucoid; Smooth Margin	MILK	5.92

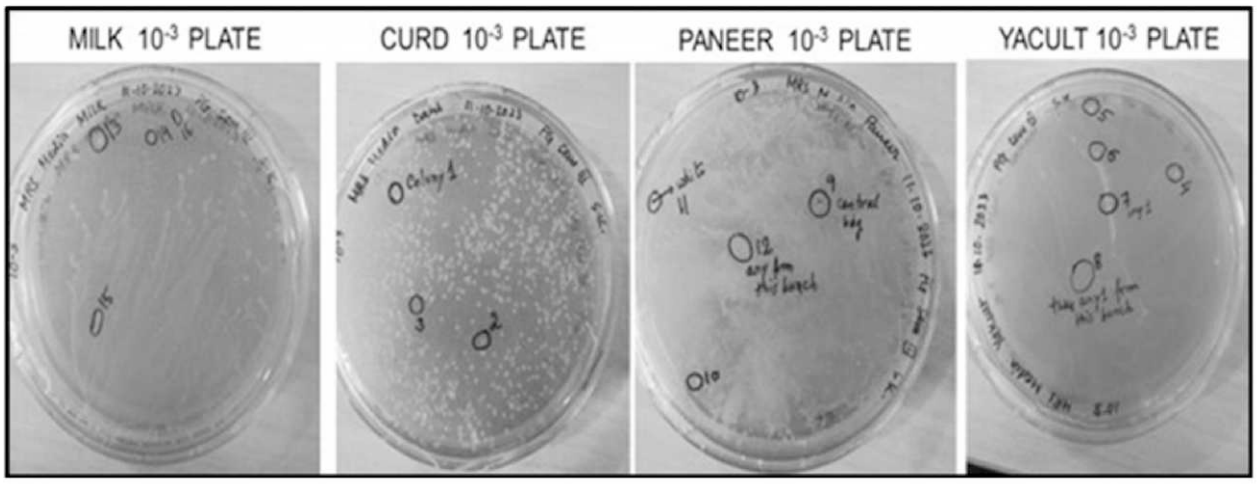


Fig. 1A. Colony morphologies on MRSA. Spread Plate of the samples (Milk, Curd, Paneer, Yakult) in MRS Media.

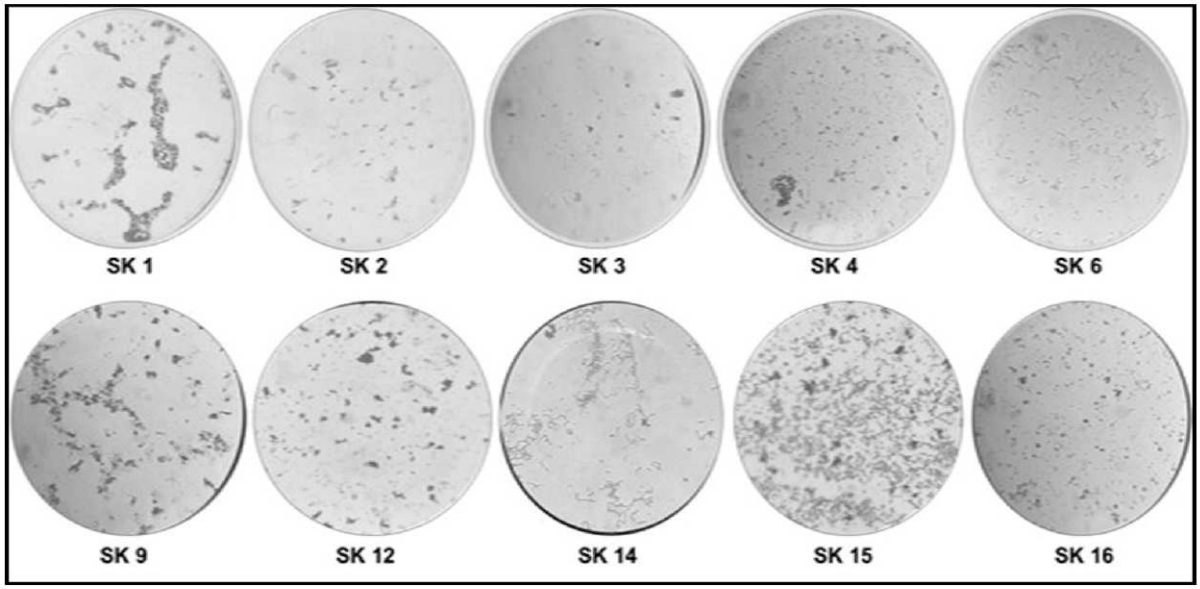


Fig. 1B: Gram staining. Gram staining of all the selected strains (SK 1-4, SK 6, SK 9, SK 12, SK 14-16).

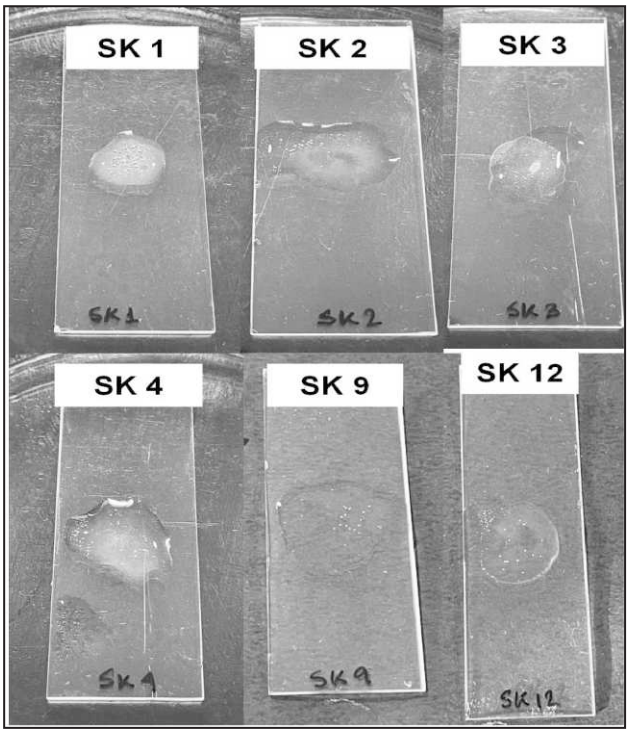


Fig. 2A. Result of Catalase test. SK 1-4, SK 9 & SK 12 showing Catalase positive result**.**

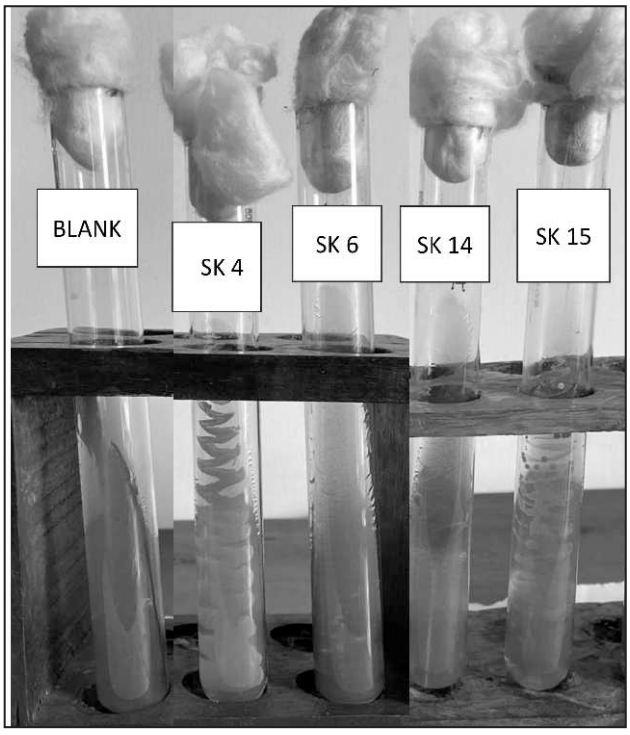


Fig. 2B: Result of Kligler's Iron test.SK 4, SK 6, SK 14, SK 15 exhibiting positive result of Kligler's Iron Test.

and SK 6 were rod-shaped. The rest were cocci in singlets or chains. SK 1 seemed like yeast with some budding.

Catalase Test

The strains were tested for catalase activity. Strains SK 1-4, SK 9, and SK 12 tested positive with fast effervescence (Fig. 2A). The Rest gave a negative result.

Kligler's Iron Test

After 48 h of observation, SK 1-3, SK 9, and SK 12 gave both dextrose -ve and lactose -ve. SK 4, SK 6, SK 14, SK 15 gave both dextrose +ve and lactose +ve (Fig. 2B). SK 16 remained the same as blank.

MR-VP Test

All the strains showed a negative result. The MR test is to detect mixed acid fermentation. Methyl red is a pH indicator that will detect mixed acid production by changing the medium to a red colour. The VR test is to detect the production of 2,3-butanediol as a byproduct of fermentation. The negative result in both cases indicates that the strains (SK 1-4, SK 6, SK 9, SK 12 neither ferment glucose through mixed acid fermentation procedure nor produce 2,3-butanediol.

Casein Hydrolysis Test

All the strains gave a negative result after 96 h of observation. The negative result thus indicates that the selected strains do not produce the caseinase enzyme and thereby do not show any hydrolysis activity.

Growth Curve at Different pH Levels

Growth curve at optimal pH 5.5

pH level plays an important role in the activity of the probiotics. One of the major characteristics of the probiotics is the pH tolerance. All the strains showed optimal growth range at pH 5.5. Though compared to others, SK 15 and SK 16 showed less growth. The growth of SK 12 also falls after 48 h (Fig. 3A).

pH tolerance at pH 2, 4, 6

Except for strain SK 9, all the strains showed tolerance in the pH range of 2-6 and showed maximum growth at pH 6. In case of SK 9, the growth rate started to fall after 20 h in pH 6 (Fig. 3B).

Growth Curve at Optimal 2.5% NaCl Concentration

Probiotics are robust enough to survive in diverse situations and can remain viable under various environmental conditions. The salinity tolerance test serves as a preliminary test for the osmotic stress tolerance of the probiotics. This mimics one of the challenges they can face in the gastrointestinal tract, especially in areas like the small intestine, where osmotic pressure varies. Salinity tolerance testing also helps to determine whether the probiotic strains that are isolated are viable candidates for commercial use, and also ensures their survival in processing, storage, and consumption.

Based on previous studies, it was observed that the strains best grow at a NaCl concentration of 2-4%, and the isolates from dairy products can show tolerance to a range of saline concentrations(2%-8%) (Mannan *et al.*, 2017).

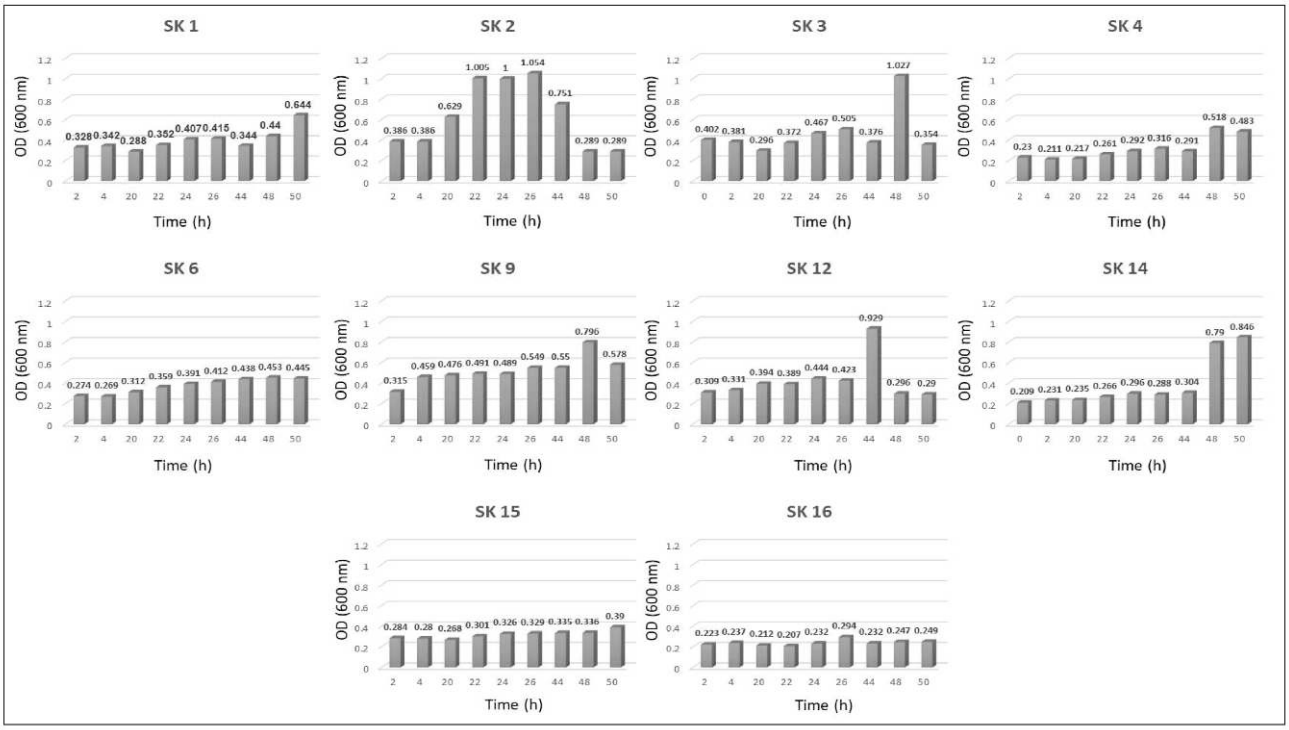
All the strains were able to grow optimally. SK 1, SK 6, SK 12 and SK 14 gradually increased with time, where SK 3, SK 4, SK 9, SK 15 and SK 16 maintained their growth rate throughout the time period (Fig. 4A).

Salinity Tolerance at Different NaCl Concentration (4.5%, 6.5%. 8.5%)

All the strain showed tolerance to salinity in the range of 4.5% NaCl concentration to 8.5% NaCl conc. Though the growth rate of SK 9 falls in the 4.5% NaCl conc. after 48 h (Fig. 4B).

Carbohydrate Fermentation Test

The carbohydrate fermentation test is used to determine whether or not a bacteria can utilize a certain carbohydrate. The basal medium used for this experiment was MRS broth. 5 different sugars at 2% conc. (dextrose, fructose, lactose, arabinose, and



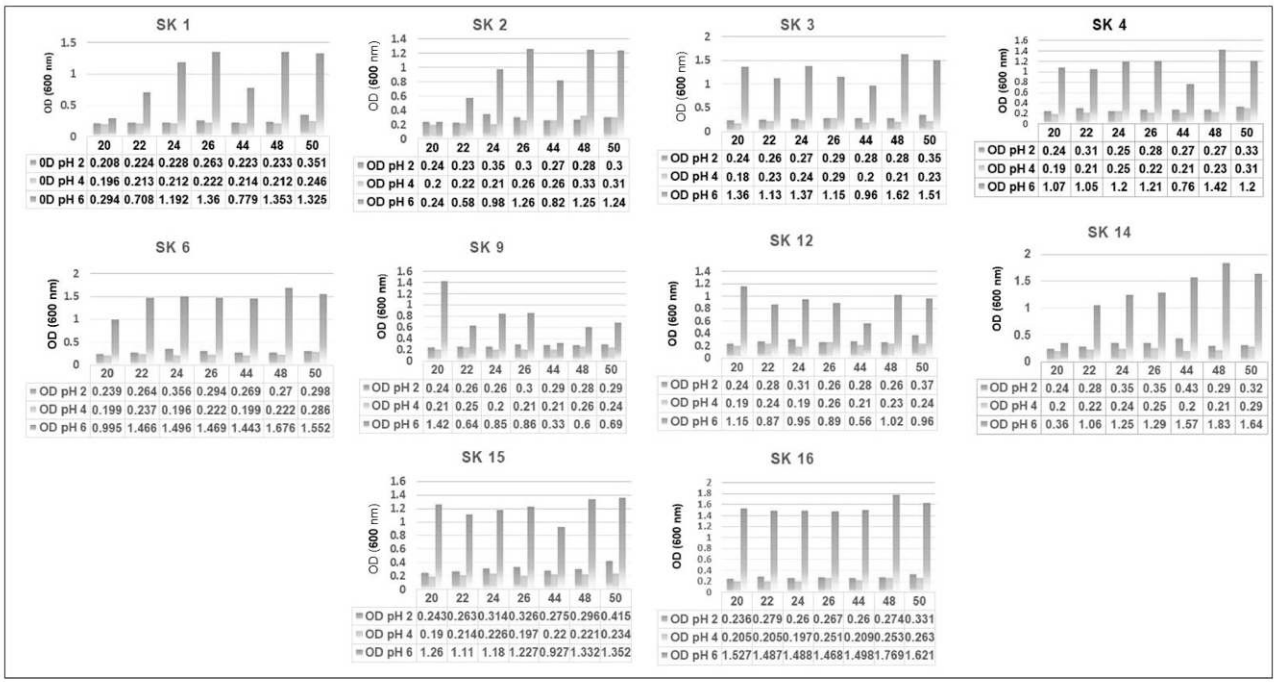
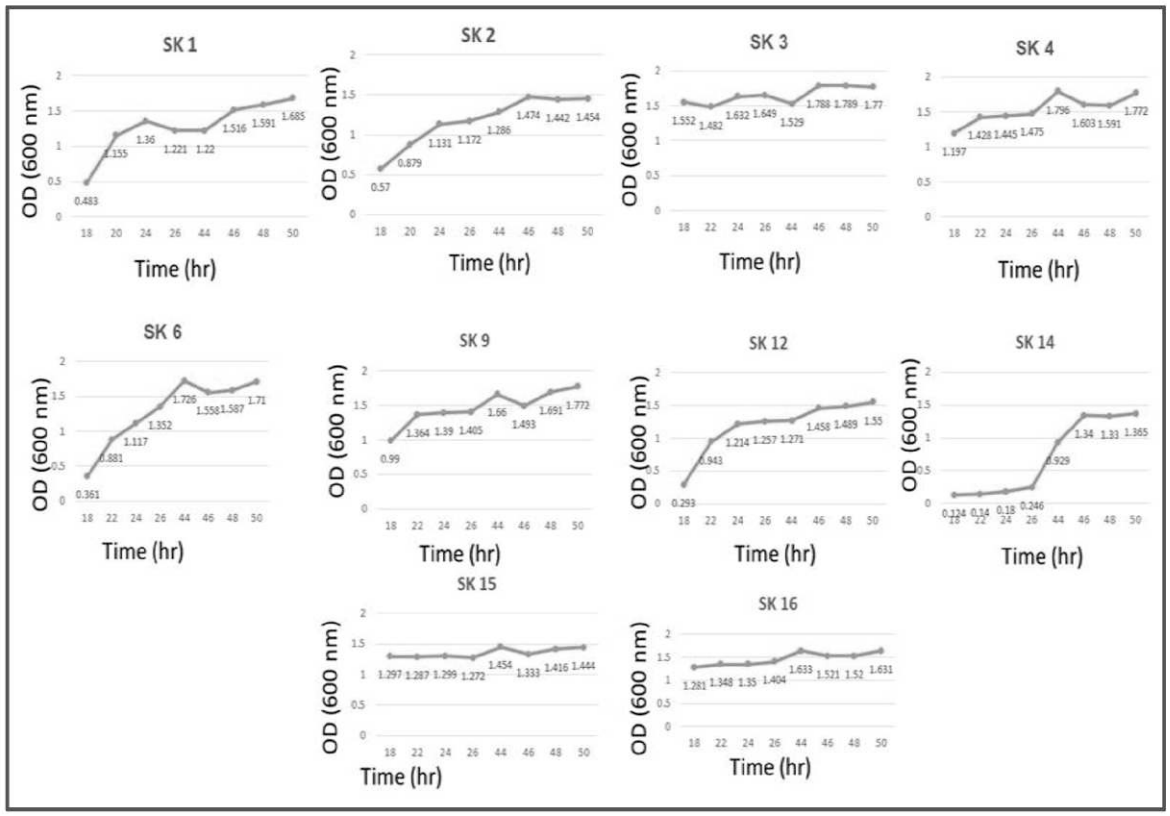


Fig. 3. Growth curve of all the strains at different pH levels. A. Growth at optimal pH 5.5 (upper panel). **B.** pH tolerance at pH 2,4,6 (lower panel)



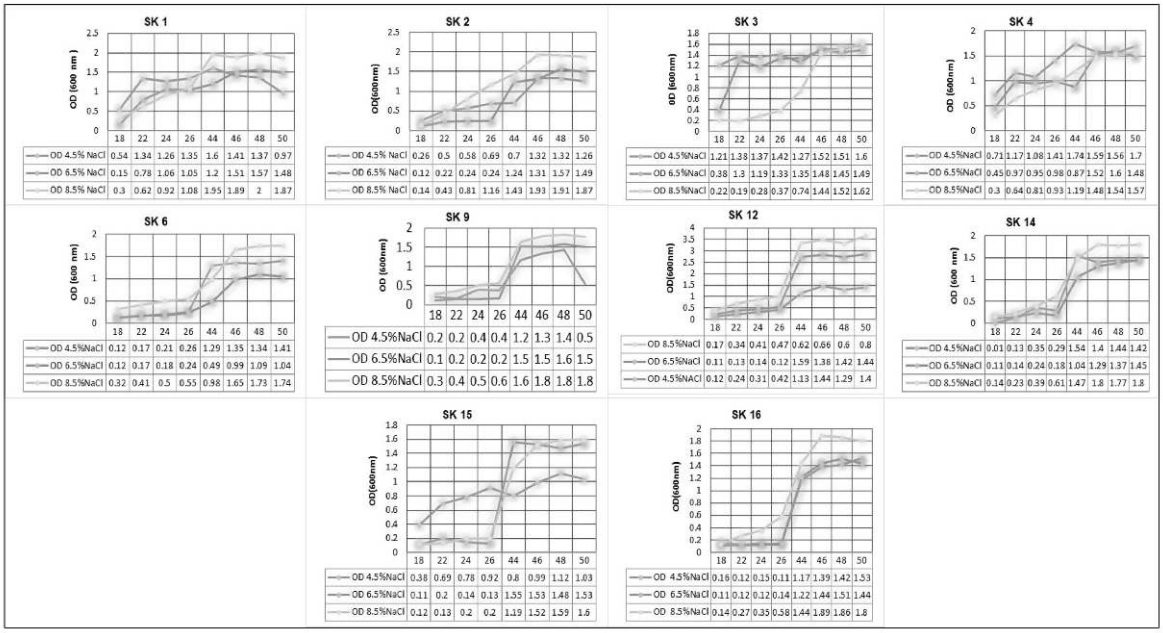


Fig. 4. Growth of all the strains at different NaCl concentration. A. growth curve at optimal 2.5% NaCl concentration (upper panel). **B.** salinity tolerance at different NaCl conc. (4.5%, 6.5%, 8.5%) (lower panel).

sucrose) were used and 0.05% phenol red was used as pH indicator (Table 7). All the strains gave mixed result (Fig. 5). Strains isolated from curd (SK 1, SK 2, SK 3) gave positive fermentation result for dextrose, fructose, lactose, arabinose and sucrose with positive gas production in case of dextrose (SK 2) and fructose (SK 1 & SK 2) and sucrose (SK 3). From Yakult SK 4 and SK 6 were isolated. It was observed that SK 4 has utilized dextrose, fructose, lactose, arabinose and sucrose and has given positive fermentation results with no gas production in case of any sugar. Whereas SK 6 has given positive results for dextrose, lactose, sucrose and showed gas production in case of dextrose. SK 9 and SK 12 were isolated from Paneer and both the strains gave positive result for all the sugars except for arabinose. SK 9 showed gas production in case of dextrose, fructose, lactose and sucrose whereas SK 12 showed gas production in case of dextrose, lactose and sucrose. From milk SK 14-16 has been isolated. SK 14 gave positive fermentation result in case of all the sugars except for fructose and showed gas production only in case of lactose. SK 15 on other hand showed positive fermentation results in case of all then sugars except for arabinose but did not exhibit gas production in any case. SK 16 fermented all the sugars except for arabinose and exhibit gas production in case of fructose, lactose and sucrose.

DISCUSSION

This study investigates the characteristics and viability of different probiotic strains from raw milk and milk products. For this, ten strains (SK 1-4, SK 6, SK 9, SK 12, SK 14-16) wereisolated from Milk, Curd, Paneer, and Yakult, selected on a preliminary basis based on the size, texture, colour, and morphology of the colonies. Then these strains were subjected to subculture multiple times, and a pure culture was established on MRSA for each strain.

All the strains were Gram-positive. SK 2 and SK 6 were rod-shaped, while the rest of the strains were

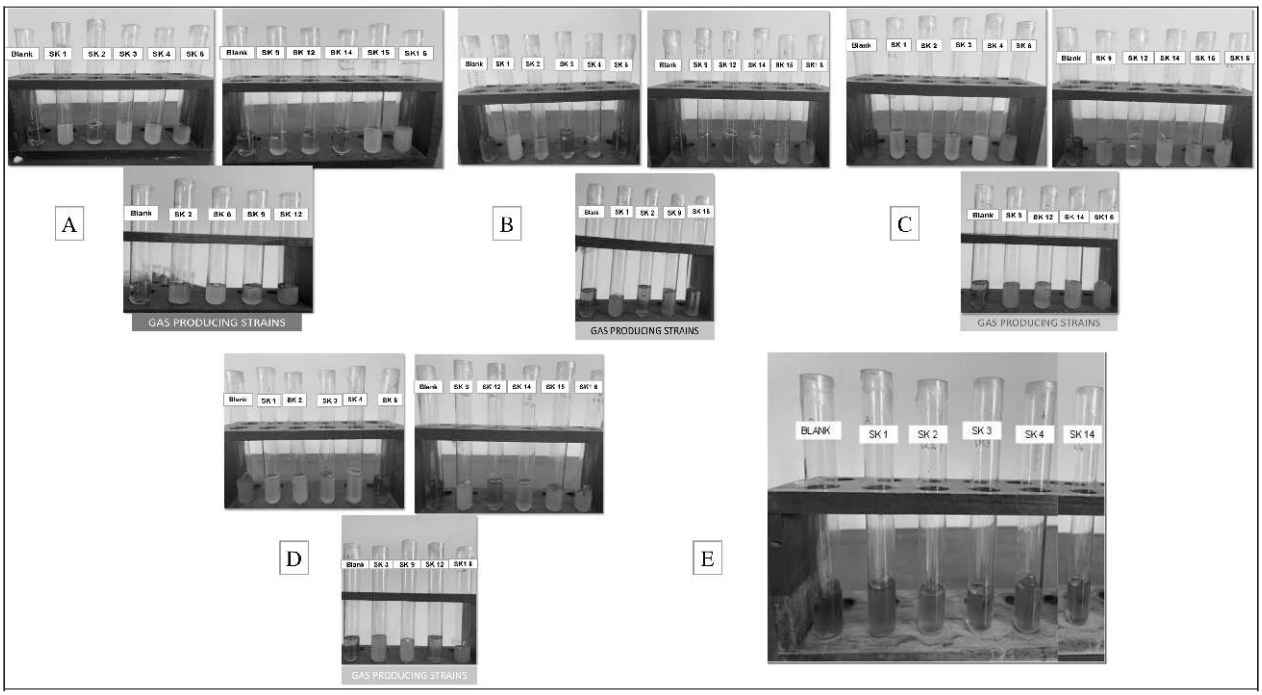


Fig. 5. Result of Carbohydrate Fermentation Test. A. All the strains showing fermentation result with dextrose. SK 2, SK 6, SK 9, SK 12 also produced gas. **B.** All the strains showing fermentation result with fructose. **C.** All the strains showing fermentation result with lactose. SK 9, SK 12, SK 14, SK 16 also produced gas**. D.** All the strains showing fermentation result with sucrose. SK 3, SK 9, SK 12, SK 16 also produced gas; **E.** SK 1-4, SK 14 only showed positive result for fermentation with arabinose but with no gas production.

Table 7. Result of Carbohydrate fermentation test

Strain No.	DEXTROSE	FRUCTOSE	LACTOSE	ARABINOSE	SUCROSE
SK 1	A^+G^-	A^+G^+	A^+G^-	A^+G^-	A^+G^-
SK 2	A^+G^+	A^+G^+	A^+G^-	A^+G^-	A^+G^-
SK 3	A^+G^-	A^+G^-	A^+G^-	A^+G^-	A^+G^+
SK 4	A^+G^-	A^+G^-	A^+G^-	A^+G^-	A^+G^-
SK 6	A^+G^+	A^-G^-	A^+G^-	A^-G^-	A^+G^-
SK 9	A^+G^+	A^+G^+	A^+G^+	A^-G^-	A^+G^+
SK 12	A^+G^+	A^+G^-	A^+G^+	A^-G^-	A^+G^+
SK 14	A^+G^-	A^-G^-	A^+G^+	A^+G^-	A^+G^-
SK 15	A^+G^-	A^+G^-	A^+G^-	A^-G^-	A^+G^-
SK 16	A^+G^-	A^+G^+	A^+G^+	A^-G^-	A^+G^+

coccus-shaped. In the catalase test, SK 1-4, SK 9, and SK 12 showed fast effervescence, giving positive results; the rest gave negative results. In the MR-VP test, all the strains exhibited a negative result. The Kligler's Iron test revealed both dextrose and lactose fermentation in the case of SK 4, SK 6, SK 14, and SK 15. All 10 strains gave optimum growth at pH 5.5. The pH tolerance test showed that all isolates except SK 9 were able to grow at pH levels 2, 4, and 6, with most growth observed at pH 6. This indicates a strong acid tolerance, which is a key trait for probiotic functionality, as the strains must survive the highly acidic environment of the stomach. The ability to grow at pH 2 suggests that the isolates have the potential to remain viable during gastric transit. The reduced growth at lower pH levels, while still significant, highlights that these conditions are suboptimal for proliferation, which is consistent with previous findings on lactic acid bacteria.The NaCl tolerance test revealed that all strains were capable of growing at 2.5%, 4.5%, 6.5%, and even 8.5% NaCl concentrations, with optimal growth at 2.5% NaCl concentration. This high level of salt tolerance indicates that the isolates are robust and can survive under osmotic stress, making them suitable as potential probiotic strains for future use. Similar type of result was found in studies by Roy *et al.*, 2019 and Vanniyasingam *et al*., 2019.

The carbohydrate fermentation test was performed with 5 different sugars: dextrose, fructose, lactose, arabinose, and sucrose. All the strains gave mixed results as described in Table 7. After analysing the results of these tests and comparing them with previously recorded data from other studies, it was found that out of ten, four species, namely SK 3 (Curd), SK 4 (Yakult), SK 9 (Paneer), and SK 12 (Paneer), were *Paediococcus sp*. SK 2 (isolated from Curd) and SK 6 (isolated from Yakult) were most likely to be *Lactobacillus sp*. SK 14, SK 15, and SK 16which were isolated from Milk, were identified as *Enterococcus sp*. SK 1 (isolated from Curd) was identified as *Saccharomyces sp*.

The preliminary characterization of probiotic isolates from milk and milk products is conducted in this study. However, the scope of the current analysis was limited to basic morphological and biochemical assessments. To build upon these findings, several areas warrant further investigation. In the future, for precise identification of the strains and analysis of probiotic potential, further tests need to be performed for bile tolerance; antagonism against pathogenic bacteria; whether it possesses clinically documented and

validated health effects; antibiotic resistance and stability during processing and storage. Also, the strains are to be identified on a molecular level using techniques such as 16S Sanger sequencing and (GTG) 5-PCR fingerprinting needs to be done. Analysing these strains and identifying the potential probiotic strains will affirm their use in the development of functional foods for the betterment of the health of the consuming public.

ACKNOWLEDGEMENT

We would like to thank The Principal, Jogamaya Devi College, University of Calcutta for providing the facilities and resources for carrying out the research work at the departmental level. Laboratory attendants, Kanai and Suchandra, are also acknowledged for their help.

DECLARATIONS

The authors declare there is no conflict of interest.

DISCLAIMER

The authors declared that there has been no conflict of interest while performing the work and preparation of the manuscript.

REFERENCE

Marco, M. L., Heeney, D., Binda, S. *et al*. 2017. Health benefits of fermented foods: Microbiota and beyond. *Current Opinion in Biotechnology,* **44**: 94-102.

Hill, C., Guarner, F., Reid, G. *et al*. 2014. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology & Hepatology,* **11**(8): 506-514.

Das, S. P., Patri, M. and Mohanty, P. K. 2019. Therapeutic uses of curd: A review. *IOSR Journal of Environmental Science,* **13**(1): 1-4.

Latif A, Shehzad A, Niazi S. *et al.* 2023. Probiotics: Mechanism of action, health benefits, and their application in food industries. *Front Microbiol*., **14**: 1216674.

Taye, Y., Degu, T., Fesseha, H. and Mathewos, M. 2021. Isolation and identification of lactic acid bacteria from cow milk and milk products. *The Scientific World Journal,* 2021, Article 4697445.

Plaza-Díaz, J., Ruiz-Ojeda, F.J., Gil-Campos, M. and Gil, A. 2019. Mechanisms of action of probiotics. *Advances in Nutrition,* **10**(Suppl. 1): S49-S66.

Ahire, J., Jakkamsetty, C., Kashikar, M.S., Lakshmi, S.G. and Madempudi, R.S. 2021. In vitro evaluation of probiotic properties of *Lactobacillus plantarum* UBLP40 isolated from traditional indigenous fermented food. *Probiotics and Antimicrobial Proteins,* **13**: 1413-1424.

Chang, Y., Jeong, C.H., Cheng, W.N. *et al.* 2021. Quality characteristics of yogurts fermented with short-chain fatty acid-producing probiotics and their effects on mucin production and probiotic adhesion onto human colon epithelial cells. *Journal of Dairy Science,* **104**(7): 7415-7425.

Bu, Y., Liu, Y., Liu, Y. *et al*. 2022. Screening and probiotic potential evaluation of bacteriocin-producing *Lactiplantibacillus plantarum* in vitro. *Foods,* **11**(11): 1575.

Chuayana, J.E., Ponce, C.V., Rivera, M.R.B. and Cabrera, E.C. 2003. Antimicrobial activity of probiotics from milk products. *Philippine Journal of Microbiology and Infectious Diseases,* **32**(2): 71-74.

Vanniyasingam, J., Kapilan, R. and Vasantharuba, S. 2019. Isolation and characterization of potential probiotic lactic acid bacteria isolated from cow milk and Milk Products. *AGRIEAST: Journal of Agricultural Sciences*, **13**(1), 32.

Mannan, S.J., Rezwan, R., Rahman, M.S. and Begum, K. 2017. Isolation and biochemical characterization of *Lactobacillus* species from yogurt and cheese samples in Dhaka metropolitan area. *Bangladesh Pharmaceutical Journal,* **20**(1): 27-33.

Reiner, K. 2010. Catalase test protocol. *American Society for Microbiology*, **1**(1): 1-9.

McDevitt, S. 2009. Methyl red and Voges-Proskauer test protocols. *American Society for Microbiology,* **8**: 1-7.

Khushboo, Karnwal, A. and Malik, T. 2023. Characterization and selection of probiotic lactic acid bacteria from different dietary sources for development of functional foods. *Frontiers in Microbiology,* 14: Article 1170725.

Roy, A., Panja, A. S., Maitra, M., & Bandopyadhyay, B. (2019). Isolation and characterizations of probiotics from bovine (cow) milk. *Department of Biotechnology, Oriental Institute of Science and Technology, Burdwan; Department of Microbiology, St. Xavier's College, Kolkata.*https://doi.org/10.6084/m9.figshare.9783911.v1

J. Botan. Soc. Bengal 79(1) : 71-79 (2025), ISSN 0971-2976

FULL LENGTH ARTICLE

A perspective on primary seed dormancy in *Clerodendrum indicum* (L.) O. Kuntze, an invaluable medicinal plant of South East Asian Tropics

Arijit Ghosh*
Triveni Devi Bhalotia College, Raniganj, Paschim Bardhaman, Pin- 713347, W. B. India

Received : 25.03.2025 Accepted : 12.05.2025 Published : 30.06.2025

The accounts of seed dormancy of the ethno-medicinally important shrub *Clerodendrum indicum* have been investigated. Freshly harvested sun-dried seeds exhibit no germination at all, though TTC test showed positive result in 100% of such seeds, reveals the presence of a sort of innate or primary dormancy in the seeds. Treatment with hot water for 20 min exhibits 60% germination, followed by 50% (15 min), 40% (10 min and 5 min) and 20% germination (30 min). Various concentrations [10(N), 18(N), 24(N), 30(N), and 36(N)] of H_2SO_4 have been used for acid scarification at varying durations (0.5 to 20 minutes). 18(N) H_2SO_4 for 5 minutes was the most successful among them, resulting in ± 98% germination. Strikingly, after 60 days of ambient storage the unscarified seeds start germinating and it increased up to 94% after 150 days of storage. The study reveals that the exalbuminous seeds of *C. indicum* possess an innate or primary dormancy which can be overcome partially (60 %) by hot water treatment and up to 98 % by the treatment with 18(N) H_2SO_4. While in ambient storage condition the primary dormancy of the freshly harvested seeds is gradually released and a maximum of 94% of the seeds become non-dormant by 150days of storage.

Keywords: *Clerodendrm indicum* (L.) O. Kuntze, exalbuminous, primary seed dormancy, scarification, hot water treatment, ambient storage.

INTRODUCTION

Seed germination begins with the uptake of water (imbibition) by the quiescent dry seed and terminates with the elongation of embryonic axis (Bewley and Black, 1994). Visible emergence of the radicle through the micropyle heralds the onset of germination which is referred to as inception. It is most important for a plant to ensure that its seeds germinate at the right time and at the right place. One of the important methods of achieving this criteria is seed dormancy. When seeds are dormant, they are unable to germinate despite the proper environmental factors, i.e., water, temperature, light, and aeration are available (Hartmann *et al*., 2002). Adormant seed possess the capacity to germinate after a certain period of time when the normal physical environmental factors are favourable for its germination (Baskin and Baskin, 2004). Dormancy has been classified by different authors in different ways. Harper (1957) distinguished three types: 'some seeds are born dormant (innate), some achieve dormancy (induced) and some have dormancy thrust upon them (enforced)'. Innate dormant seeds are dormant upon shedding from the mother plant. This is commonly also referred to as primary dormancy.

**Corresponding author* : arijit.botany@gmail.com

Induced dormancy is similar to what is commonly called secondary dormancy (Saha and Takahashi, 1986). Dormancy is also divided into several classes: morphological, physical and physiological dormancy (Nikolaeva, 1977; Baskin and Baskin, 1998; Baskin and Baskin, 2004). A certain period of differentiation is needed in morphological dormancy as the seed is immature after shedding from the mother plant. In case of physical dormancy seeds have impermeable seed coats (Saha and Takahashi, 1981). Therefore, the embryo is dry until the seed coat is somehow opened, and then the water can enter. The third class, physiological dormancy, is related to a physiological inhibiting mechanism of the germination process (Sarkar *et al*., 2001). Several researches have been conducted on the nature of seed dormancy because of its important implications in plant propagation. The causes of seed dormancy have been grouped into five types: i) Rudimentary embryos, ii) Physiologically immature embryos with inactive enzyme system, iii) Mechanically resistant seed coats, iv) Impermeable seed coats and v) Presence of germination inhibitors**.**

The moisture content of seeds is a determining factor because it regulates how long the seed will remain viable. The seed moisture content is the amount of water present in seeds. The moisture content of seeds is expressed usually as a percentage. The seeds' storage life is significantly affected by a slight variation in their moisture content. Therefore, it is important to know the moisture content of seeds immediately after harvest, then prior to storage and at various intervals during storage.

Clerodendrum indicum (L.) O. Kuntze is a medicinally important plant of Lamiaceae (Kirtikar and Basu, 1935; Chopra *et al.,* 1958). The present paper deals with the report of seed dormancy of the species, which had been hitherto unknown. The present work reveals that the seeds of the species represent a sort of innate or primary dormancy and the several techniques were deployed to overcome it.

MATERIAL AND METHODS

Clerodendrum indicum is a sparsely branched shrub up to 4.5 m tall. It has two flowering seasons, i.e., May-June and September-October. Ripened fruits of *C. indicum* were randomly collected for germination experiments from two wild habitats, Amarun Village (almost 18 km North of Burdwan Town in Burdwan District, West Bengal, India) and Rambhadrapur Village (South-West of Sabang Town, almost 2.5 km, in Paschim Midnapur District) of West Bengal. Healthy mature fruits produced by five different individuals in each of the two flowering seasons were plucked and kept within separate borosil glass vials to ensure that no seeds were lost. Seeds were isolated from each fruit after removing its hard pericarp. The work was repeated in three consecutive years (2018-2020). The number of seeds was counted, after sorting out the healthy seeds from the aborted ones. Freshly collected seeds were sun-dried for 2-3 hours on each of 3 consecutive days. Healthy seeds were sorted out, divided into a number of sets of 50 seeds each in borosil glass containers with bake lite caps and stored under laboratory conditions. As mentioned earlier, *C. indicum* produces flowers twice in a calendar year. Therefore, fresh seed lots were obtained twice a year and the seed lots of the two flowering seasons were stored separately and their germination behaviour was studied separately in order to detect the presence of any seasonal variation.

Moisture content of seeds at harvest was determined from the difference in weight between the freshly harvested seeds and dry seeds. Seeds were made totally moisture-free by keeping in a Hot Air Oven for 14 consecutive days at 50°C. Percentage of seed moisture content with respect to both fresh weight and dry weight of seeds were determined from the formulae below:

$$\frac{\text{(Weight of freshly harvested seeds - Weight of dry seeds) x 100}}{\text{Weight of freshly harvested seeds (before drying)}} = \text{\% of moisture content with respect to fresh weight}$$

$$\frac{\text{(Weight of freshly harvested seeds - Weight of dry seeds) x 100}}{\text{Weight of dry seeds}} = \text{\% of moisture content with respect to dry weight}$$

Germination experiments were performed as per the guidelines of International Seed Testing Association (1996). Before germination experiments, seeds were surface sterilized with 0.1% $HgCl_2$ for 90 seconds and washed properly with double distilled water. For imbibition, the seeds were soaked in double-distilled water for the entire night. The water-imbibed seeds were allowed to germinate in sterile Petri dishes under standard laboratory conditions. Germination data were recorded at 24 hour intervals, during the following consecutive days. Each experiment was repeated four times during the entire course of study. The well-known scarification methods, viz., hot water treatment and acid scarification with concentrated H_2SO_4, were employed (Copeland and Mc Donald, 2001). For the hot water treatment, dry seeds were first immersed in hot water at 50°C for five, ten, fifteen, twenty, and thirty minutes.

For acid scarification experiment different concentrations of sulphuric acid [10(N), 18(N), 24(N), 30(N) and 36(N)] were prepared with concentrated H_2SO_4 [36(N)] and double distilled water. To deduce the optimum scarification strategy those concentrations of H_2SO_4 solutions were applied for different time durations (0.5 to 20 min). After the scarification treatment, seeds were made acid-free by repeated washing in distilled water. Finally, the seeds were soaked in distilled water followed by sowing in Petri dishes for germination.

Germination experiments of individual seed lots under ambient storage were performed at regular intervals (15 days interval). The experiment was performed with the stored seeds, with and without applying the acid scarification methods.

RESULTS

Seed structure and seed moisture content

C. indicum produces dry, fibrous drupes with ±11-20 mm in diameter. The ovary is internally four-chambered with single ovule in each chamber. In majority of the fruits, out of the four ovules only one develops into healthy seeds and thereby those are 1-seeded (Fig. 1B). Often the fruit is 2-seeded, less often 3-seeded and 4-seeded. Seeds (6-9 mm x 4-6 mm) are nearly elliptical in outline, creamish in colour with a dark brown patch at the chalazal end, little curved with laterally a concave and a convex side (Figs. 1A, C, D). The chalazal end is broad and rounded while the micropylar end is narrow and pointed. The hilum is situated on the concave side in between the chalaza and the micropyle. The overall seed surface is rough and somewhat rugged (Fig. 1C-E). The seed is exalbuminous, the seed coat encloses the embryo with two fleshy cotyledons (Fig. 1F).

Seeds of the 1st flowering season of *C. indicum* exhibit a higher moisture content (i.e., 7.26%) than the seeds of the 2nd flowering season which show relatively low moisture content (i.e., 3.80%).

Dormancy and scarification

Freshly harvested sun-dried seeds of *C. indicum* successfully imbibe water but exhibit no germination at all. However, TTC test showed positive result in 100% of such seeds indicating the presence of viable embryos in them. The observations reveal the presence of a sort of primary dormancy in the seeds.

Freshly harvested sun-dried seeds were initially treated with hot water at 50°C for various time durations (5-30 min). Treatment with hot water for 20 min was found to be the most effective, bringing about 60% germination, followed by 50% (15 min), 40% (10 min and 5 min) and 20% germination (30 min). Thus, the hot water treatment can bring about a maximum of 60% germination in opposed to the presence of viable embryo in 100% of seeds as revealed by TTC test. Therefore, the hot water can break the dormancy partially. This partial breaking of dormancy by hot water treatment indicates the probable role of some chemical inhibitor(s) which is leached out as a result of the treatment. The results are given in Table-1.

As hot water treatments did not yield more than 60% germination, scarification of the freshly harvested sun-dried seed lots with different concentrations of H_2SO_4 [10(N), 18(N), 24(N), 30(N) and 36(N)] for different durations (0.5 to 20 min) was also carried out. The most successful method was acid scarification

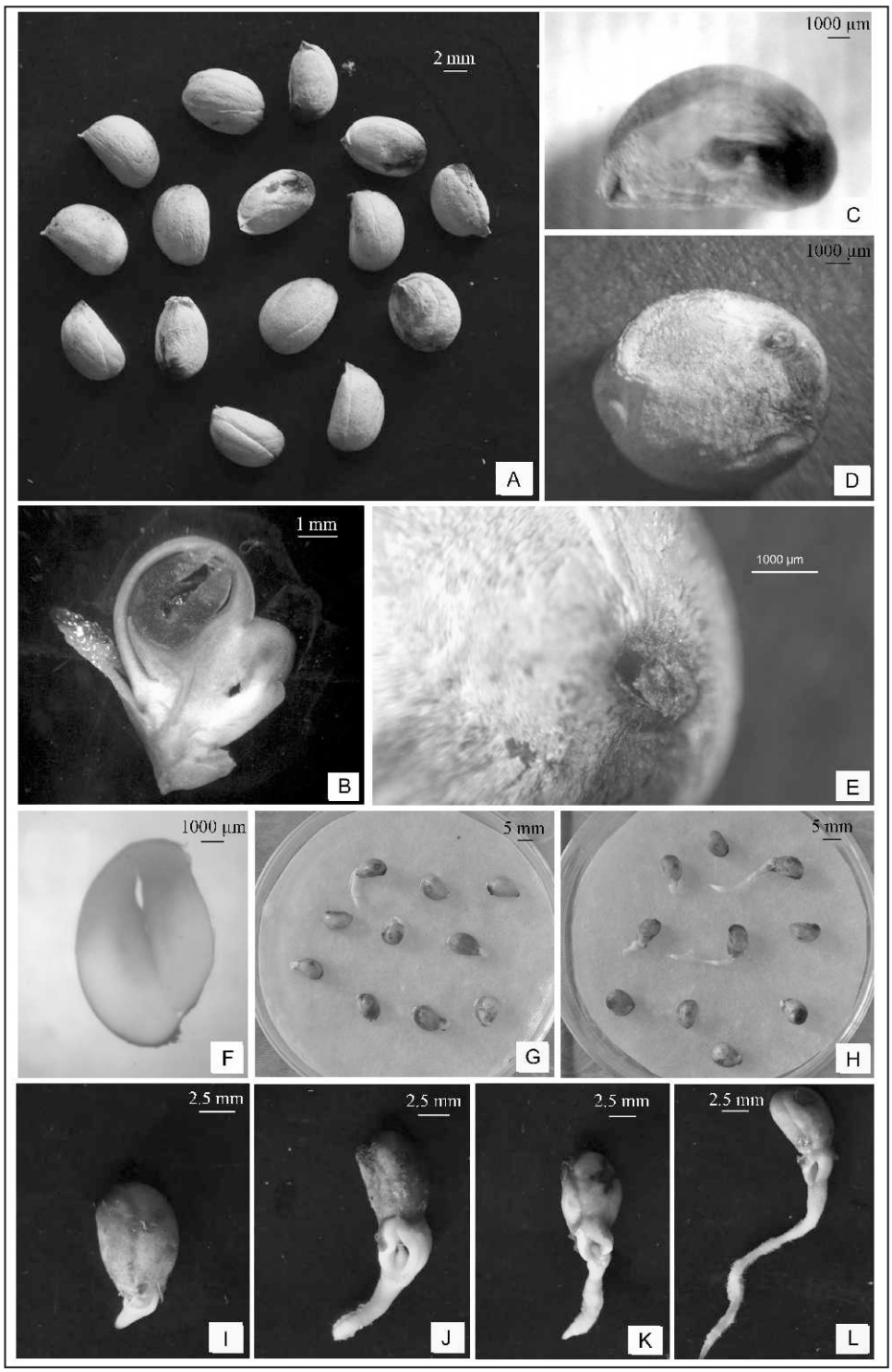


Fig. 1. A. Freshly harvested sun-dried seeds. **B**. Longitudinal section of a young fruit, containing the developing seed showing its embryo with two cotyledons. **C**. Ventral view of a seed showing the hilum, the narrow micropylar end, a broad chalazal end with a dark brown patch. **D**. A seed showing its rough and somewhat rugged surface. **E**. The hilum magnified. **F**. Longitudinal section of a water-imbibed seed, showing the seed coat enclosing the embryo with two fleshy cotyledons. **G&H**. Germinating seeds after the scarification with H_2SO_4. **I-L**.Germinating seeds showing progressive stages of radicle elongation since the inception of germination.

Table 1. Percentage of germination after hot water treatment at 50ºC for different durations in first and second flowering seasons

	50ºC for different time durations (min)				
Hot water treatment	5	10	15	20	30
Germination percentage	40	40	50	60	20

with 18(N) H_2SO_4 for five minutes, resulting in ± 98% germination, followed by 80% germination with the same concentration for 10 minutes (Fig. 1G-L). The results are given in Table-2.

Germination experiments of seeds stored under ambient conditions were performed at 15 day intervals. Seeds stored under natural laboratory conditions were scarified with 18(N) H_2SO_4 for 5 min and sowed in Petri-dishes. Also, stored seeds (control) were sowed in Petri-dishes without scarification.

In case of seeds obtained in the 1st flowering season, after 15 days, 30 days, 45 days and 60 days of storage, H_2SO_4 scarified seeds yielded 96-98% germination whereas unscarified seeds exhibited no germination. A striking phenomenon was observed after 75 days of storage. 22% germination was noticed in case of unscarified seeds. After 90 days of storage, the germination percentage of the unscarified seeds increased up to 44% followed by 90% after 165 days. In all cases, germination percentage for the H_2SO_4 treated seeds was 92-94%. After 195 days of storage, germination percentage dropped to 64% for the unscarified seeds and 80% for the H_2SO_4 treated seeds followed by 42% and 64% germination respectively by the unscarified seeds and the H_2SO_4 treated seeds after 210 days of storage and after 255 days, no germination was found in the unscarified seeds and 16% germination was noticed in case of the H_2SO_4 treated seeds. No germination was observed after 270 days of ambient storage for both the unscarified seeds and the H_2SO_4 treated seeds.

In case of seeds obtained in the 2nd flowering season, after 15 days, 30 days and 45 days of storage, the H_2SO_4 scarified seeds yielded 98% germination whereas the unscarified seeds exhibited no germination. However, the unscarified seeds exhibited 20% germination after 60 days of storage followed by 40%, 58%, 82% and 94% after 75, 105, 135 and 150 days of storage respectively. In all cases, germination percentage for the H_2SO_4 treated seeds was 92-96%. After 195 days of storage, germination percentage dropped to 72% in case of unscarified seeds and 82% in case of H_2SO_4 treated seeds. 40% and 66% germination were noticed respectively for unscarified seeds and H_2SO_4 treated seeds after 225 days months of storage. After 270 days of storage, no germination was found in the unscarified seeds and 14% germination was noticed in case of the H_2SO_4 treated seeds. Finally, no germination was observed after 285 days of ambient storage for both the control seeds and H_2SO_4 treated seeds. The results are given in Table-3.

Table 2. Percentage of germination of freshly harvested seeds of *Clerodendrum indicum* (1st and 2nd flowering seasons) after scarification with different concentrations of H_2SO_4 for different durations

Conc. of H_2SO_4	**30 sec**	**1 min**	**2 min**	**5 min**	**10 min**	**15 min**	**20 min**
10(N)	0	0	0	34	50	66	36
18 (N)	0	26	74	**98**	80	44	22
24 (N)	38	56	24	0	0	0	0
30 (N)	6	0	0	0	0	0	0
36(N)	0	0	0	0	0	0	0

Table 3. Germination performance of seeds stored under natural laboratory condition

Germination (%)	Unscarified seeds (control)		Scarified seeds [18(N) H_2SO_4 (5 min)]	
Storage duration (days)	1st flowering season	2nd flowering season	1st flowering season	2nd flowering season
Freshly harvested seeds	0	0	98	98
15	0	0	98	98
30	0	0	98	98
45	0	0	96	98
60	0	20	96	96
75	22	40	94	96
90	44	40	94	96
105	50	58	94	94
120	50	58	92	94
135	72	82	92	92
150	72	**94**	92	92
165	90	94	92	92
180	90	94	92	92
195	64	72	80	82
210	42	72	64	82
225	20	40	62	66
241	14	22	38	42
255	00	12	16	34
270	00	00	00	14
285	00	00	00	00

DISCUSSION

Freshly harvested seeds of *Clerodendrum indicum* possess an innate or primary dormancy. As the primarily dormant seeds successfully imbibe water and up to 60% and 98% germination are obtained by treatments with hot water and H_2SO_4[18(N) H_2SO_4 for 5 min], the dormancy is imparted possibly by the presence of some inhibitory chemicals. The treatment with higher concentrations for varying durations exhibited negligible results, demonstrating the detrimental effects on embryos. The inhibitors are leached out or broken down to some extent by hot water and to a greater extent by H_2SO_4. In the induction of primary dormancy, the role of inhibitors, i.e., abscisic acid (ABA), is now firmly established in so many species and the dormancy can be overcome when the inhibitor is eliminated or promoters overcome it (Black, 1991; Bewley and Black, 1994; Hilhorst and Karssen, 1992; Hilhorst, 1995).

The primary dormancy of the freshly harvested seeds *C. indicum* is gradually released in course of a few months of storage in ambient condition. The process by which mature, dormant seeds become non-dormant after a period of dry storage is known as 'after-ripening'. During this period they acquire the ability to germinate. In after-ripening period there are several possibilities of breaking of primary dormancy: 1. Inhibitory chemicals might be removed by leaching or inactivation. 2. Inhibitors were not so effective on embryos as they came out of physiological dormancy. 3. Germination promoting chemicals (i.e., GA) were produced and counteracted the effects of the inhibitor (Baskin and Baskin, 2004).

After-ripening reduces seed ABA content of wild-type tomato seeds (Groot and Karssen, 1992). Seeds of Tabasco pepper (*Capsicum frutescens*) require a post-harvest after-ripening treatment of dry storage at room temperature to overcome primary dormancy (Edwards and Sundstrom, 1987; Randle and Honma, 1981). Dry after-ripening treatments have also been shown to

alleviate or reduce dormancy in a number of other species (Bewley and Black, 1983). Endogenous short-chain volatile fatty acids (C_6-C_m) have been implicated in maintaining the dormancy of oats (Berrie *et al*., 1976, 1979) and rice (Majumder *et al*., 1989). Successful after-ripening was related to loss of endogenous volatile fatty acids that inhibit germination in case of *Capsicum frutescens* (Ingham *et al.*, 1993). Volatile compounds have also been found to inhibit the germination of other seeds including onion *(Allium cepa),* carrot (*Daucus carota), Amaranthus* sp., tomato *(Lycopersicon esculentum)* (Bradow and Connick, 1990), lettuce (Reynolds, 1989), soyabean (Gardner *et al*., 1990) and species of weed seed (French and Leather, 1979; Leather and French, 1990).

Seeds of the 1st flowering season of *C. indicum* exhibit a higher moisture content (i.e., 7.26%) than the seeds of the 2nd flowering season which show relatively low moisture content (i.e., 3.80%). A relatively lower moisture content of seeds and a lower after-ripening temperature at the time of their storage is found to be more beneficial. This is evident from the fact that seeds of the 2nd flowering season (September-October) of a calendar year lose their primary dormancy earlier than those of the 1st flowering season (May-June). Also, the former has been found to maintain their viability for a longer period. Seeds of the 1st flowering season stored during the rainy season are obviously subjected to a higher after-ripening temperature than those of the 2nd flowering season stored during the winter.

The rate of seed dormancy is alleviated during dry storage might vary depending on temperature and seed moisture content (Probert, 2000). Various studies have shown that dry after-ripening of seeds is effective at moisture contents from *ca*. 5–18% (on a fresh weight basis) (Leopold *et al.*, 1988; Foley, 1994; Probert, 2000; Steadman *et al*., 2003; Bair *et al*., 2006). They have suggested that the mechanisms for dormancy alleviation are inoperative beyond this range of moisture content. Freshly harvested *Striga asiatica* L. seeds germinate in response to a stimulant only after the passage of time, an after-ripening period, and exposure to moisture at a suitable temperature. Seeds at moisture contents of less than 10% had a germination of greater than 93% while a moisture content of 17% caused about 3% germination (Mohamed *et al*., 1998). Therefore, in this species, germination decreased with increasing moisture content. In weed seed biology, the requirement for a dry condition to transform seeds from one physiological state to another is not unusual. For example, dormancy is lost by drying in seeds of *Avena fatua* L. (Quailand Carter, 1969), *Draba verna* L. (Baskin and Baskin, 1979), *Datura ferox* (Miguel and Soriano, 1974), *Oryza sativa* L. (Leopold *et al*., 1988) and *Xanthium pennsylvanicum* Wallr. (Esashi *et al*., 1983a,b).

From this study it can be concluded that the ellipsoidal exalbuminous seeds of *Clerodendrum indicum* possess an innate or primary dormancy imparted by inhibitory chemicals. The innate dormancy can be overcome partially (60%) by hot water treatment while treatment with 18(N) H_2SO_4 gives rise to 98% germination. In course of storage in ambient condition, the primary dormancy of the freshly harvested seeds is gradually released and a maximum of 94 % of the seeds become non-dormant by 150 days of storage.

DISCLAIMER

The author(s) declare no conflict of interest in the work.

REFERENCE

Bair, N.B., Meyer, S.E. and Allen, P.S. 2006. A hydrothermal after-ripening time model for seed dormancy loss in *Bromus tectorum* L. *Seed Science Research*, **16**: 17-28.

Baskin, C.C. and Baskin, J.M. 1998. *Seeds: Ecology, biogeography, and evolution of dormancy and germination*. California: Academic Press.

Baskin, J.M. and Baskin, C.C. 1979. Effect of relative humidity on after ripening and viability in seeds of the winter annual *Draba verna. Botanical Gazette*, **140**: 284-287.

Baskin, J.M. and Baskin, C.C. 2004. A classification system for seed dormancy. *Seed Science Research*, **14**: 1-16.

Berrie, A., Buller, D., Don, R. and Parker, W. 1979. Possible role of volatile fatty acids and abscisic acid in the dormancy of oats. *Plant Physiol.*, **63**: 758-764.

Berrie, A., Don, R., Buller, D., Alam, M. and Parker, W. 1976. The occurrence and function of short chain length fatty acids in plants. *Plant Sci. Lett.,* **6**: 163-173.

Bewley, J.D. and Black, M. 1983. *Physiology and Biochemistry of Seeds in Relation to, Development, Germination, and Growth. Vol. I.* Berlin : Springer-Verlag.

Bewley, J.D. and Black, M. 1994. *Seeds, Physiology of Development and Germination*. New York: Plenum Press, 2ed.

Black, M. 1991. Involvement of ABA in the physiology of developing and mature seeds. *In* Davies, W.J., Jones H.G. (Ed.), *Abscisic acid. Physiology and biochemistry.* Oxford: BIOS Scientific Publishers, 99-124.

Bradow, J. and Connick, W. 1990. Volatile seed germination inhibitors from plant residues. *J. Chem. Ecol.*, **16**: 645-666.

Chopra, R.N., Chopra, I.C., Handa, K.L. and Kapur, L.D. 1958. *Chopra's Indigenous Drugs of India, 2nd ed.* Calcutta: U. N. Dhur and Sons Private Ltd.

Copeland, L.O. and McDonald, M.B. 2001. *Principles of Seed Science and Technology*, Ed. 4. U.S.A : Kluwer Academic Publishers.

Edwards, R. and Sundstrom, F. 1987. Afterripening and harvesting effects on Tabasco pepper seed germination performance. *Hort-Science*, **22**: 473-475.

Esashi, Y.M., Kuriashi, R., Tanka, R. and Satoh, S. 1983a. Transition from primary dormancy to secondary dormancy in cocklebur seeds. *Plant Cell and Environment*, **6**: 47-54.

Esashi, Y.M., Ogasawara, M., Gerecki, R. and Leopold, C.A. 1983b. Possible mechanism of after-ripening in *Xanthium* seeds. *Physiologia Plantarum*, **87**: 359-64.

Foley, M.E. 1994. Temperature and water status of seeds affect after-ripening in wild oat (*Avena fatua*). *Weed Science*, **42**: 200-204.

French, R. and Leather, G. 1979. Screening of nonanal and related volatile flavor compounds on the germination of 18 species of weed seed. *J. Agric. Food Chem.*, **27**: 828-832.

Gardner, H., Dombos, D. and Desjardins, A. 1990. Hexanal, trans-2-hexend, and trans-2-nonenal inhibit soybean, *Glycine max*, seed germination. *J. Agric. Food Chem.*, **38**: 1316-1320.

Groot, S.P.C. and Karssen, C.M. 1992. Dormancy and germination of abscisic acid-deficient tomato seeds: studies with the sitiens mutant. *Plant Physiology*, **99**: 952-958.

Harper, J.L. 1957. The ecological significance of dormancy and its importance in weed control. *Proceedings of the IVth International Congress of Crop Protection*, **1**: 415-420.

Hartmann, H.T., Kester, D.E., Davies, F.T. Jr. and Geneve, R.L. 2002. *Hartmann and Kester's plant propagation: Principles and practices 7th ed*. New Jersey : Prentice-Hall, Inc., Englewood Cliffs.

Hilhorst, H.W.M. and Karssen, C.M. 1992. Seed dormancy and germination: the role of abscisic acid and gibberellins and the importance of hormone mutants. *Plant Growth Regulation*, **11**: 225-238.

Hilhorst, H.W.M. 1995. A critical update on seed dormancy. I. Primary dormancy. *Seed Science Research*, **5**: 61-73.

Ingham, B.H., Hsieh, TC-Y., Sundstrom, F.J. and Coh, M.A. 1993. Volatile compounds released during dry afterripening of Tabasco Pepper seeds. *J. Agric. Food Chem.*, **41**: 951-54.

International Seed Testing Association. 1996. International Rules for Seed Testing, *Seed Science and Technology*, **24**: 335.

Kirtikar, K.R. and Basu, B.D. 1935. *Indian Medicinal Plants*. Vol. III. Allahabad: L.M. Basu.

Leather, G. and French, R. 1990. Evaluation of volatile compounds on the germination of seventeen species of weed seeds. *J. Agric. Food Chem.*, **38**: 856-859.

Leopold, C.A., Gleninster, C.R. and Cohn, M.A. 1988. Relationship between water content and after-ripening in red rice. *Physiologia Plantarum*, **74**: 659-662.

Majumder, M., Seshu, D. and Shenoy, V. 1989. Implication of fatty acids and seed dormancy in a new screening procedure for cold tolerance in rice. *Crop. Sci.*, **29**: 1298-1304.

Miguel, L.C. and Soriano, A. 1974. The breaking of dormancy in *Datura ferox* seeds as an effect of water absorption. *Weed Research*, **14**: 265-270.

Mohamed, A.H., Ejeta, G., Butler, L.G. and Housley, T.L. 1998. Moisture content and dormancy in *Striga asiática* seeds. *Weed Research*, **38**: 257-265.

Nikolaeva, M.G. 1977. Factors controlling the seed dormancy pattern. *In* Khan, A.A. (Ed.), *The*

physiology and biochemistry of seed dormancy and germination, 51-74.

Probert, R.J. 2000. The role of temperature in the regulation of seed dormancy and germination. *In* Fenner, M. (Ed.), *The ecology of regeneration in plant communities*. Oxon: CABI Publishing, 261-292.

Quail, P.H. and Carter, O.G. 1969. Dormancy in seeds of *Avena ludoviciana* and *A. fatua. Australian Journal of Agriculture Research*, **20**: 1-11.

Randle, W. and Honma, S. 1981. Dormancy in peppers. *Sci. Hortic.* (Amsterdam), **14**: 19-25.

Reynolds, T. 1989. Comparative effects of heterocyclic compounds on inhibition of lettuce fruit germination. *J. Exp. Bot*., **40**: 391-404.

Saha, P.K. and Takahashi, N. 1981. Seed dormancy and water uptake in *Crotalaria sericea* Retz. *Ann. Bot.*, **47***:* 423-425.

Saha, P.K. and Takahashi, N. 1986. Chilling effect on seed longevity and secondary dormancy in *Shorea robusta* Gaertn. f. *Environ. Control. Biol.*, **24**: 27-32.

Sarkar, S., Chattopadhyay, S. and Saha, P.K. 2021. *J. Bot. Soc. Bengal*., **75**(1): 1-12.

Steadman, K.J., Crawford, A.D. and Gallagher, R.S. 2003. Dormancy release in *Lolium rigidum* seeds is a function of thermal after-ripening time and seed water content. *Functional Plant Biology*, **30**: 345-352.

Wareing, P. and Foda, H. 1957. Growth inhibitors and dormancy in *Xanthium* seed. *Physiol. Plant.*, **10**: 266-280.

J. Botan. Soc. Bengal 79(1) : 80-100 (2025), ISSN 0971-2976

FULL LENGTH ARTICLE

Pharmacognostic, phytochemical and antioxidant studies of leaf and bark parts of *Xylia xylocarpa* (Roxb.) W. Theob.

Riya Bhadury, Samik Hazra and Chowdhury Habibur Rahaman*

Ethnopharmacology Laboratory, Department of Botany (UGC-DRS-SAP & DST-FIST sponsored), Visva-Bharati, Santiniketan- 731235, India.

Received : 11.04.2025 Accepted : 17.06.2025 Published : 30.06.2025

The current research encompasses the studies in pharmacognostic, phytochemical, and antioxidant profiles of the leaf and bark parts derived from *Xylia xylocarpa* (Roxb.) W. Theob., an under explored medicinal plant of the family Fabaceae. Traditionally, the leaf and bark of this plant have been utilized for treating the health conditions such as diarrhea, ulcers, piles, worms, vomiting, rheumatism, leprosy, etc. The pharmacognostic investigation revealed that in leaf, the epidermal cells were irregular in shape, with straight walls found in both upper and lower epidermal surfaces. Stomata are of paracytic type, exclusively present on the lower epidermis of leaf. The stomatal index was determined to be 10.06±0.63%. The palisade ratio was 8.91±0.31. Glandular trichomes were multicellular, sessile, observed in both surfaces of the leaf. The trichome index was 7.02±0.15% for the upper surface and it was 6.41±0.69% on the lower surface. The physicochemical characteristics of the leaf material, including moisture content and total ash, were 9.0±0.5% and 7.2±0.2%, respectively, which were significantly higher than the values estimated for the same parameters incase of the bark part. Microchemical tests confirmed the existence of alkaloids, flavonoids, tannins, glycosides, and reducing sugars in the methanolic leaf and bark extracts. The amount of total phenolic content was higher in the bark (138.02± 0.08 mg of GAE/g tissue) than the leaf (105.02± 0.08 mg of GAE/g tissue), which correlates with superior antioxidant activity of the bark part. The HPLC analysis of the leaf and bark parts revealed the presence of three flavonoid and four phenolic compounds in substantial amount. The IC_{50} values for DPPH and ABTS radical scavenging assays of the bark extract were 65.43±0.9 µg/mL and 95.11±0.10 µg/mL, respectively, which were lower than the IC_{50} values of the leaf extract. This study highlights the bark of this plant highly potent in terms of the phytochemical content and antioxidant activity. Additionally, the study provides diagnostic pharmacognostic features that can be used to accurately identify the leaf and bark crude drugs of *X. xylocarpa*. This study highlights the need for further phytochemical and pharmacological research on leaf and bark parts of the studied plant species to fully understand its therapeutic potential.

Keywords: *Xylia xylocarpa,* Pharmacognosy, Total Phenolics, Total Flavonoids, Antioxidant activity.

**Corresponding author :* habibur_cr@visva-bharati.ac.in

INTRODUCTION

Plants offer an extensive variety of chemical compounds that exhibit unique structural and physicochemical properties, as well as a wide range of significant biological activities. Medicinal plants have long been acknowledged as dependable sources of medicaments used for treating various health conditions, such as cancer, diabetes, obesity, autoimmune disorders, and varied range of inflammations. Many of the herbal drugs used in traditional medicine have been developed into commercially viable effective medicaments, including teniposide, digoxin, reserpine, silymarin, atropine, aescin, vincristine, huperzine A, and others. Comprehending the traditional uses of raw plant materials is crucial for the development of innovative medicines. Many therapeutically important plants have been extensively studied through phytochemical and pharmacognostical research. However, a huge number of such medicinal plants still remain untouched for evaluation of their therapeutic abilities. Standardizing and documenting the characteristics of the crude drugs utilized in preparation of herbal medicines is of utmost importance to verify the authenticity of these herbal ingredients. Essentially, pharmacognostic standards supply all the critical data required for the accurate identification and maintenance of the quality of crude drugs (Ray and Rahaman, 2018). Pharmacognostic investigations of a number of medicinal plants have so far been carried out by different workers to develop the scientific standards which are successfully employed in identification and authentication of the crude drugs procured from the respective plant sources (Murti *et al*., 2010; Saha and Rahaman, 2013; Pal and Rahaman, 2014; Singh *et al.*, 2014; Ray and Rahaman, 2018; Sultana *et al*., 2023).

Xylia xylocarpa is an ethnomedicinally important tree belonging to the family Fabaceae. Traditionally, different parts of this plant, such as bark, leaves and seeds have been employed to treat a wide range of health problems, including rheumatism, piles, worm infestation, leprosy, diarrhea, ulcers, and many other conditions (Manimegalai and Prithiba, 2021). As a result, leaf and bark parts have been the focus of scientific research aimed at assessing their chemical and pharmacological properties. Previous studies have indicated that the methanolic extract derived from the leaves of this plant exhibits notable biological activities, such as anti-inflammatory and anti-nociceptive effects (Chowdhury *et al*., 2021). The ethanolic extracts from both the leaves and bark have also shown potential in improving memory, particularly in the context of Alzheimer's disease (Lam *et al*., 2016). Despite the existing research on the phytochemistry and bioactivity of *X. xylocarpa*, there are still gaps in the comprehensive evaluation of phytochemical, pharmacological and pharmacognostic attributes of its leaf and bark. No previous studies have specifically examined their pharmacognostic parameters. Therefore, the main objective of this study was to investigate the pharmacognostic, phytochemical, and antioxidant properties of the leaf and bark of *X. xylocarpa*, as these parts are commonly used as crude drugs for various medicinal purposes.

MATERIALS AND METHODS

Material

Scientific name: *Xylia xylocarpa* (Roxb.)W.Theob.

Synonyms: *Mimosa xylocarpa* Roxb., *Inga xylocarpa* (Roxb.) DC., *Acacia xylocarpa* (Roxb.) Willd., *Xylia dolabriformis* Benth.

Common English name: Burma Ironwood.

Bengali vernacular name: Loha-kath.

Parts used: Leaf, bark and seed.

Botanical characters: A large tree; branches many, thick, young twig brown-tomentose. Leaves bi-pinnate, 10-32.5 cm; pinnae 2, terminal on a petiole 2.5-5.7 cm long. Leaflets usually 5-6 pairs, rarely 2-3 or 7 pairs, 5-20 x 2.5-5.5 cm, diminishing in size downwards, ovate, oblong, elliptic-oblong or obovate-oblong, acute to acuminate, subcoriaceous, glabrous, glands 4-5 on rachis between the upper leaflets; petiolule 4-5 mm. Flowers in a globose head, sessile, unisexual, small, 1.5 cm long; peduncles 7.5 cm, slender, thickening in fruit. Calyx tubular, 5-

toothed, 4 mm, valvate. Corolla tubular, yellowish white, 6 mm long; petals 5, linear, slightly connate at base, valvate. Stamens10, filaments free, 13 mm, exserted. Ovary sessile; ovules many. Fruits legume,10-15 x 2.5-6.3 cm, dehiscent, large, flat, woody, oblong, falcate, rusty-tomentose, septate between the seeds. Seeds 6-10, oblong-ellipsoid, compressed, 13-16x 6-10 mm, brown, smooth, polished (Saxena and Brahmam, 1994).

Flowering time: April-May.

Fruiting time: November-April.

Distribution: It is widely distributed in India, Bangladesh, China, Myanmar, Malaysia, Philippines. In India, the plant is commonly found in Andhra Pradesh, Bihar, Kerala, Madhya Pradesh, Orissa, and West Bengal (Saxena and Brahmam, 1994; https://indiaflora-ces.iisc.ac.in/Flora Peninsular).

Medicinal uses:

Bark and Leaf: Decoction of leaf and bark is used in leprosy, vomiting, diarrhea, gonorrhea ulcers and expelling of worms (Manimegalai and Prithiba, 2021).The bark and leaf of this tree are also used to address respiratory disorders and skin conditions by Phu Tai community of Thailand (Junsongduang *et al.,* 2025).

Seeds: Oil from the seeds is given in rheumatism, piles and leprosy (Kirtikar and Basu, 1991).

METHODS

Plant identification and herbarium preparation: The twigs were collected from a matured plant grown in Ballabhpur forest, Santiniketan (Fig. 1A) (23°24' 17.28"N, 87°24'17.28"E) in the month of April, 2024. The plant has been identified with the help of available literature (Saxena and Brahmam, 1994) and identification was confirmed through consultation with an expert taxonomist. The nomenclature of the species has been updated following the standard website like 'Plants of the world online' (http://powo.science.kew.org/). After collection, the plant specimen has been processed and herbarium was prepared following the techniques suggested by Jain and Rao (1977). For future reference, the herbarium specimen deposited in the departmental Herbarium, Department of Botany, Visva-Bharati, Santiniketan [Voucher specimen number: INDIA, West Bengal, Birbhum district, Santiniketan, 21.04.2024, RB 102 (VBH)].

Plant extract preparation: The collected bark (Figs. 1B and C) and leaf of *X. xylocarpa* were washed thoroughly and then sliced into small thin pieces, shade-dried, and ground into fine powder. The leaf and bark powder were stored in an airtight vessel at 4°C for future use. The 10 g of powder sample of each plant partwas extracted with 150 mL of 80% aqueous methanol in a 250 mL conical flask keeping in a mechanical shaker at 28±2°C for 36 h. For a single extraction, the whole process was repeated three times. The resulting pull-out was mixed and filtered with Whatman's No.1 filter paper. The filtrate was subjected to evaporation at room temperature (28±2°C)for 48 h. The ultimate extract-yield was stored at 4°C and dissolved in dimethyl sulfoxide (DMSO) to make the stock solution of extract before use.

Study of foliar micromorphology: Leaf samples were cleared off the chlorophylls following the Bokhari's method (Bokhari, 1970). The cleared leaf samples were then mounted on the slide with a drop of 10% glycerine and 1% safranin and observed under compound light microscope (ZEISS, AXIOSTAR plus, model number 176045). For field emission scanning electron microscope (FE-SEM) analysis, the leaf specimens were prepared following the methodology of Yuan *et al*. (2020), and subsequently examined under a FE-SEM (Gemini SEM 450; Serial No. 8216010130), from which suitable micrographs were captured.

Vegetative anatomy: An anatomical study was conducted by cutting freehand sections of the freshly collected leaf (lamina, petiole) and bark of this tree. The sections were subsequently stained following double staining method (Johansen, 1940). The stained sections were then examined under a compound light microscope (ZEISS, AXIOSTAR plus, model number 176045). Transverse sections of the midrib and petiole

of the studied plant species were prepared for FE-SEM analysis following the method described by Yuan et al. (2020), and examined using a field emission scanning electron microscope (Gemini SEM 450; Serial No. 8216010130).

Bark maceration study: Small pieces of bark (1 cm long) were macerated following the standard method (WHO, 1998). The macerated samples were washed several times with distilled water. A bit of macerated bark was taken on the slide, teased with needles, stained in 1% safranin, and mounted with 10% glycerine by putting a cover glass. The mounted samples were then observed under a microscope (ZEISS, AXIOSTAR plus, model number 176045).

Organoleptic study: The study was conducted on the powdered leaf and bark samples with the help of sensory organs. This involved assessing various properties, including color, odor, taste and texture of the crude drugs (WHO, 1998).

Physicochemical evaluation: The physicochemical characteristics of the leaf and bark powder were evaluated (Evans, 2008; Harborne and Williams, 1994). This evaluation included the determination of moisture content, ash value (total ash, acid-insoluble ash, and water-soluble ash), and extractive value of the plant samples.

Determination of extractive value: The powdered plant samples of 10g each were extracted separately with 100 mL of each of five solvents namely, water, methanol, chloroform, ethyl acetate, and hexane for 48 h, with continuous shaking at room temperature (28 ± 2°C). The extracts were filtered and dried under vacuum using a rotary evaporator at 45°C. Once dried, the weight of each solvent extract was recorded, and the extractive value was calculated using the following formula (Evans, 2008):

$$\text{Extractive value}(\%) = \frac{\text{Weight of extracted residue obtained}}{\text{Weight of the plant material taken}} \times 100$$

Phytochemistry

Preliminary phytochemical screening: In accordance with standard protocols, methanolic extracts derived from powdered leaf and bark samples were subjected to a series of chemical color reaction tests utilizing various reagents. The objective of this study was to identify different phytochemical groups present in the powdered samples (Swain and Hillis, 1959; Zhishen *et al.,* 1999).

Estimation of total phenolic content: A 0.5 g sample of powdered plant material was weighed and transferred into a centrifuge tube. To this, 5 mL of 80% methanol was added. The mixture was then homogenized at 10,000 rpm for 20 min at room temperature. Following homogenization, and the supernatant was carefully collected and transferred to a Petri dish. The supernatant was allowed to evaporate at room temperature (24–26°C) for 5–6 h. Once the solvent had completely evaporated, the resulting residue was scraped off and transferred to a test tube. The residue was then dissolved in 5 mL of distilled water. To this solution, 0.5 mL of Folin-Ciocalteu reagent was added, followed by the addition of distilled water to bring the total volume to 10 mL. The composition of the Folin-Ciocalteu reagent per litre of stock solution includes 10% phosphomolybdic acid and 2.5% phosphotungstic acid, dissolved in 0.5 N hydrochloric acid. After allowing the mixture to react for 3 min, 2 mL of 20% sodium carbonate solution was added, and the mixture was thoroughly mixed. The solution was then boiled for one minute in a water bath and subsequently cooled to room temperature. Finally, the absorbance of the solution was measured at 650 nm using a spectrophotometer (Shimadzu UV-Vis 1800). A blank solution, containing Folin-Ciocalteu reagent, distilled water, and sodium carbonate but without the plant extract, was used as a reference for the absorbance measurement (Ray *et al*., 2018).

Estimation of total flavonoid content: The aluminum chloride method was utilized for the estimation. To begin, a stock solution was prepared by dissolving 100 mg of extract from each plant part in 5 mL of methanol, which was then diluted to a final volume of 10 mL with additional methanol. For the assay, 0.5 mL of the sample extract was pipetted into a test tube containing 1.5 mL of methanol, 0.1 mL of a 10% aluminum chloride solution, 2.8 mL of distilled water, and 0.1 mL of a 1M potassium acetate solution. The test tube was thoroughly shaken to ensure that the components were uniformly mixed. The absorbance

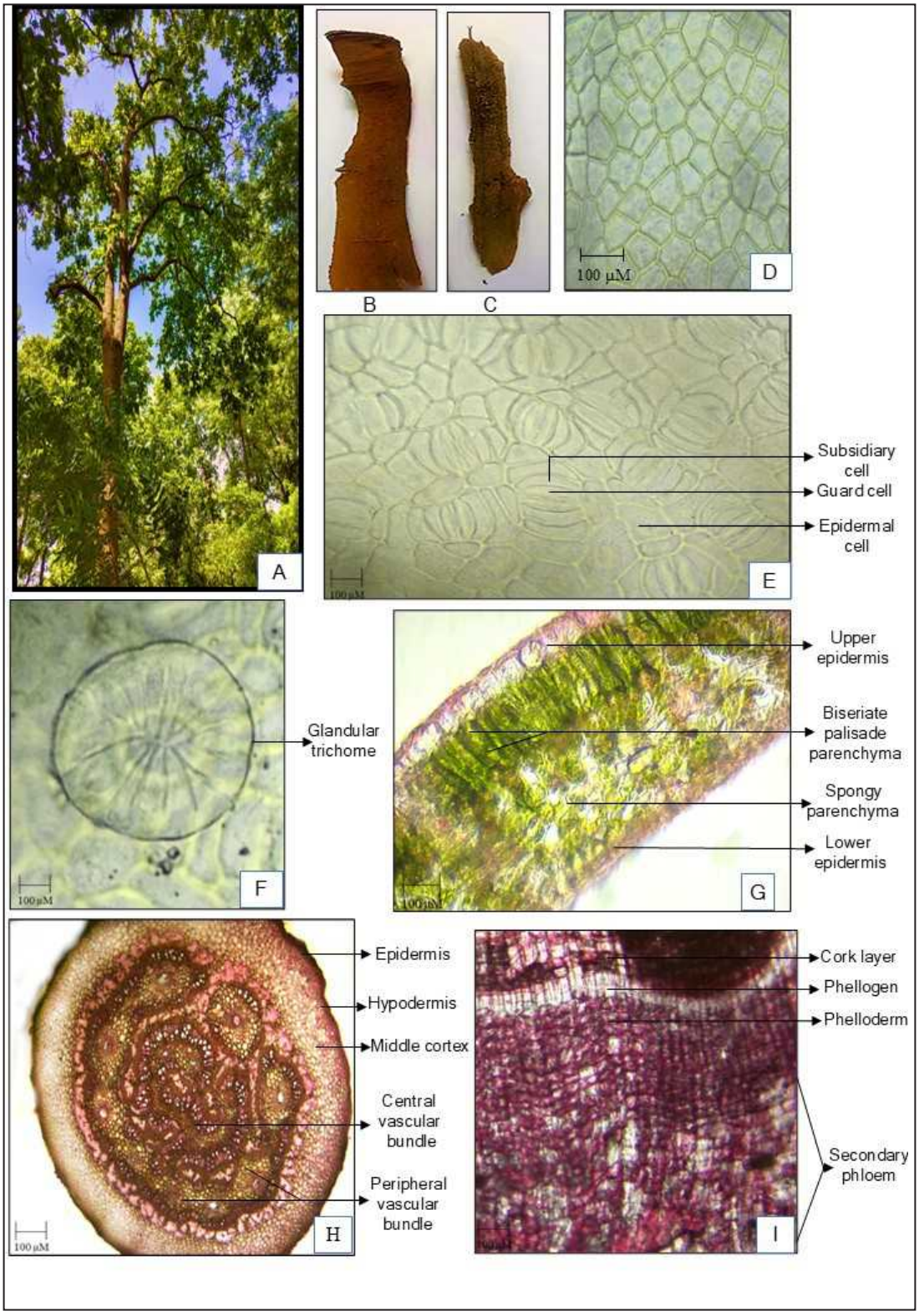


Fig. 1. **A.** Plant habit, **B.** Inner surface of stem bark, **C.** Outer surface of stem bark, **D.** A portion of epidermis of upper leaf surface, **E.** A portion of epidermis of lower leaf surface, **F.** Glandular trichome, **G.** L.T.S. of leaf lamina, **H.** T.S. of petiole, **I.** T.S. of bark.

of the resulting solution was measured at 415 nm using a Shimadzu UV-Vis 1800 double-beam spectrophotometer, with an appropriate blank solution serving as the reference (Zhishen *et al.*, 1999).

Estimation of total tannin content: For tannin estimation, a conical flask was charged with 500 mg of powdered plant sample and 75 mL of distilled water. The mixture was boiled for 30 min. After cooling to room temperature, the mixture was centrifuged at 2000 rpm for 20 min. The residue was discarded, and the volume of the supernatant was adjusted to 100 mL with distilled water. This prepared extract was then used for tannin estimation. The reagent used for tannin estimation was Folin–Denis reagent. This reagent was prepared by dissolving 100 gm of sodium tungstate dihydrate and 20 gm of phosphomolybdic acid in approximately 700 mL of distilled water. To this mixture, 50 mL of concentrated orthophosphoric acid (85%) was added, followed by refluxing for 2 h. After cooling, the solution was diluted to a final volume of 1 L with distilled water. To perform the estimation procedure, 1 mL of the plant extract was transferred to a volumetric flask containing 75 mL of distilled water. Subsequently, 5 mL of Folin-Denis reagent and 10 mL of sodium carbonate solution were added to the flask. The volume was then adjusted to 100 mL with distilled water. The mixture was thoroughly mixed and allowed to stand for 30 min. The absorbance of the solution was measured at 700 nm using a double-beam spectrophotometer (Shimadzu UV-Vis 1800). A blank solution, prepared using distilled water instead of the plant extract, was used as the reference for the absorbance measurement. The tannin content was calculated using a standard curve of tannic acid (0.1 mg/mL) and expressed as milligrams of tannic acid equivalents (TAE) per gram of plant sample (Afify *et al.*, 2012).

Estimation of total alkaloid content: The total alkaloid content in the plant samples was assessed using a modified version of the 1,10-phenanthroline method, as described by Singh *et al*. (2004). To begin, 100 mg of powdered plant material was weighed and extracted with 10 mL of 80% ethanol. The mixture was then filtered through filter paper, and the filtrate was centrifuged at 5000 rpm for 10 min. The resulting supernatant was collected for detailed alkaloid estimation. The reaction mixture was prepared by combining 1 mL of the leaf extract, 1 mL of 0.025 M $FeCl_3$ in 0.5 M HCl, and 1 mL of 0.05 M 1,10-phenanthroline in ethanol. This mixture was incubated in a hot water bath at 72°C for 30 min. The absorbance of the resulting red-colored compound was measured at 510 nm using a reagent blank for reference. The alkaloid content was quantified using a standard curve of pilocarpine. A standard solution was prepared by dissolving 10 mg of pilocarpine in 10 mL of ethanol and diluting it to 100 mL with distilled water to achieve a concentration of 0.1 mg/mL. The alkaloid content was expressed as milligrams of alkaloid per gram of plant extract, based on the pilocarpine standard curve.

HPLC analysis of leaf and bark extracts: The phytochemical analysis of the methanolic leaf and bark extracts of *X. xylocarpa* was conducted using an Agilent 1260 Infinity II HPLC device (USA) coupled with Open Lab data processing software. For compound separation, a reversed-phase Luna C18 column (25 cm length, 4.6 mm inner diameter, and 5 µm thickness) (Phenomenex, USA) was employed. Stock solutions (1 mg/mL) were prepared for both the standard and the plant extracts. To prepare the stock solution of the leaf and bark extracts, 1 mg of each extract was mixed with 0.5 mL of HPLC-grade methanol and sonicated continuously for 10 min. The total volume was then adjusted to 1 mL by adding the HPLC mobile phase solvent, which consisted of a mixture of 1% aqueous acetic acid and acetonitrile in a 9:1 ratio. A 20 µL volume of the plant extract was injected at a flow rate of 0.7 mL/min, and the column temperature was maintained at 28 °C. Gradient elution was performed by varying the percentage of solvent B (acetonitrile) to solvent A (1% aqueous acetic acid). Over the first 28 min, the gradient ratio was linearly increased from 10% to 40% of solvent B, then from 40% to 60% up to 39 min, and finally from 60% to 90% over 50 min. The mobile phase composition was returned to its initial state of 10% solvent B and 90% solvent A after 55 min, followed by an additional 10 min before the next sample injection. The total analysis time for each sample was approximately 65 min. The HPLC chromatograms were recorded at three different wavelengths (272, 280, and 310 nm) using a photodiode UV detector. Phenolics and

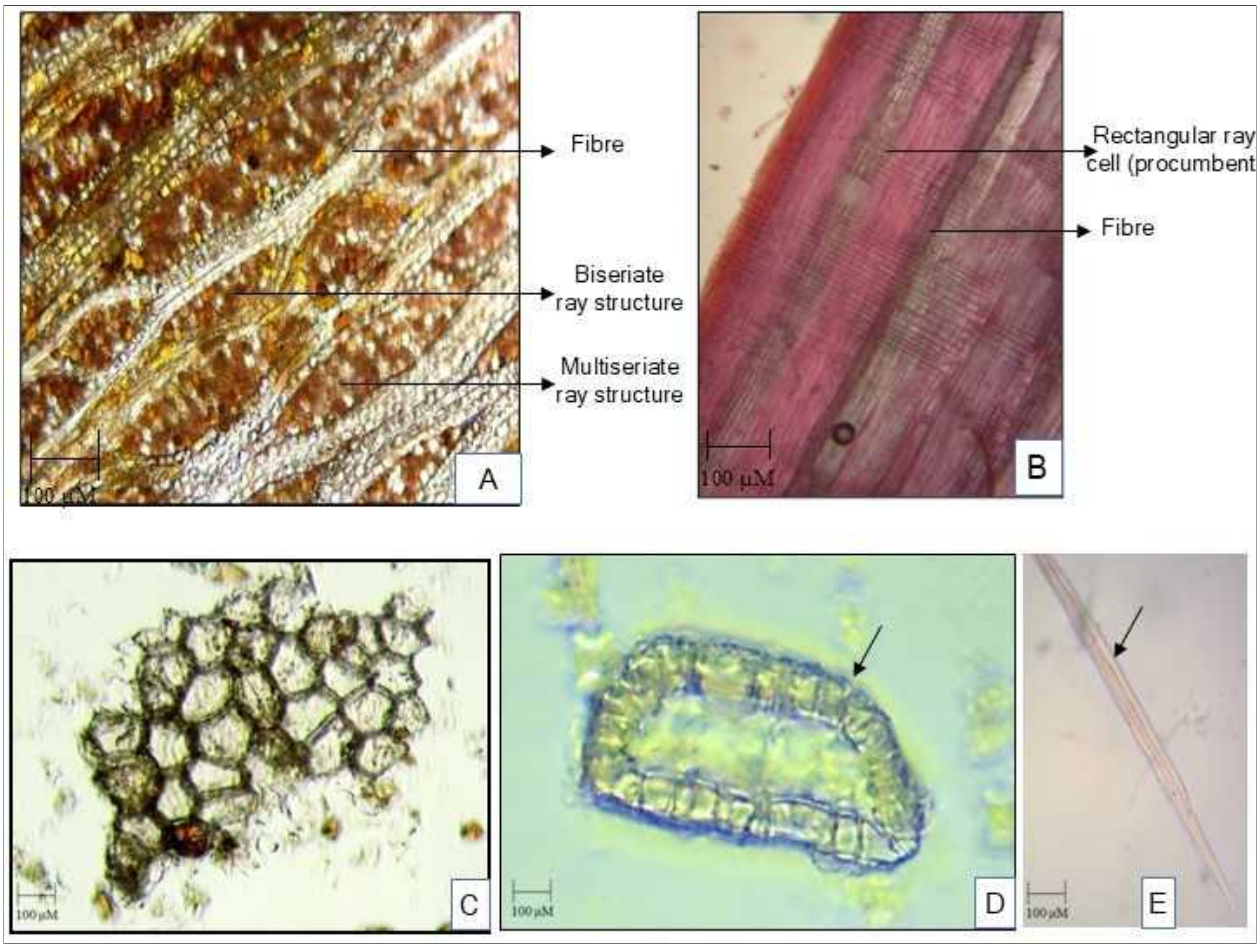


Fig. 2. A. T.L.S. of bark, **B.** R.L.S. of bark, **C.** Cork cells, **D.** Brachysclereid, **E.** A portion of fibre.

flavonoids were identified based on their retention times and by comparison with the applied standard compounds. A calibration curve was constructed using various concentrations of the relevant standard samples to quantify the detected compounds. Here in the HPLC study, the utilized seven standard compounds were three from flavonoids (catechin, naringenin, and quercetin) and four from the phenolics (p-coumaric acid, curcumin, ellagic acid, and gallic acid).

Antioxidant study

DPPH radical scavenging activity: The DPPH radical scavenging activity was assessed using the method described by Karadag *et al.* (2009). To prepare the stock solution, 24 mg of DPPH was dissolved in 100 mL of methanol. The working solution was then prepared by mixing 10 mL of the stock solution with 45 mL of methanol and adjusting the absorbance to 1.1 ± 0.02 at 515 nm using a UV-Vis spectrophotometer. To evaluate the scavenging activity, 0.015 mL of plant extract was combined with 0.85 mL of the DPPH working solution and stored in the dark for 24 hours. The absorbance of the resulting mixture was measured at 515 nm. Ascorbic acid was used as the standard compound to generate a standard curve. The results were expressed as the percentage of scavenging activity, and the experiment was performed in triplicate. The percentage of DPPH radical scavenging activity was calculated using the following formula:

DPPH Radical Scavenging Activity (%) = $(A_0 - A_t / A_0) \times 100$

Where A_t is the absorbance of the leaf extracts or standard, and A_0 is the absorbance of the control.

ABTS radical scavenging Assay: The ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging activity was assessed using the standard method described by Thaipong *et al*. (2006). To prepare the stock solution, 7.4 mM $ABTS^+$ was dissolved in a 2.6 mM potassium persulfate solution. The working solution was prepared by mixing 10 mL of the stock solution with 40 mL of methanol. The absorbance of the working solution was adjusted to 1.1 ± 0.02 at 734 nm using a UV-Vis spectrophotometer (Shimadzu UV1800). A standard curve was generated using ascorbic acid as the reference compound. The results were expressed as the percentage of scavenging activity. Each experiment was conducted in triplicate. The percentage of ABTS radical scavenging activity was calculated using the following formula:

ABTS radical scavenging activity(%)= $(A_0 - A_t / A_0) \times 100$

Where, A_t is the absorbance of the plant extract or standard and A_0 is the absorbance of the control.

RESULTS

Foliar micromorphology: It offers a comprehensive description of the epidermal cells, trichomes, and stomata, encompassing their dimensions.

Epidermal cells: In the investigated plant, the epidermal cells exhibited an irregular shape on both the upper and lower surfaces of the leaf. The cell wall outline appeared straight on both surfaces. The frequency of epidermal cells was 1061.33/mm² on the upper leaf surface and it was1694.5/mm² on the lower leaf surface. The sizes of the epidermal cells were 202.87±0.77 µm × 78.00±1.30 µm on the upper leaf surface and 127.04±1.06 µm × 55.21±1.64 µm on the lower leaf surface. The palisade ratio was determined to be 8.91±0.31(Figs. 1D and E).

Stomatal complex: Stomata are exclusively found on the lower leaf surface and are of the paracytic type. The stomatal frequency on the lower epidermis is 12.82±0.66 per mm². The dimensions of the stomata were 91.41±2.0 µm in length and 17.04±0.62 µm in breadth. The stomatal index was 10.06±0.63 % (Fig. 1E; Figs. 3A and B).

Trichomes: The multicellular, sessile, glandular trichomes were observed on both the upper and lower epidermises of the leaf. The trichomes on the upper epidermal surface were 118.64±3.25 µm in length and 18.17±1.08 µm in width. In the lower epidermis, they are slightly smaller, with 78.31±2.45 µm in length and 18.28±0.77 µm in width. The trichome index was 7.02±0.15 % on the adaxial leaf surface and it was 6.41±0.69 % on the abaxial surface (Fig. 1F; Figs. 3A-C).

Leaf anatomy: The leaf lamina's lateral tangential section (L.T.S.) revealed the uniseriate upper and lower epidermis, both featuring thick cuticles on the outer walls of the epidermal cells. The mesophyll consists of two distinct zones: the upper zone of columnar palisade cells, arranged compactly in two layers, and the lower zone of spongy parenchyma cells present beneath the palisade zone in 2-3 layers. The spongy parenchyma cells are loosely arranged, thin-walled, and more or less spherical in shape (Fig. 1G).

The midrib exhibits a single-layered, cuticularized epidermis on both its upper and lower surfaces. Beneath the epidermis, the ground tissue consists of two layers of collenchyma followed by five to six layers of parenchyma cells. A semilunar vascular bundle is present at the centre (Fig. 3D).

Petiole anatomy: In transverse section (T.S.), the petiole outline appears circular. The epidermis is composed of a single layer of compactly arranged cells, featuring a thick cuticle on the outer wall. Beneath the epidermis lies a ground tissue zone consisting of 7-8 layers of parenchyma cells. At the center, a large vascular bundle is surrounded by six smaller vascular bundles (Fig. 1H; Fig. 3E).

Bark anatomy: The surface of the stem bark is relatively smooth and has a whitish-grey color. In this mature stem bark, the two main constituent tissues areperiderm and secondary phloem. The sections of

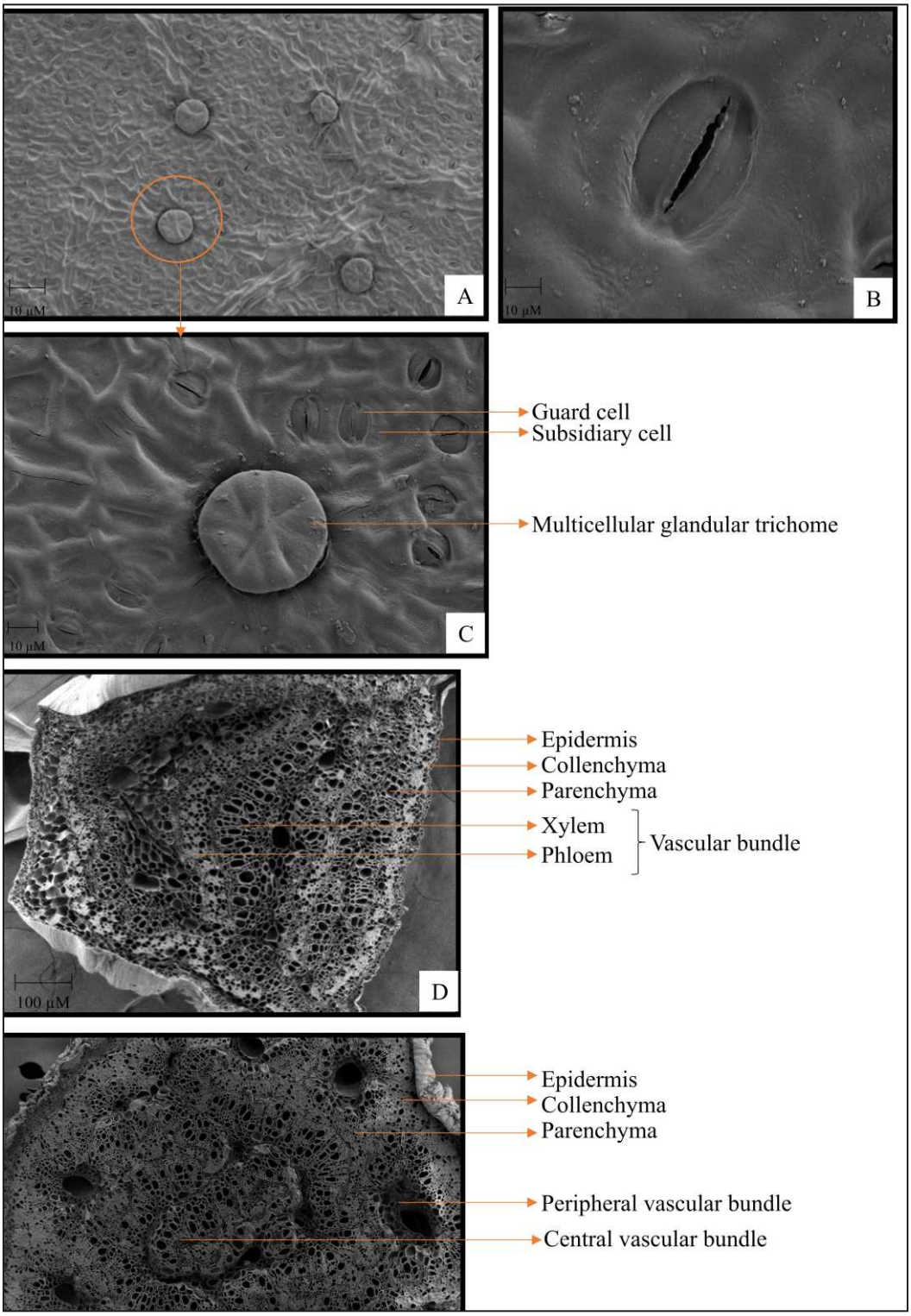


Fig. 3. FE-SEM microphotographs. **A.** A portion of lower epidermis with stomata and trichomes, **B.** A stoma, **C.** Enlarged view of a multicellular glandular trichome and paracytic stomata, **D.** T.S. of leaf midrib, **E.** T.S. of petiole.

Table 1. Organoleptic features of leaf and bark powders of *X. xylocarpa.*

Organoleptic features	Leaf powder	Bark powder
Colour	Greenish	Brownish
Odour	Odourless	Odourless
Taste	Acrid	Acrid
Texture	Powdery	Fibrous

the bark cut in transverse (T.S.), tangential longitudinal (T.L.S.), and radial longitudinal (R.L.S.) planes, revealed the following anatomical features.

T.S.- The bark is differentiated into the cork layer, phellogen, phelloderm, and secondary phloem. The cork layer is composed of multiple rows of radially arranged polygonal dead cork cells. The phellogen consists of 3-4 layers of rectangular, narrow, thin-walled meristematic cells. The phelloderm is made up of 7-8 layers of rectangular, thick-walled parenchymatous cells, also arranged in radial rows. Secondary phloem zone is massive found beneath the phelloderm, the last layer ofperiderm, with groups of thick-walled phloem fibers interspersed throughout (Fig. 1I).

T.L.S.- The tangential longitudinal section (T.L.S.) of bark exhibited the elliptical ray structures and phloem fibres mainly. The structures of ray were predominantly multiseriate, with a few being biseriates. The frequency of multiseriate and biseriate ray structures was 2.37±0.40 per mm² and 1.25±0.50 per mm², respectively. The height and width of the multiseriate ray structure was 345.20±42.49µm and 39.44±9.03µm, respectively. Biseriate rays are smaller in size compared to multiseriate ones. The biseriate ray structures have a height of 275.10±22.29 µm and a width of 28.44±8.13 µm. The number of ray parenchyma cells per multiseriate ray ranging from 28-45 cells, whereas biseriate ray structure comprises of 20-36 cells. The phloem fibres are lignified and have very thick cell walls, densely packed on either side of the ray structures (Fig. 2A).

R.L.S.- In the radial longitudinal section (R.L.S.), the internal structure of the ray structures are visible. The ray cells are rectangular in shape,thin-walled, parenchymatous, living and procumbently arranged. Thus, the rays are homogeneous in nature. The fibres elongated, with lignified wall and longitudinally arranged (Fig. 2B).

Bark elements study: The macerated bark sample contains several distinct cell types. The most common cell type found in the bark was cork cells. These are polygonal in shape and have moderately thick walls with suberin deposition. Another main type was phloem fibres. These are elongated cells, with moderately thick lignified wall and a broad cell lumen. Fibres aretapered at both ends, featuring blunt tips. Among the phloem fibres a few septate structures were also observed. The average measurement of the phloem fibres was 785.23±20.36 µm in length and 36.59±2.36 µm in width. The frequency of fibres in the bark was 32.54±1.25 per mm^2. Sclereids are of the brachysclereid type, characterized by highly thickened, lignified cell walls and polygonal, isodiametric in shape. They have a length of 48.56±0.38 µm and a breadth of 45.27±0.15 µm (Figs. 2C-E).

Table 2. Moisture content and ash values of powdered leaf and bark drugs of the studied plant.

Powdered plant sample	Moisture content (%)	Total ash (%)	Water soluble ash (%)	Acid insoluble ash (%)
Leaf powder	9.0 ± 0.5	7.2 ± 0.2	3.21 ± 0.10	2.4 ± 0.06
Bark powder	7.0 ± 0.1	4.2 ± 0.21	3.35 ± 0.10	2.8 ± 0.06

Table 3. Extractive values of powdered leaf and bark drugs.

Plant parts	Extractive values (%)				
	Water	Methanol	Ethyl acetate	Chloroform	Hexane
Leaf	5.8 ± 0.08	5.1 ± 0.06	2.12 ± 0.04	1.01 ± 0.02	0.99 ± 0.05
Bark	6.5 ± 0.02	5.6 ± 0.03	2.25 ± 0.05	1.13 ± 0.06	0.94 ± 0.04

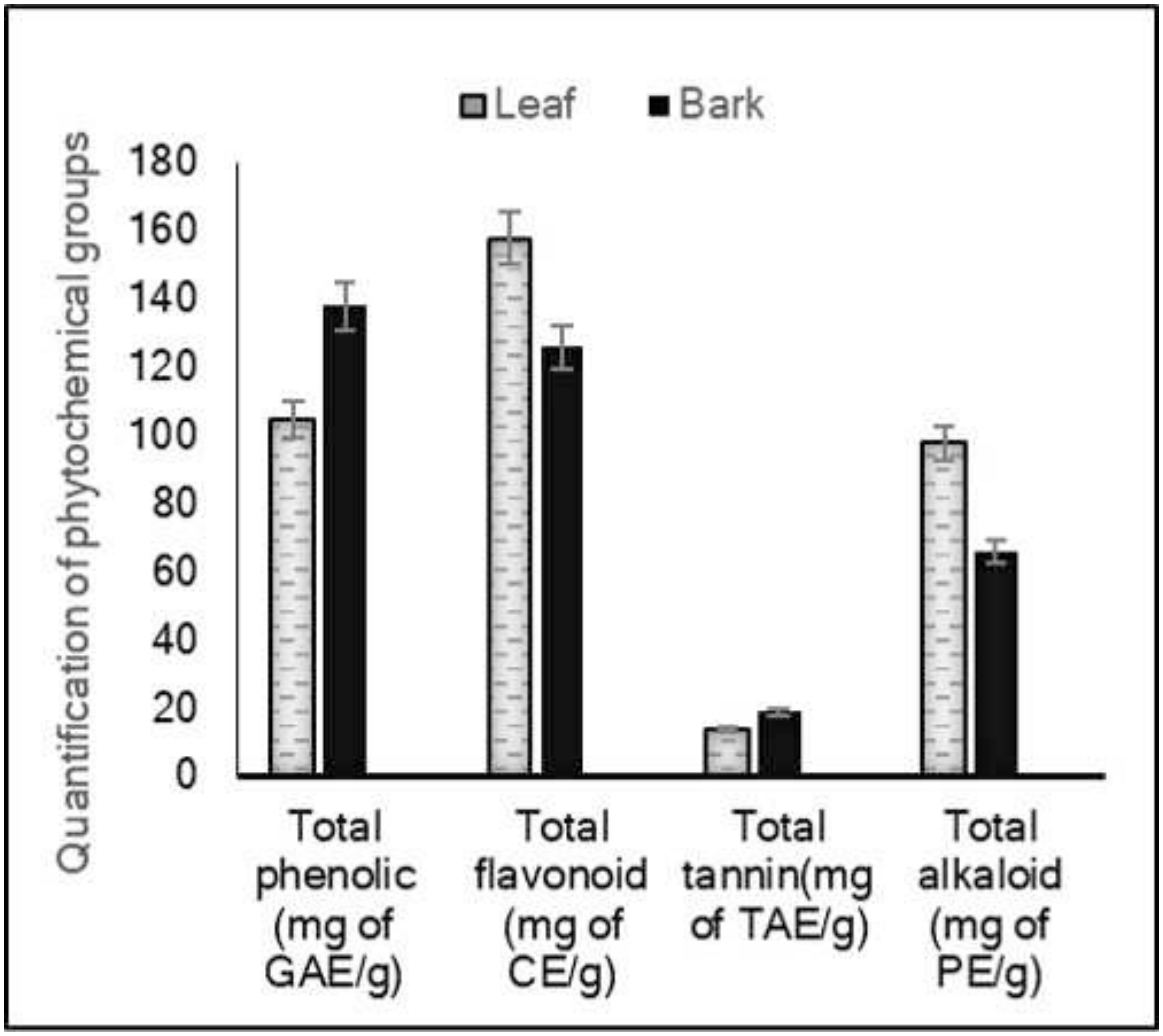


Fig. 4. Phytochemical profile of the methanolic extracts of leaf and bark.

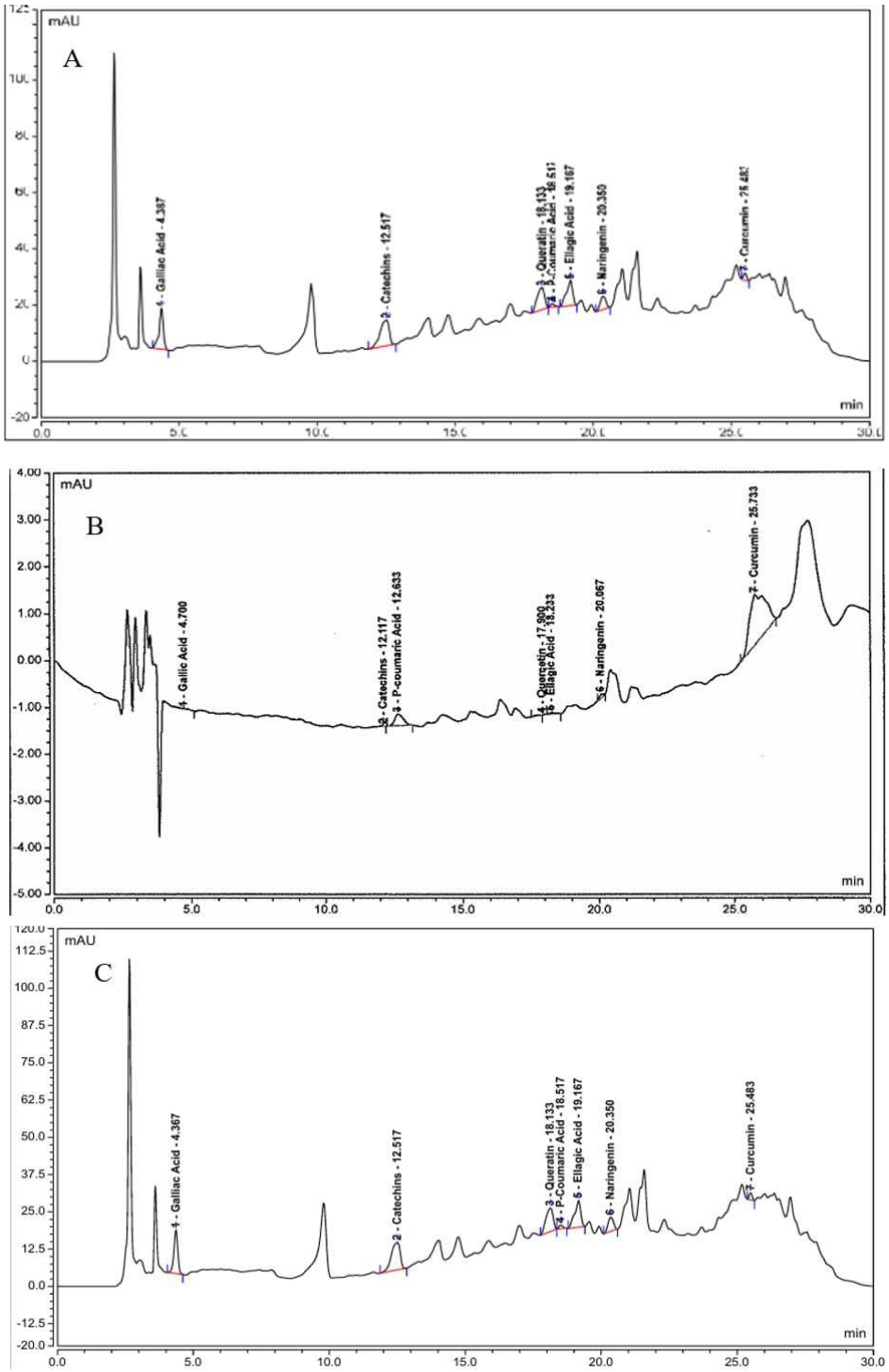


Fig. 5. HPLC chromatograms. **A.** Standards; **B.** Methanolic leaf extract; **C.** Methanolic bark extract.

Organoleptic study: The organoleptic features of powdered samples of leaf and bark of the investigated plant species has been tabulated in Table 1.

Physicochemical evaluation: The moisture content of the leaf powder was estimated to be 9.0 ± 0.5%, while bark powder had 7.0 ± 0.1% of moisture content. The total ash value for the leaf powder was 7.2 ± 0.2%, and in case of bark powder, it was 4.2 ± 0.21%. The acid-insoluble ash percentage was 2.4 ± 0.06% for the leaf powder and it was 2.8 ± 0.06% for the powdered sample of bark. The percentage of water-soluble ash was 3.21 ± 0.10% in case of leaf powdered sample and 3.35 ± 0.10% was the estimated value for the bark powder (Table 2).

Extractive value: The extractive value of a crude drug is significantly influenced by the type of solvent used. In the present investigation, for leaf and bark samples, water yielded the highest extractive value compared to other solvents like methanol, ethyl acetate, chloroform, and hexane (Table 3).

Phytochemistry

Preliminary phytochemical screening: To identify new sources of chemicals that hold both therapeutic and commercial significance, it is essential to conduct a phytochemical examination of medicinal plants. The initial chemical analysis of methanol extracts from leaves and bark has revealed the presence of therapeutically important chemical groups, including alkaloids, flavonoids, tannins, glycosides, reducing sugars. The anthraquinones and saponins were not detected in both leaf and bark extracts (Table 4).

Total phenolic content: The amounts of total phenolics estimated in the leaf and bark were 105.02± 0.08 and 138.02±0.08 mg of GAE/g tissue, respectively **(**Fig. 4).

Total flavonoid content: The total flavonoid content in the methanol extract of the leaf and bark was quantified to be 158.70 ± 0.77 and 126.78 ± 0.77 mg CE/g tissue, respectively **(**Fig. 4).

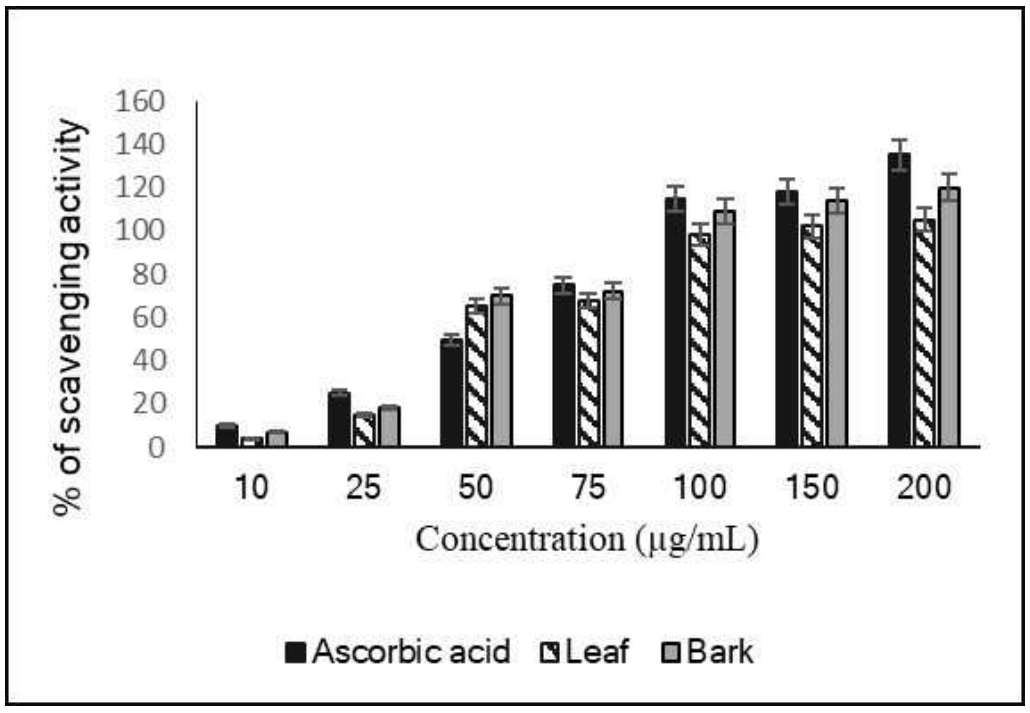


Fig. 6. DPPH radical scavenging activity of the methanolic leaf and bark extracts.

Table 4. Microchemical tests of leaf and bark methanolic extract of *X. xylocarpa.*

Chemical groups	Tests	Colour change	Leaf	Bark
Alkaloids	Dragendorff's reagent Wagner's reagent Mayers reagent	Cream Colour Orange brown Reddish brown	+++ +++ +	+++ +++ +
Reducing sugars	Fehlings reagent Benedict's reagent	Brick red Brick red	++ +	++ +
Steroids	Salkowaski test	Reddish blue green fluorescence	+	–
Anthraquinones	Bontrager's test	Upper red lower pink	–	–
Proteins	Lugol's reagent Millon's reagent	Faint yellow White	++ +	++ +
Saponins	1% Lead acetate solution	White	–	–
Amino acids	Ninhydrin solution	Violet colour	+	+
Lignin	Phloroglucinol+HCL	Red	++	++
Tannins	10% NH_4OH solution 10% lead acetate solution 5% $FeCl_3$ solution	Yellow fluorescence White Blackish green	++ + +	++ + +
Flavonoids	Shinoda test	Crimson red	+++	+
Glycosides	10% NaOH	White	+	+

Total tannin content: The tannin content in the leaf and bark extract was estimated to be 14.5± 0.78 and 19.25±0.36mg of TAE/g, respectively **(**Fig. 4).

Total alkaloid content: The leaf exhibited a higher alkaloid content of 98.42±1.08 mg PE/g, while the alkaloid content of the bark was estimated to be 66.38 ± 1.35 mg PE/g **(**Fig. 4).

HPLC analysis of leaf and bark extracts:

A total of seven phenolic and flavonoid compounds have been identified and their amount was quantified through HPLC analysis (Figs. 5A-C). Quercetin was the most abundant compound overall, with the highest concentration observed in the bark (69.3 mg/g dry tissue). In contrast, quercetin level in the leaf was much lower (3.92 mg/g dry tissue). Curcumin ranked second in abundance but its amount differs in leaf and bark. It was present in high amounts in the leaf extract (32.58 mg/g dry tissue), while only a small amount was detected in the bark (0.04 mg/g dry tissue).The amount of ellagic acid was also higher in the bark (4.79 mg/g dry tissue) compared to the leaf (0.58 mg/g dry tissue). Similarly, catechin concentration was greater in the bark (1.8 mg/g dry tissue) than in the leaf (0.08 mg/g dry tissue). p-Coumaric acid was detected at 1.15

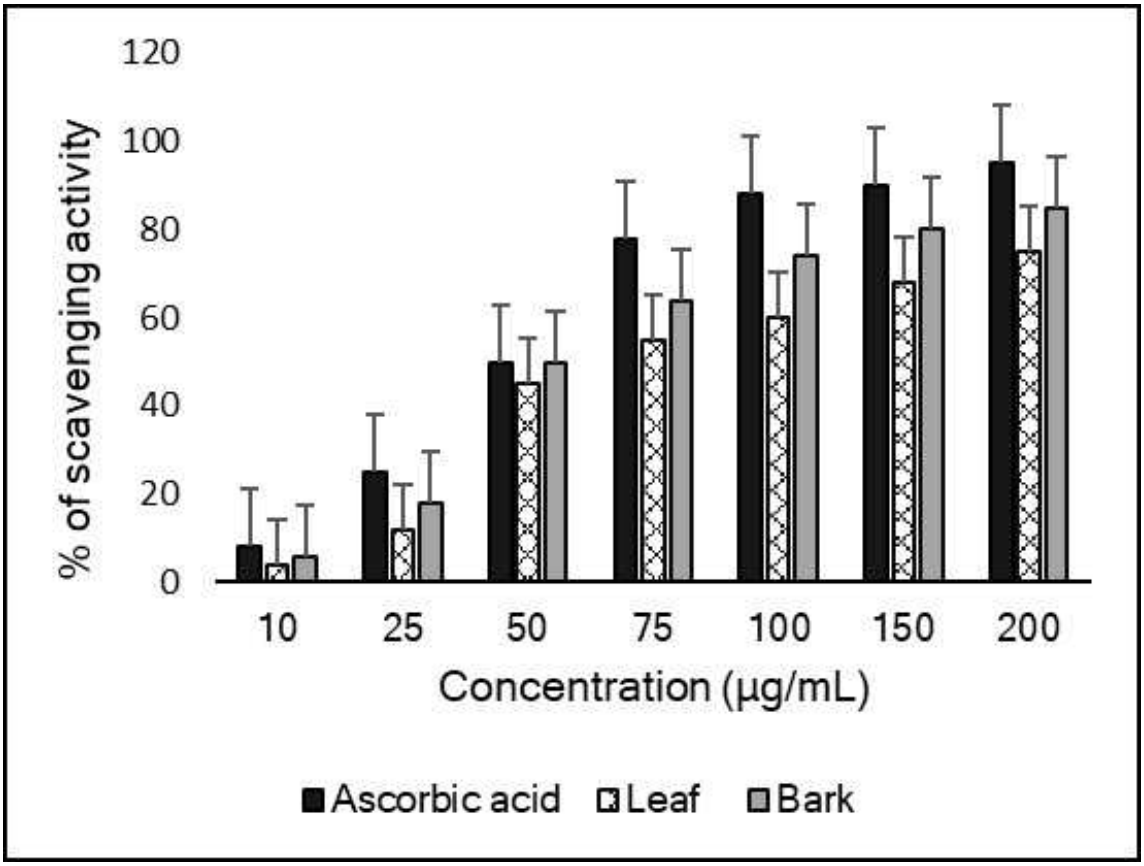


Fig. 7. ABTS radical scavenging activity of methanolic leaf and bark extracts.

mg/g dry tissue in the bark and it was 0.56 mg/g dry tissue in the leaf. Naringenin was found in both bark and leaf, with amounts of 1.13 mg/g dry tissue and 0.44 mg/g dry tissue, respectively. Gallic acid was the least abundant compound overall, with 0.76 mg/g dry tissue in the bark and 0.16 mg/g dry tissue in the leaf.These results indicate that the bark generally contains higher concentrations of compounds such as quercetin, ellagic acid, and catechin, suggesting its stronger therapeutic potential. Conversely, the leaf uniquely accumulates a significant amount of curcumin.

Antioxidant activity

DPPH radical scavenging activity: The reduction of DPPH radicals, indicated by decreased absorbance, was used to measure the free radical quenching ability of plant extracts. The leaf extract exhibited an IC_{50} value of 78.48±0.2 µg/mL, which is higher than the bark extract's IC_{50} value of 65.43±0.9 µg/mL. At a concentration of 100 µg/mL, the leaf extract's scavenging activity was 98.82±1.8%, lower than the bark extract's 109.23±2.6%, when compared to the reference compound ascorbic acid (Fig. 6).

ABTS radical scavenging activity: The scavenging activity of the leaf extract was found to be 60.17±0.16% at a concentration of 100 µg/mL, which is lower than the bark extract, which exhibited 74.13±0.48% scavenging activity at the same concentration. The IC_{50} values for the leaf and bark extracts were determined to be 103.41±0.10 µg/mL and 95.11±0.10 µg/mL, respectively. The ABTS radical scavenging activity of the bark methanolic extract was notably high. In this study, ascorbic acid was used as a standard for comparison (Fig.7).

DISCUSSION

In present investigation, an attempt has been made to ascertain the diagnostics characters of crude drugs obtained from leaf and bark parts of *X. xylocarpa* through pharmacognostic tools. The findings of this study encompass a range of pharmacognostic features, procured from the foliar micromorphology, leaf and bark anatomy, bark elements studies, powder drug microscopy, organoleptic features and physicochemical constants of the plant samples. Many of these characteristics are found distinct that will be instrumental for authentication of the leaf and bark drugs of this medicinal tree in their dried as well as fresh forms.

Foliar micromorphological investigation does have considerable pharmacognostic importance for distinguishing various leaf materials used as crude drug (Ray *et al*., 2018). In the present study, the stomatal index (SI) was 10.06 ± 0.63%, a value that is notably distinct from other taxa within the Fabaceae family. For example, the lower leaf epidermis of *Pisum sativum* has an SI value of 8.3% (Khandelwal and Sindhu, 2012), while *Bauhinia acuminata* exhibits an estimated SI value of 13.05 ± 0.1% (Sultana *et al*., 2023). The variation in stomatal indices among different Fabaceae species underscores the uniqueness of the stomatal index and also reinforces the use of stomatal index as a marker for identifying leaf drugs derived from *Xylia xylocarpa*. In the present study, the stomata are exclusively of paracytic type. This finding is consistent with previous reports, as the paracytic type is observed in many other taxa of the Fabaceae family (Metcalfe and Chalk, 1950).The palisade ratio, measured at 8.91 ± 0.31, serves as a highly distinctive characteristic of this medicinal plant as other species within the Fabaceae family exhibit different values such as in *Melilotus officinalis* the palisade ratio was 6.5 ± 0.58 (Sheikh *et al*., 2016).

The anatomical distinction of the petiole often plays a crucial role in identifying plant taxa. In our research, the petiole manifested a circular shape in cross-section, featuring a large central vascular bundle and six peripheral ones, which might be a trait specific to this species. By contrast, the petiole of *Derris malaccensis*, another species in the Fabaceae, has a planoconvex shape in cross-section with two lateral wings and an approximately circular continuous vascular cylinder (Metcalfe and Chalk, 1950). These distinctions underscore the value of petiole anatomy in differentiating the *X. xylocarpa* plant from other Fabaceae species. Additionally, trichome characteristics serve as marker for identifying leaf drugs. A broad range of structural variations in both glandular and non-glandular trichomes can be seen across different Fabaceae species (Albert and Sharma, 2013). In this study, multicellular glandular trichomes were detected on both surfaces of the leaf in the studied plant. The multicellular glandular trichomes are also found in many genera including *Xylia* genus of the family Leguminosae (Metcalfe and Chalk, 1950).

Physicochemical parameters are essential for the standardization and quality control of genuine herbal drugs, including loss on drying and ash content. Loss on drying is a widely used test procedure to determine the moisture content in powdered drug samples. Maintaining low moisture content in drugs is crucial to prevent the growth of bacteria and fungi during storage. In this study, the higher moisture content in leaves compared to bark suggests that the shelf-life of the leaf-based drug would be shorter than bark drug. The high moisture content in leaves leads to quicker degradation of its quality assurance compared to that of bark sample.

Ash value, a key physicochemical parameter, is utilized to evaluate the quality and purity of crude drugs. It offers a unique quantitative measure that is specific to each crude drug. This value adheres to an official standard set for the crude drug material. The total ash value reflects the presence of various impurities, primarily in the form of silicate matter from dirt and mud, as well as other mineral groups like carbonates and phosphates present in the cellsandsilica-substances present in cell walls of some plants. In our study, the total ash value of *Xylia xylocarpa* leaves was found to be 7.2 ± 0.2%, which is distinct from other members of Fabaceae family. For instance, *Cajanus scarabaeoides* leaves exhibit a higher ash value of 10.75% (Ray and Rahaman, 2018), while *Acacia catechu* leaves have a distinctly higher ash value of 12.75% (Trishala and Thangavelu, 2018).The total ash value of bark powder (4.2±0.21%)

was lower than the total ash value (7.2 ± 0.2%) of leaf part of the studied plant. This difference in percentage of total ash makes these two crude drugs distinguished from each other. The water-soluble ash, which is part of the total ash, indicates the quantity of inorganic salts like phosphates and carbonates present in the drug sample. These salts have been identified with various therapeutic properties such as anti-cancer, anti-obesity, protection against cardiovascular diseases, bone and dentin formation (Yu *et al*., 2020). In present study, content of water-soluble ash in leaf (3.21±0.10%) is slightly lower than the bark part (3.35±0.10%) which gives an impression about presence of significant amount of therapeutically important inorganic salts like phosphate and carbonate in both drug samples of this medicinal plant. Thus, in terms of the bioactivity of minerals, both bark and leaf drugs appear to be therapeutically effective. However, the therapeutic efficacy of a crude drug is not determined solely by its mineral content, but also influenced by various other bioactive compounds present within it. Like total ash, the value of acid insoluble ash also provides a very confirmatory character that helps in authentication and quality control of the herbal crude drug. The results of our study showed that acid insoluble ash of the leaf part is very distinct, i.e., 2.4±0.06% from the value of acid insoluble ash (5.5%) of *Bauhinia variegata* leaf (Khare *et al*., 2017). The quantitative differences in ash content at all three levels i.e., total ash, acid-insoluble ash, and water-soluble ash makes *X. xylocarpa* plant distinctive among other studied species of Fabaceae.

Extractive value refers to the amount ofchemical constituents extracted out in a given amount of plant material with a particular solvent. The extraction of any crude drug with a particular solvent yields a fixed amount of different phytoconstituents. The compositions of these chemical constituents in the extract depend mainly upon the solvent used and also upon the nature of crude drug. It also provides an indication whether the crude drug is exhausted or not. Here in our study, water solvent showed the greatest extractive value for both leaf and bark parts i.e., 5.8±0.08% and 6.5±0.02%, respectively among other four solvents used. Such trends in extractive values are noticed in many previous works where highest extractive values of crude drugs had been recorded in water (Tatiya *et al*., 2012). The specific quantitative scores of extractive values in different solvents are also considered one of the diagnostic features used for raw drug sample identification. Here bark exhibited greater extractive value than the leaf sample in case of all five solvents used except hexane, where leaf exhibited higher extractive value (0.99±0.05%) than the bark (0.94±0.04%). Such difference in extractive value highlights the leaf and bark samples very unique in respect of variation in phytochemicals, and provides the diagnostic attributes useful for identification of these two drug samples of the studied medicinal plant.
Preliminary phytochemical screening helps in predicting the chemical nature of crude drugs and detecting the chemical constituents present in them (Pal and Rahaman, 2014). In the present study, important phytochemical groups were identified in both leaf and bark extracts, including alkaloids, flavonoids, tannins, steroids, glycosides, and others. The detection of therapeutically significant chemical groups in the bark and leaf of this plant indicates their various medicinal properties.

From the quantitative analysis it is evident that both the drug parts of *X. xylocarpa* contained good amounts of phenolics (105.02±0.08 to 138.02±0.08 mg GAE/g), and flavonoids (158.70±0.77 to 126.78±0.77 mg CE/g), which highlight the medicinal properties of this plant used as an antioxidant and anti-inflammatory agent. Phenolic contents of both leaf and bark parts are nearly equal or in some cases higher than the phenolic contents of some common fruits like grapes (126-195 mg GAE/g), cherry (94.3 mg GAE/g), and pear (69.2 mg GAE/g) (Wu *et al*., 2004; Brat *et al*., 2006). It is also well established that phenolics, flavonoids and tannins are very much effective against various types of inflammatory conditions, diabetes, cancer, wounds and body pain (Tungmunnithum *et al*., 2018; Das *et al*., 2020; Mutha *et al*., 2021). Presence of good amount of these therapeutically potent compounds also elucidates the future prospective of this plant to be an effective antioxidant and anti-inflammatory drug source.

The HPLC analysis of the studied plant's leaf and bark extracts revealed significant concentrations of the bioactive compounds, including three flavonoids

(catechin, naringenin, quercetin) and four compounds of phenolics (p-coumaric acid, curcumin, ellagic acid, gallic acid). These compounds are known for their diverse biological activities, such as antioxidant, anti-inflammatory, cardioprotective, antiviral, and anticarcinogenic properties. The high levels of curcumin (32.58 mg/g dry tissue in leaf) and quercetin (69.3 mg/g dry tissue in bark) suggest potent antioxidant and anti-inflammatory potential of this studied medicinal plant. Several earlier studies have demonstrated that curcumin can effectively neutralize reactive oxygen species (ROS) and inhibits pro-inflammatory enzymes such as cyclooxygenase-2 (COX-2) and nuclear factor-kappa B (NF-κB) (Guo *et al*., 2020). The quercetin, a flavonoid found in many fruits and vegetables, is reported for its ability to scavenge free radicals and reduce oxidative stress (Alharbi *et al*., 2025). The contents of ellagic acid (0.58 mg/g dry tissue in leaf, 4.79 mg/g dry tissue in bark) and gallic acid (0.16 mg/g dry tissue in leaf, 0.76 mg/g dry tissue in bark) further support the plant's anti-microbial and anticarcinogenic properties. The experimental studies evidenced that ellagic acid significantly inhibited the cancer cell proliferation and gallic acid showed antitumor and antimicrobial activities (Malik *et al*., 2011; Keyvani-Ghamsari *et al*., 2023). The presence of p-coumaric acid (0.56 mg/g dry tissue in leaf, 1.15 mg/g dry tissue in bark) and naringenin (0.44 mg/g dry tissue in leaf, 1.13 mg/g dry tissue in bark) highlights additional health benefits, including cardio protection and antiviral activity (Roy and Prince, 2013; Tutunchi *et al*., 2020). The catechin, a flavonoid with significant antioxidant, anti-inflammatory, antidiabetic properties, was also detected in the leaf and bark parts (0.08 mg/g dry tissue in leaf, 1.8 mg/g dry tissue in bark) that further elucidates the plant's varied therapeutic promise including as antidiabetic agent (Grzesik *et al*., 2018). These findings to some extent validate the rationale of the plant's traditional medicinal uses by elucidating the presence of considerable amounts of seven therapeutically potent phenolic and flavonoid molecules and suggest its potential for treating wide range of ailments.

Here in this study, antioxidant potentials of the methanol extracts of leaf and bark parts were assessed using the DPPH and ABTS methods and their free radical scavenging activity was estimated in terms of IC_{50} values. The IC_{50} values were 78.48±0.2 µg/mL for leaf and 65.43±0.9 µg/mL for bark in the DPPH assay. In the ABTS assay, the estimated IC_{50} values for bark and leaf part were 95.11±0.10 µg/mL and 103.41±0.10µg/mL, respectively. In both antioxidant assays, the leaf was estimated to have a higher IC_{50} value than bark part of the investigated plant. It is due to presence of more amounts of phenolics in the bark (138.02± 0.08 mgof GAE/g tissue) than leaf part (105.02±0.08 mg of GAE/g tissue) which further indicates a positive correlation between the antioxidant activity of plant extract and its total phenolic content. The antioxidant property of phenolics is related to their redox properties. Presence of hydroxyl groups in phenolics allows them to act as reducing agents, metal chelators, and single oxygen quencher. The phenolic content of leaf and bark of the *X. xylocarpa* was found to be notably higher compared to several other medicinal plants. For instance, the phenolic content of the fruit of *Phyllanthus emblica* is reported to be 81.5 mg of GAE/g (Ali *et al*., 2018), while the phenolic content of *Zingiber officinale* rhizome was reported to be 60.34 mg of GAE/g (Yadav *et al*., 2015). Similarly, the bark of *Saraca asoca* and leaf of *Andrographis paniculata* have phenolic contents of 55 mg (Liu *et al*., 2008) and 58.78 mg of GAE/g (Sani *et al*., 2016), respectively. These comparisons suggest that the leaf of studied plant is a rich source of phenolic compounds, which may contribute to its superior antioxidant activity (Chen *et al*., 2020). Given these findings, *X. xylocarpa* appears to be a promising and accessible resource for natural antioxidants in the pharmaceutical sector. Therefore, it is crucial to conduct further scientific research on both the leaf and stem bark to identify and standardize their potent antioxidant compounds. Moreover, the pharmacognostic characteristics identified in this study will serve as key markers for accurately identifying crude drugs derived from the leaf and bark of *X. xylocarpa.*

CONCLUSION

The novelty of present study is marked by the comprehensive pharmacognostic investigation of leaf and bark parts of *Xylia xylocarpa*, which has not been reported earlier. This study explored certain

pharmacognostic standards that are highly diagnostic for the authentication and quality control of crude drugs derived from leaf and bark of the investigated plant. The FE-SEM analysis was employed here for the first time, to examine the foliar micromorphology and vegetative anatomy of this species, revealing some distinctive structural features. Moreover, pharmacognostic characters of the crude drugs will enrich the database of the studied medicinal plant's pharmacopoeia.

Phytochemical analysis of *X. xylocarpa* plant has shown that both its bark and leaf contain substantial amounts of therapeutically important chemical groups, including phenolics, flavonoids, tannins, and alkaloids. Earlier chemical studies were limited to qualitative and quantitative phytochemical screening of the leaf only, where no advanced analytical technique has been used. However, the present study advances this work by conducting HPLC analysis of both leaf and bark parts, providing novel insights into their chemical composition. The HPLC profiling of *X. xylocarpa* leaf and bark extracts revealed presence of seven bioactive phenolic and flavonoid compounds in substantial amounts, particularly curcumin and quercetin. The notable antioxidant activity of both the stem bark and leaf correlates with their considerable content of phenolics and flavonoids. The strong antioxidant, anti-inflammatory, antimicrobial, and anticancer properties attributed to these compounds provide scientific support for the traditional therapeutic uses of this plant species. These findings not only validate its ethnomedicinal uses but also indicate its potential for further development into plant-based formulations targeting oxidative stress-related and inflammatory disorders.

Collectively, these findings make a significant contribution to the pharmacognostic and phytochemical profiling of this species. Ultimately, this study has identified several promising avenues for future research into the chemical and pharmacological profiles of *X. xylocarpa*.

ACKNOWLEDGEMENT

Grateful acknowledgment is extended to the Department of Botany, Visva-Bharati (sponsored by UGC-DRS-SAP and DST-FIST), for providing the essential laboratory facilities.

DISCLAIMER

The author(s) declare no conflict of interest in the work.

REFERENCES

Afify, A.M., El-Beltagi, H.S., El-Salam, S.M. and Omran, A.A. 2012. Biochemical changes in phenols, flavonoids, tannins, Vitamin E, beta carotene and antioxidant activity during soaking of three white Sorghum varieties. *Asian Pac. J. Trop. Biomed.,* **2**(3): 203-209.

Albert, S. and Sharma, B. 2013. Comparative foliar micromorphological studies of some *Bauhinia* (Leguminosae) species. *Turk. J. Bot.,* **37**: 276-281.

Alharbi, H.O.A., Alshebremi, M., Babiker, A.Y. and Rahmani, A.H. 2025. The Role of Quercetin, a Flavonoid in the Management of Pathogenesis Through Regulation of Oxidative Stress, Inflammation, and Biological Activities. *Biomolecules,* **15**(1): 151.

Ali, M., Khan, T., Fatima, K. *et al.* 2018. Selected hepatoprotective herbal medicines: Evidence from ethnomedicinal applications, animal models, and possible mechanism of actions. *Phytother Res.* **32**(2):199-215.

Bokhari, M.H. 1970. Morphology and taxonomic significance of foliar sclereids in Limonium. *Notes Roy Bot. Gard Edinburgh,* **30**: 43-53.

Brat, P., George, S. and Bellamy, A. 2006. Daily polyphenol intake in France from fruit and vegetables. *J. Nutr.,* **136**(9): 2368-2373.

Chen, J., Yang, J. and Ma, L. 2020. Structure-antioxidant activity relationship of methoxy, phenolic hydroxyl, and carboxylic acid groups of phenolic acids. *Sci. Rep.,* **10**(1): 2611.

Chowdhury, K.H., Chowdhury, R. and Hasan, M. 2021. *Xylia xylocarpa* (Roxb.) Taub. leaves ameliorates inflammation and pain in experimental mice and computer-Aided Model. *Walailak J. Sci. Tech.,* **18**(15): 1-13.

Das, A.K., Islam, M.N., Faruk, M.O., Ashaduzzaman, M. and Dungani, R. 2020. Review on tannins:

Extraction processes, applications and possibilities. *South Afr. J. Bot.,* **135**: 58-70.

Evans, W.C. 2008. *Pharmacognosy*. Singapore: Saunders Comp. Ltd. (Elsevier), pp.21-132.

Flora of Peninsular India, Centre for Ecological Sciences (CES), Indian Institute of Science (IISc), Bangalore - 560012. https://indiaflora-ces.iisc.ac.in/FloraPeninsular/contact.php.

Grzesik, M., Naparło, K., Bartosz, G. and Sadowska-Bartosz, I. 2018. Antioxidant properties of catechins: Comparison with other antioxidants. *Food Chem.,* 241: 480-492.

Guo, J., Cao, X., Hu, X., Li, S. and Wang, J. 2020. The anti-apoptotic, antioxidant and anti-inflammatory effects of curcumin on acrylamide-induced neurotoxicity in rats. *BMC Pharmacol. Toxicol.,* **21**: 1-10.

Harborne, J.B. and Williams, C.A. 1994. Recent advances in the chemosystematics of the monocotyledons. *Phytochem.,* **37**(1): 3-18.

Haridas, R., Sharma, D., Singh, D. *et al*. 2024. Pharmacognostical Evaluation of Selected Seeds from Fabaceae Family. *Pharmacogn. Res.,* **16**(3): 508-517.

Johansen, D.A. 1940. *Plant Microtechnique*. New York, USA: McGraw-Hill, pp. 523-538.

Junsongduang, A., Saensouk, S. and Balslev, H. 2025. Amnat Charoen healers in Thailand and their medicinal plants. *Plants,* **14**(4): 602.

Karadag, A., Ozcelik, B. and Saner, S. 2009. Review of methods to determine antioxidant capacities. *Food Anal. Methods,* **2**(1): 41-60.

Keyvani-Ghamsari, S., Rahimi, M. and Khorsandi, K. 2023. An update on the potential mechanism of gallic acid as an antibacterial and anticancer agent. *Food Sci. Nutr.,* **11**(10): 5856-5872.

Khandelwal, A. and Sindhu, S.S. 2012. Expression of 1-aminocyclopropane-1-carboxylate deaminase in rhizobia promotes nodulation and plant growth of clusterbean (*Cyamopsis tetragonoloba* L.). *Res. J. Microbiol.,* **7**(3): 158.

Khare, P., Kishore, K. and Sharma, D.K. 2017. A study on the standardization parameters of *Bauhinia variegata. Asian J. Pharm. Clin. Res.,* **10**(4): 133-136.

Kirtikar, K.R. and Basu, B.D. 1991. *Indian Medicinal Plants*. Vol 2. Delhi, India: Periodical Experts Book Agency, pp. 905-906.

Lam, L.M.T., Nguyen, M.T.T., Nguyen, H.X. *et al.* 2016. Anti-cholinesterases and memory improving effects of Vietnamese *Xylia xylocarpa*. *Chem. Cent. J.,* **10**: 1-10.

Liu, X., Dong, M., Chen, X., Jiang, M., Lv, X. and Zhou, J. 2008. Antimicrobial activity of an endophytic *Xylaria* sp. YX-28 and identification of its antimicrobial compound 7-amino-4-methylcoumarin. *Appl. Microbiol. Biotechnol.,* **78**: 241-247.

Malik, A., Afaq, S., Shahid, M., Akhtar, K. and Assiri, A. 2011. Influence of ellagic acid on prostate cancer cell proliferation: A caspase-dependent pathway. *Asian Pac. J. Trop. Med.,* **4**(7): 550-555.

Manimegalai, S. and Prithiba, A. 2021. Qualitative and Quantitative Phytochemical Screening of Leaves of *Xylia xylocarpa* (Fabaceae). *Int. J. Innov. Re. Multidiscip. Field,* **7**: 241-245.

Metcalfe, C.R. and Chalk, L. 1950. *Anatomy of the Dicotyledons.* Vol 1. Oxford, UK: Clarendon Press, pp.1002-1011.

Mutha, R.E., Tatiya, A.U. and Surana, S.J. 2021. Flavonoids as natural phenolic compounds and their role in therapeutics: An overview. *Future J. Pharm. Sci.,* **7**: 1-13.

Murti, Y., Yogi, B. and Pathak, D. 2010. Pharmacognostic standardization of leaves of *Calotropis procera* (Ait.) R. Br. (Asclepiadaceae). *Int. J. Ayurveda Res.,* **1**(1): 14-17.

Pal, K. and Rahaman, C.H. 2014. Studies on foliar epidermal micromorphology, vegetative anatomy and xylem elements of four members of Portulacaceae. *Int. J. Curr. Res.,* **6**(02): 4968-4975.

Ray, A.S., Mandal, S.K. and Rahaman, C.H. 2018. Pharmacognostical fingerprinting and selective bioactivity studies of *Solanum glaucophyllum* Desf. *J. Trad. Folk Pract.,* **6**(1): 3-25.

Ray, A.S. and Rahaman, C.H. 2018. Pharmacognostic standardization and phytochemical investigation of *Cajanus scarabaeoides* (L.) Thouars. *Res. J. Pharmacogn. Phytochem.,* **10**(1): 120-131.

Roy, A.J. and Prince, P.S.M. 2013. Preventive effects of p-coumaric acid on cardiac hypertrophy and

alterations in electrocardiogram, lipids, and lipoproteins in experimentally induced myocardial infarcted rats. *Food Chem. Toxicol.,* **60**: 348-354.

Saha, S. and Rahaman, C.H. 2013. Pharmacognostic and anatomical studies of *Antigonon leptopus* Hook. and Arn.: A promising medicinal climber. *Int. J. Ayurveda Pharma. Res.,* **4**(2): 186-191.

Sani, M.H., Ibrahim, G., Danmalam, U.H., Muhammad, Z.A. and Kachallah, M. 2016. Phytochemical study and antibacterial properties of the leaf extracts of *Swartzia madagascariensis* Desv (Fabaceae). *Br. Microbiol. Res. J.,* **11**(6): 1-6.

Saxena, O.H. and Brahmam, M. 1994. *The flora of Orissa*. Vol. 1 (Ranunculaceae to Fabaceae). Bhubaneswar, India: Regional Research Laboratory and Orissa Forest Department Corporation Limited, pp. 435-436.

Sheikh, N.A., Desai, T.R. and Patel, R.D. 2016. Pharmacognostic Evaluation of *Melilotus officinalis* Linn. *J. Pharmacog.,* **8**(3): 239-242.

Singh, D.K., Srivastava, B. and Sahu, A. 2004. Spectrophotometric determination of *Rauwolfia* alkaloids: estimation of reserpine in pharmaceuticals. *Anal. Sci.,* **20**(3): 571-573.

Singh, A., Saharan, V.A. and Bhandari, A. 2014. Pharmacognostic standardization with various plant parts of *Desmostachya bipinnata*. *Pharm. Biol.,* **52**(3): 298-307.

Sultana, Z., Begum, S.N. and Rahaman, C.H. 2023. Pharmacognostic study and evaluation of antioxidant property of *Bauhinia acuminate* L.– an important folk medicinal plant. *J. Bot. Soc. Bengal,* **77**(1): 9-24.

Tatiya, A., Surana, S., Bhavsar, S., Patil, D. and Patil, Y. 2012. Pharmacognostic and preliminary phytochemical investigation of *Eulophia herbacea* Lindl. tubers (Orchidaceae). *Asian Pac. J. Trop. Dis.,* **2**: S50-S55.

Thaipong, K.U., Boonprakob, K., Crosby, L., Cisneros, Z. and Byrne, D.H. 2006. Comparison of ABTS, DPPH, FRAP and ORAC assays for estimating antioxidant activity from guava fruit extracts. *J. Food Comp. Anal.,* **19**: 669-675.

Tungmunnithum, D., Thongboonyou, A., Pholboon, A. and Yangsabai, A. 2018. Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. *Medicines,* **5**(3): 93.

Tutunchi, H., Naeini, F., Ostadrahimi, A. and Hosseinzadeh-Attar, M.J. 2020. Naringenin, a flavanone with antiviral and anti-inflammatory effects: A promising treatment strategy against COVID-19. *Phytother. Res.,* **34**(12): 3137-3147.

Wu, X., Beecher, G.R., Holden, J.M., Haytowitz, D.B., Gebhardt, S.E. and Prior, R.L. 2004. Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *J. Agric. Food Chem.,* **52**(12): 4026-4037.

World Health Organization. 1998. Quality control methods for medicinal plant materials. WHO, Geneva. http://www.who.int/iris/handle/10665/41986.

Yadav, G. and Ganguly, S. 2015. Structure activity relationship (SAR) study of benzimidazole scaffold for different biological activities: A mini-review. *Eur. J. Med. Chem.,* **97**: 419-443.

Yuan, J., Wang, X., Zhou, H. *et al.*, 2020. Comparison of sample preparation techniques for inspection of leaf epidermises using light microscopy and scanning electronic microscopy. *Front Plant Sci.,* **11**: 492104.

Zhishen, J., Mengcheng, T. and Jianming, W. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem.,* **64**(4): 555-559.

J. Botan. Soc. Bengal 79(1) : 101-109 (2025), ISSN 0971-2976

FULL LENGTH ARTICLE

Pollen analysis of *Apis florea* F. honey from plains of West Bengal, India

Amit Kumar Gayen[1,2], Shreyasi Basak[3] and Narayan Ghorai[1]*

[1]Department of Zoology, West Bengal State University, Berunanpukuria, Malikapur, Barasat, North 24 Parganas, Kolkata 700126.

[2]Department of Zoology, Vidyanagar College, South 24 Parganas, Pin 743503.

[3]Centre of Advanced Studies, Department of Botany, University of Calcutta, 35 B.C. Road, Kolkata-700019, India.

Received : 07.05.2025 Accepted : 30.05.2025 Published : 30.06.2025

Palynological analysis of six *Apis florea* F. honey samples collected from the three districts of West Bengal (Purulia, Birbhum and North 24 Parganas) during the period of 2021 to 2023 was carried out to assess the botanical origin of honey. A total of 19 pollen types belonging to 11 families were recovered in these six samples, of which four were found to be unifloral and two multifloral. In terms of frequency, Myrtaceae represents the major pollen family in Purulia; Apiaceae represents the major pollen family in Birbhum; Brassicaceae and Apiaceae represent the major pollen family in North 24 Parganas. In multifloral honey samples Myrtaceae and Apiaceae were found to have the highest frequency. The present study indicates that West Bengal is a suitable place for beekeeping ventures especially for *A. florea*, because of its high plant diversity. Present study also indicates that the *A. florea* is a good pollinator for wide range of agricultural crops.

Keywords: Melissopalynology, Pollination behavior, *Apis florea*, West Bengal.

INTRODUCTION

Apis florea F. is an economically important, smallest, wild bee out of 11 species of the genus *Apis* which constructs a single, open, aerial nest. *A. florea* is distributed in a wide range of habitats, such as, tropical rainforests, subtropical steppes, savannas and semi-deserts climate in and around South East Asia and Africa (Hepburn and Hepburn, 2005). Honeybees are well known for their pollination services. Like other *Apis* species, *A. florea* is a very important pollinator for various plant species. Very little work was carried out on this dwarf honey bee and the local people also ignore this small bee species for their small size, low quantity of honey production etc., but the advantage of their small size is that they pollinate enormous number of flowers within a short period (Abrol, 2020).

Melissopalynological investigations for determining the pollen and nectar source of a particular area have been practiced in different states of India. Including Andhra Pradesh (Jhansi *et al.*, 1991; Ramanujam and Khatija, 1992; Ramanujam and Kalpana, 1992; Lakshmi and Suryanarayana 2004), Maharashtra (Deodikar and Thakar, 1953; Bhusari *et al.*, 2005),

**Corresponding author :* nghorai@gmail.com

Karnataka (Bhargava *et al*., 2009; Sivaram *et al*., 2012), Himachal Pradesh (Attri, 2010), Bihar (Suryanarayana *et al*., 1992), Assam (Bera *et al.*, 2007; Tripathi *et al.*, 2017), Uttar Pradesh (Chauhan and Trivedi, 2011; Sahney *et al.,* 2016, 2018; Shukla and Rao, 2021), Haryana (Chaudhary, 2003), Orissa (Upadhyay and Bera, 2012), Uttarakhand (Dixit *et al*., 2013) and Hyderabad (Kalpana *et al*., 1990).

In West Bengal, earlier melissopalynological studies have been done in the Sundarbans (Kamble *et al*., 2013), Baruipur, South 24 Parganas (Naiya *et al.*, 2016), Salt Lake City (Bhattacharya *et al*., 1983), Murshidabad (Jana *et al.*, 2002), Nadia (Bhattacharya, 2014), Bankura and Paschim Mednipur (Layek and Karmakar, 2016), Howrah (Chakraborty *et al.*; 2023) and Northern districts of West Bengal (Mukhopadhyay *et al*., 2007; Saha *et al.*, 2023; Basak *et al*., 2025).

Melissopalynological works on *A. florea* honey are sparsely done in India, such as in south-western Satpura, Maharashtra (Mahajan *et al.*, 2000), Andhra Pradesh (Ramanujam and Kalpana, 1992). Recently, Rateb *et al.* (2020) identified the foraging plants and pollen spectrum of *A. florea* in Jordan. In West Bengal Layek *et al.* (2015) studied the foraging behavior of *A. florea* in Bankura and Paschim Medinipur districts during winter and spring-summer seasons. Moreover, the foraging activity of *A. florea* and their nesting parameter was studied by Gayen and Ghorai (2023) in West Bengal.

According to local people number of *A. florea* colony is declining day by day due to various stresses like loss of habitat for anthropogenic activity, heavy uses of pesticides, climate changes and other factors. Thus the species needs to be conserved. The present study is designed to provide basic information on pollen and nectar sources of *A. florea* in different parts of West Bengal plains, India.

MATERIALS AND METHODS

Among the six collected samples, two were collected from Purulia district (23.0254ºN, 86.5938ºE), two from Birbhum district (23.7613ºN, 87.6619ºE) and two from North 24 Parganas district (22.6003ºN, 88.4832ºE) West Bengal. Honey samples were collected during the period of December, 2021 to September, 2023. Honey bearing portion of the wild *A. florea* hive was collected carefully, squeezed and stored in a clean container for further analysis. In laboratory, 1 ml pure, natural honey sample was dissolved in 10 ml of distilled water, mixed properly and centrifuged. Sediment was collected and mixed with 5 ml glacial acetic acid. After that glacial acetic acid was decanted and the treated sample was acetolysed following Erdtman (1960) with a few modifications and finally the recovered pollen pellets were stored in 25% glycerin. Pollen grains were then mounted in glycerin jelly, examined and photographed using Zeiss Axioskop 40. Reference pollen slides of local flora and relevant literatures were used for pollen identification as far as possible. Unidentified pollen types were designated as 'unknown'. For frequency analysis approximately 300 pollen grains were counted for each sample. Four pollen frequency groups were suggested by "International Commission for Bee Botany" (Louveaux *et al.*, 1978) viz., predominant pollen type (>45%), secondary pollen type (16-45%), important minor pollen type (3-15%) and minor pollen type (<3%). The absolute pollen count (APC) of each honey sample was done following the protocol recommended by Suryanarayana *et al.*, 1981. The honey dew elements (fungal spores, algal filaments etc.) were identified from unacetolysed honey samples.

RESULTS AND DISCUSSION

A total of 19 different pollen taxa were recovered of which 16 belongs to 11 families and remaining 3 pollen taxa represented as 'Unidentified'. Out of six samples four samples are found to be unifloral (P H-2, B H-1, N H-1, N H-2) and two multifloral (P H-1, B H-2). Pollen analytical data of the six samples are shown in Table 1 and Table 2.

P H-1: The sample was collected from Bhutadi tribal village of Purulia district. It was multifloral honey with secondary pollen types Myrtaceae (>30%), Apiaceae (>30%) and Rhamnaceae (15-20%). Important minor pollen types Asteraceae (5-10%) and

Table 1. List of natural honey samples from six study areas

*Sample number	Spot of collection	Date of collection	Number of pollen type	h-index	Honey type	*HDE/P
P H-1	BHUTADI	20/03/2022	10	2.00	Multifloral	0.005
P H-2	MANBAZAR 2	16/02/2023	6	1.44	Unifloral	0.008
B H-1	KAMARDANGA	08/04/2023	10	1.38	Unifloral	0.01
B H-2	KASHBA	29/07/2023	11	2.11	Multifloral	0.04
N H-1	NEWTOWN	18/09/2023	7	1.38	Unifloral	0.006
N H-2	SANDESHKHALI	14/12/2021	9	1.40	Unifloral	0.03

(*Note: P.H.- Purulia Honey; B.H.- Birbhum Honey; N.H.- North 24-pgs Honey; HDE/P- Honeydew elements / total number of pollen grains)

Ulmaceae (5-10%) were also found in this sample.

P H-2: The sample was collected from Manbazar-2 of Purulia district. It was unifloral honey with predominant pollen types Myrtaceae (>70%) followed by important minor pollen types Asteraceae (10-15%) and Brassicaceae (5-10%) and minor pollen types Cannabaceae (<3 %).

B H-1: The sample was collected from Kamardanga tribal village of Birbhum district. It was unifloral honey with predominant pollen types Apiaceae (>70%), important minor pollen types Myrtaceae (3-5%), Arecaceae (3-5%) and minor pollen types Asteraceae (<3 %), Poaceae (<3 %).

B H-2: The sample was collected from Kashba tribal village of Birbhum district. It was multifloral honey with secondary pollen types Apiaceae (>30%) and Rubiaceae (15-20%) followed by important minor pollen types Asteraceae (3-5%), Arecaceae (5-10%), Myrtaceae (5-10%), Brassicaceae (5-10%) and minor pollen types Ulmaceae (<3%).

N H-1: The sample was collected from Newtown of North 24 Parganas district. It was unifloral honey with predominant pollen types Apiaceae (>50%). Secondary pollen types Arecaceae (15-20%), Brassicaceae (15-20%) and minor pollen types Asteraceae (<3%), Poaceae (<3%), Acanthaceae (<3%) were also found in this sample.

N H-2: The sample was collected from Sandeshkhali of North 24 Parganas district. It was unifloral honey with predominant pollen types Brassicaceae (>55 %) followed by secondary pollen types Apiaceae (>25 %), important minor pollen types Myrtaceae (5-10 %), minor pollen types Asteraceae (<3 %) and Poaceae (<3%).

Pollen diversity indicates the floral makeup of the particular area (Fig. 1 and Fig. 2). In the study area, the following nectariferous plants like *Syzygium cumini* (Myrtaceae), *Tridax procumbens* (Asteraceae), *Brassica juncea* (Brassicaceae) etc. were reported. Some of these nectariferous plants were already established as foraging plant of *A. florea* (Layek *et al*., 2015; Layek and Karmakar 2016). The absence of any toxic pollen grains and low HDE/P of honey samples indicates that the honey samples are of good quality and safe for human consumption (Table 1). The present study also represents the local foraging plant species of *A. florea* in the study area.

Bhattacharya (2014) reported that bees preferred weed for collection of nectar or pollen or both. This study

Table 2. Different parameters of collected honey samples from six study areas

Sl. No.	Sample Code	**APC/ml	Predominant pollen type (>45%)	Secondary pollen type (16-44%)	Important minor pollen type (3-15%)	Minor pollen type (1-<3%)
1.	PH-1	326200	–	Myrtaceae, Apiaceae and Rhamnaceae	Asteraceae and Ulmaceae	–
2.	PH-2	114600	Myrtaceae	–	Asteraceae and Brassicaceae	Cannabaceae
3.	BH-1	78000	Apiaceae	–	Myrtaceae and Arecaceae	Asteraceae and Poaceae
4.	BH-2	94000	–	Apiaceae and Rubiaceae	Asteraceae, Arecaceae, Myrtaceae and Brassicaceae	Ulmaceae
5.	NH-1	137000	Apiaceae	Arecaceae and Brassicaceae	–	Asteraceae, Poaceae and Acanthaceae
6.	NH-2	98000	Brassicaceae	Apiaceae	Myrtaceae	Asteraceae and Poaceae

(**APC - Absolute Pollen Count)

also noted that *A. florea* prefers to forage on *Parthenium* sp. This ultimately hinders the pollination of target crop (Bhattacharya, 2014). This is also corroborated by the present study. The present study shows that *Tridax procumbens* (Asteraceae) and *Phoenix sylvestris* (Arecaceae) were the predominant nectar and pollen source of *A. florea* which is also supported by the findings of Kalpana *et al*. (1990). The present study also mentioned that *Brassica* sp. (Brassicaceae) is the major source of nectar of *A. florea* but uses of insecticides affect the floral fidelity of bees which is corroborate the findings of Jana *et al.* (2002). They also draw a relationship and suggest the simultaneous growth of agriculture and apiculture. The present study also supports this view. Previously, Mahajan *et al.* (2000) reported that Anacardiaceae, Asteraceae, Myrtaceae, Fabaceae and Combretaceae represent the reliable nectar source of *A. florea* in south-western Satpura, Maharashtra. Roy *et al.* (2014) established *Brassica* sp. as a major foraging plant of *A. florea* which is corroborated by the present study. Hazra *et al.* (2021) reported that Asteraceae represents the maximum flora in the honey sample of Purulia district West Bengal, which is also observed in the present study. Hazra *et al.* (2021) also mentioned that *Terminalia arjuna* is an important source of pollen of *A. florea*. Rateb *et al.* (2020) reported that Asteraceae (*Centaurea hylolepis*) represents the maximum flora in the honey sample of *A. florea* in Jordan. Rateb *et al.* (2020) also mentioned that pollen diversity of *A. florea* depends on the floral makeup and the sampling area.The present study also supports this view. Chaya (2022) reported that *Alternanthera sessile* (Amaranthaceae), *Sphaeranthus indicus* (Asteraceae), *Carum copticum* (Apiaceae), and *Ageratum conyzoides* (Asteraceae) represent the major pollen and nectar source of *A. florea* in Telangana.

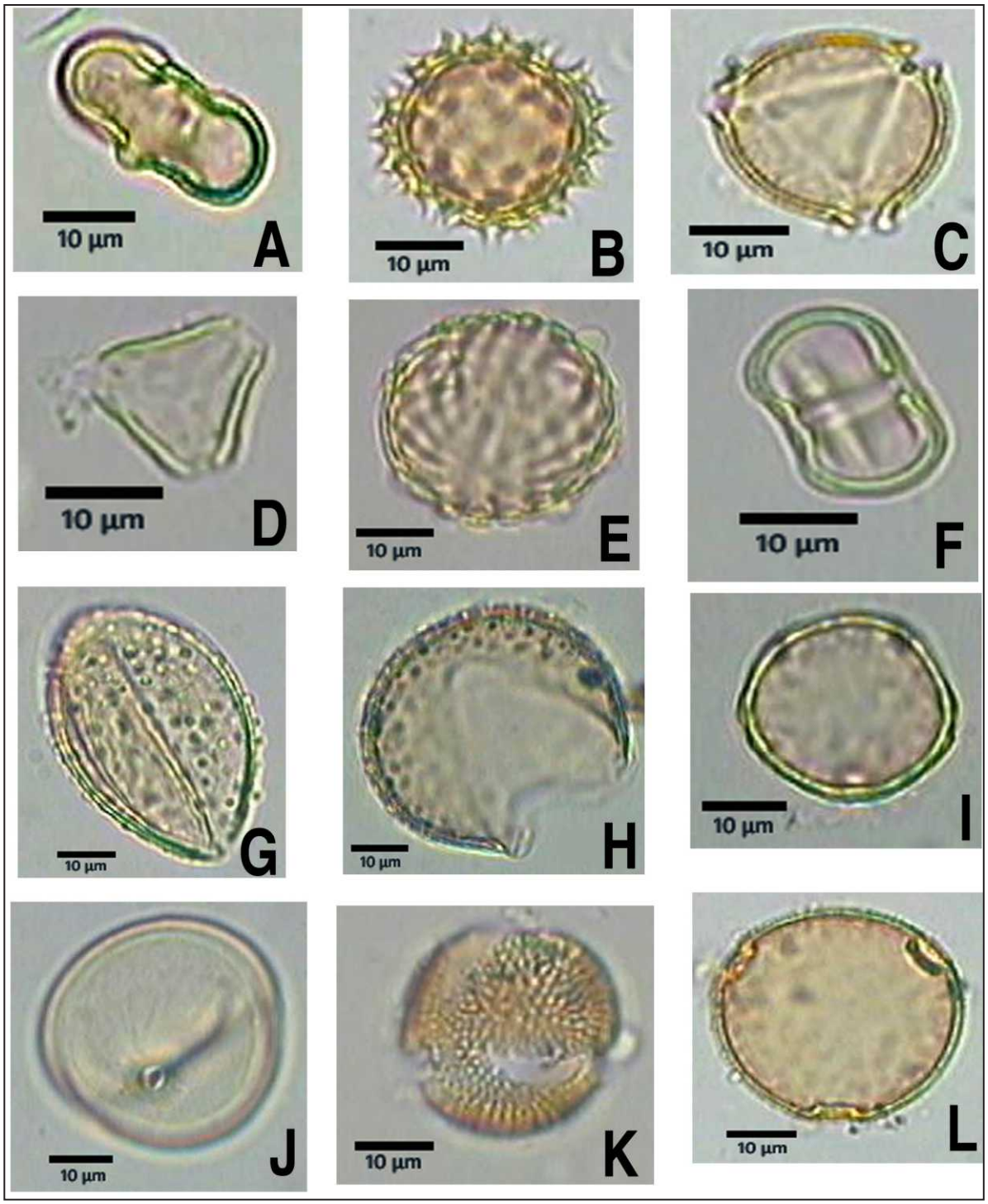


Fig.1. Light microscopic photographs of stored pollen grains collected by *Apis florea*, recovered from honey samples **A**. Apiaceae, **B**. Asteraceae, **C** & **D**. Myrtaceae, **E**. Acanthaceae, **F**. Apiaceae, **G** & **H**. Arecaceae, **I**. Ulmaceae, **J**. Poaceae, K. Rubiaceae, **L**. Cannabaceae

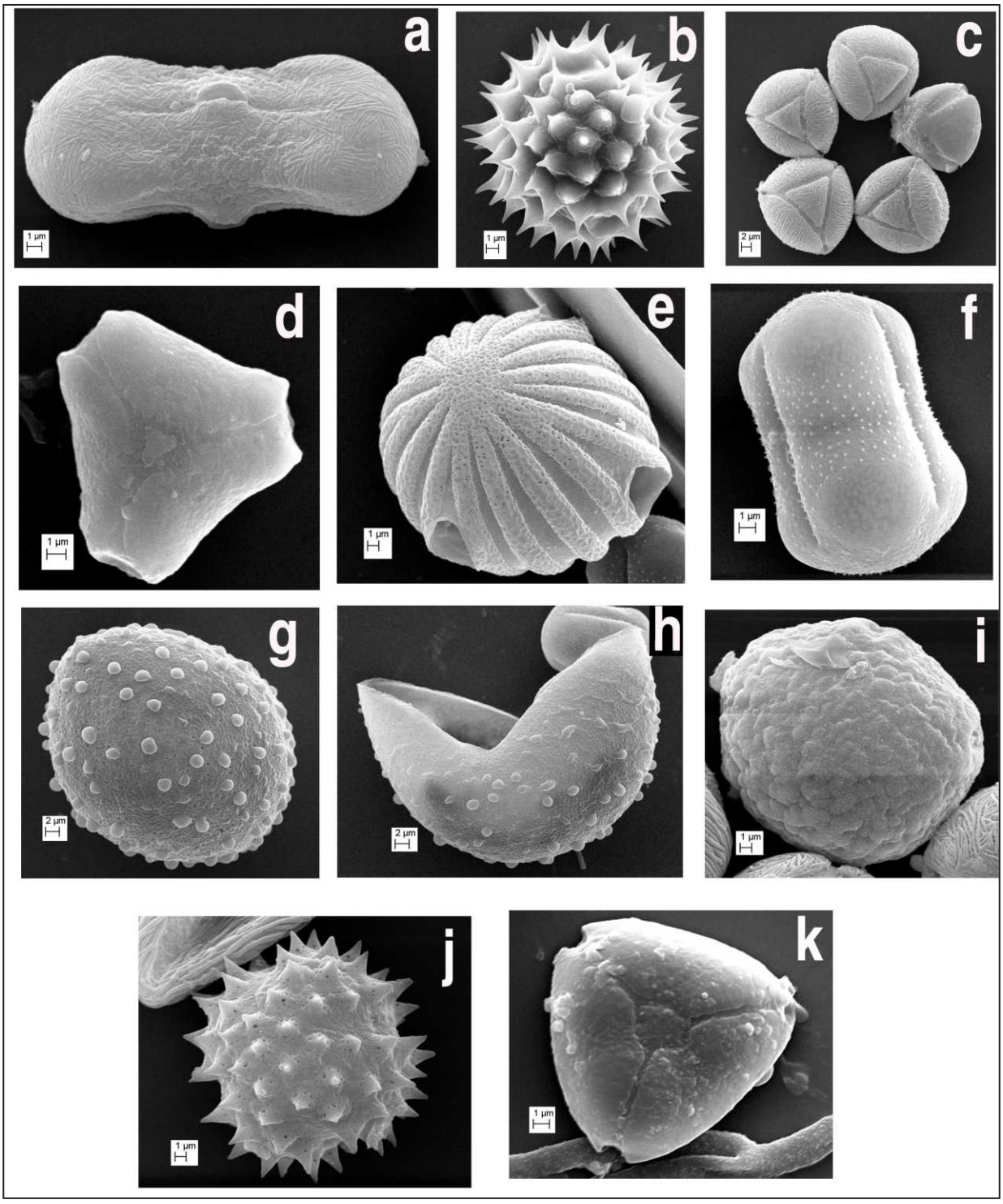


Fig. 2. Scanning electron microscopic photographs of stored pollen grains collected by *Apis florea*, recovered from honey samples

a. Apiaceae, **b.** Asteraceae, **c** & **d.** Myrtaceae, **e.** Acanthaceae, **f.** Apiaceae, **g** & **h.** Arecaceae, **i.** Ulmaceae, **j.** Asteraceae, **k.** Myrtaceae

Apis florea is a good pollinator because they easily access to the flower for their small size and can pollinate large number of flower within a short timeas compared to other *Apis* species (Free, 1981; Singh *et al*., 1990; Gubartalla, 1997; Abrol, 2020; Gayen and Ghorai, 2023). Layek *et al*. (2015) mentioned that *A. florea* helps our society by their effective pollination services. Dalio (2018) reported that foraging activity of *A. florea* depends on the hours of the day. Unplanned applications of insecticides in foraging plants of honeybee interfere with pollination processes and consequent crop production. There seems to be a lack of awareness about the importance of this dwarf honeybee and their role in pollination in different agricultural fields. So, this study provides baseline information to the farmers and beekeepers about the foraging behaviour of red dwarf honey bee (*Apis florea*).

ACKNOWLEDGEMENT

Authors are thankful to Dr. Shamee Bhattacharjee, Head, Department of Zoology, West Bengal State University for her continuous encouragement. Authors are highly indebted to Prof. Subir Bera, for permitting the use resources in the Palaeobotany-Palynology Laboratory, Department of Botany, University of Calcutta and helped in pollen identification. Authors are also grateful to all faculty members of Department of Zoology, WBSU.

DISCLAIMER

The authors declared that there is no conflict of interest while performing the work and preparation of the manuscript.

REFERENCE

Abrol, D.P. 2020. The Future Role of Dwarf Honeybees in Natural and Agricultural Systems. *CRC Press*.1st edition:322 pp.

Attri, P.K. 2010. Melittopalynological investigations on *Apis cerana* autumn honey collected from Chamba District, Himachal Pradesh. *International Journal of Science and Nature*, **1**(1): 67-72.

Basak, S., Biswas, O., Chhetri, B., Rai, S., Paruya, D.K., Patra, D.C. and Bera, S. 2025. Nectar and pollen source of natural honey produced by *Apis ceranaskorikovi* Engel (=*himalaya*): Palynological analysis from tropical to sub-tropicalforests of eastern Himalaya. *Review of Palaeobotany and Palynology*, **332**: 105210.

Bera, S., Dixit, S., Basumatary, S. and Sharma, G.C. 2007. Pollen analysis of honey from Kamrup Reserve forests, Assam. *Journal of Palynology*, **43**(1&2): 57-65.

Bhargava, H.R., Jyothi, J.V.A., Bhushanam, M. and Surendra, N.S. 2009. Pollen analysis of *Apis* honey, Karnataka, India. *Apiacta*, **44**(5): 14-19.

Bhattacharya, A. 2014. Melittopalynological study of Nadia district, West Bengal, India. *International Journal of Current Research*, **6**(5): 6521-6526.

Bhattacharya, K., Chanda, S., Gupta, S. and Ganguly, P. 1983. Analysis of the pollen load from a honey sample from Salt Lake City, Calcutta. *Science & Culture*, **49**(7): 222-224.

Bhusari, N.V., Mate, D.M. and Makde, K.H. 2005. Pollen of *Apis* honey from Maharashtra. *Grana*, **44**(3): 216-224.

Chakraborty, P., Saha, A. and Chakraborty, A. 2023. Pollen diversity in the winter honey from Howrah district, West Bengal, India. *Ind. J. Aerobiol*, **36**(2): 29-38.

Chaudhary, O.P. 2003. Evaluation of honeybee flora of the northeastern region of Haryana. *Journal of Palynology*, **39**: 127-141.

Chauhan, M.S. and Trivedi, A. 2011. Pollen analysis of honey from Lucknow District, Uttar Pradesh. *Journal of Applied Bioscience*, **37**(1): 48-51.

Chaya, P. 2022. *Apis florea* honeys from Vikarabad district of Telangana State of South India–A pollen based study. *International Journal of Botany Studies*, **7**(3): 218-222.

Dalio, J.S. 2018. Foraging Frequency of *Apis* Species on Bloom of *Brassica Napus* L. *The International Journal of Engineering and Science (IJES)*, **7**(2): 28-33.

Deodikar, G.B. and Thakar, C.V. 1953. A pollen study of major honey yielding plants of Mahabalashwar hills. *Apicultural Lab Bulletin*, **1**: 1-6.

Dixit, S., Basumatary, S.K., Singh, H. and Bera, S.K. 2013. Melissopalynological studies of western part of Almora District, Uttarakhand. *The Palaeobotanist*, **62**(1-2): 39-46.

Erdtman, G. 1960. The Acetolysis method: A revised description. *Seven Bot Tidskr*, **54**: 561-564.

Free, J.B. 1981. Biology and behaviour of the honeybee *Apis florea*, and possibilities for beekeeping. *Bee World*, **62**: 2; 46-59.

Gayen, A.K. and Ghorai, N. 2023. Foraging behavior of *Apis florea* F. on Wax Apple *Syzygiumsamarangense* and their role in pollination of West Bengal, India. *Uttar Pradesh J. Zool.*, **44**(21): 198-203.

Gayen, A.K. and Ghorai, N. 2023. Studies on Foraging and Pollinating Activity of Dwarf Honey Bee (*Apis florea* F.) on Blooms of *Brassica juncea* Linnaeus in West Bengal, India. *Bionotes*,**25**(1 &2): 39-44.

Gayen, A.K. and Ghorai, N. 2023. Study on nesting parameters of dwarf honey bee, *Apis florea* F. in arid zone of W.B, India. *International Journal of Entomology Research*, **8**(7): 24-27.

Gubartalla, A.E. 1997. Studies on some aspects of the biology of the dwarf honeybee *Apis florea* F. in Khartoum state. M.Sc. Faculty of Agriculture, University of Khartoum, Sudan.

Hazra, T., More, S., Mahato, S. and Bera, S. 2021. *Terminalia arjuna*: An Important Subsistence Pollen Source for *Apis florea* During Spring Season in Southern West Bengal. Proceedings of the National Academy of Science, India-Section B: Biological Science **91**(2).

Hepburn, H.R. and Hepburn, C. 2005. A bibliography on *Apis florea*, Apidologie (in press).

Jana, D., Bandyopadhyay, A. and Bera, S. 2002. Pollen analysis of winter honey samples from Murshidabad District, West Bengal. *Geophytology*, **30**: 91-97.

Jhansi, P., Kalpana, T.P. and Ramanujam, C.G.K. 1991. Pollen analysis of rock bee summer honeys from the Prakasam district of the Andhra Pradesh, India. *Journal of Apicultural Research*, **30**(1): 33-40.

Kalpana, T.P., Fatima, K. and Ramanujam, C.G.K. 1990. Pollen analysis of *Apiscerana* and *Apis florea* honeys from Adikmet area, Hyderabad. *Proc. Indian Acad. Sci. (Plant Sci.)*,**100**(3): 183-193.

Kamble, K.D., Pandit, R.S. and Rao, K.L. 2013. Melittopalynological investigations of honey from sunderban region, West Bengal, India. *Proceeding of National Academy of Sciences, India*, **85**(1): 101-106.

Lakshmi, K. and Suryanarayana, M.C. 2004. Melittopalynological investigation of *Apis dorsata* honeys from forest area of Cuddaph district, Andhra Pradesh. *Journal of Palynology*, **40**: 189-198.

Layek, U. and Karmakar, P. 2016. Bee plants used as nectar sources by *Apis florea* Fabricius in Bankura and Paschim Medinipur districts, West Bengal. *Geophytology*, **46**(1): 1-14.

Layek, U., Bhakat, R.K. and Karmakar, P. 2015. Foraging behavior of *Apis florea* Fabricius during winter and spring-summer in Bankura and Paschim Medinipur districts, West Bengal. *Global Journal of Bio-Science and Biotechnology*, **4**(3): 255-263.

Louveaux, J., Maurizio, A. and Vorwhol, G. 1978. Methods of melissopalynology. *Bee World*, **59**: 139-153.

Mahajan, D.M., Salunkhe, I.B. and Gunale, V.R. 2000. Pollen analysis of *Apis florea* Fabr. Honeys from the South-western Satpura (Maharashtra). *The Indian Forester*, **126**(2): 159-164.

Mukhopadhyay, S.K., Gupta, S., Das, A.P. and Bera, S. 2007. The beekeeping potential of Sub-Himalayan West Bengal, India: A palynological assessment of honey. *Journal of Apicultural Research*, **46**(3): 165-180.

Naiya, S., Sengupta, P. and Ghorai, N. 2016. A study on the long-term variation in pollen Foraging by *A. cerana* (Fabr.) [*Apidae* : Insecta]. *International Journal of Entomology Research*, **1**(5): 12-18.

Ramanujam, C.G.K. and Kalpana, T.P. 1992. *Tamarindus indica* L. an important forage plant for *Apis florea* F. in south central India. *Apidologie*, **23**(5): 403-413.

Ramanujam, C.G.K. and Khatija, F. 1992. Summer pollen sources of *Apis dorsata* honeybees in deciduous forest of Mahboobnagar District, Andhra Pradesh. *Geophytology*, **21**: 155-161.

Rateb, S.H., Haddad, N. and Hussein, M.H. 2020. Pollen spectrum and foraging plants for the red dwarf bee in Jordan. *Egyptian Academic Journal of Biological Science*, **13**(4): 239-245.

Roy, S., Gayen, A.K., Mitra, B. and Duttagupta, A. 2014. Diversity, foraging activities of the insect visitors of Mustard (*Brassica juncea* Linnaeus) and their role in pollination in West Bengal. *The Journal of Zoology Studies*, **1**(2): 7-12.

Saha, S., Nath, R., Dey, A., Laskar, N. and Kundu, A. 2023. Melissopalynological analysis of *Apis* honey from northern districts of West Bengal. *Grana*, **62**(5-6): 352-368.

Sahney, M., Kumar, A. and Rahi, S. 2016. Pollen analysis of honey from Varanasi district, Uttar Pradesh, India. *Biological Forum- An International Journal*, **8**: 126-133.

Sahney, M., Rahi, S., Kumar, A. and Jaiswal, R. 2018. Melissopalynological studies on winter honeys from Allahabad, Uttar Pradesh. *Palynology*, **42**(4): 540-552.

Shukla, V. and Rao, K.S. 2021. Pollen analysis of summer honeys from Prayagraj district, Uttar Pradesh, India. *Acta Palaeobotanica*, **61**(1): 20-31.

Singh, M.P, Verma, L.R. and Daly, H.V. 1990. Morphometric analysis of the Indian honeybee in the northeast Himalayan Region. *J. Apic. Res*., **29**: 3-4.

Sivaram, V., Roopa, P., Shubharani, R. and Suwannapong G. 2012. Pollen analysis in honeys collected from Karnataka region of Nilgiri Biosphere, South India. *Journal of Apiculture*, **27**: 223-231.

Suryanarayana, M.C., Rao, G.M. and Singh, T.S.M.S. 1992. Studies on pollen sources for *Apis cerana* Fabr. and *Apis mellifera* L. bees at Muzaffarpur, Bihar, India. *Apidologie*, **23**(1): 33-46.

Tripathi, S., Basumatary, S.K., Bera, S.K., Brahma, M. and Sharma, G.C. 2017. A palynological study of natural honeys from the Bongaiga on district of Assam, northeast India. *Palynology*, **41**(3): 389-400.

Upadhyay, D. and Bera, S. 2012. Pollen spectra of natural honey samples from a Coastal district of Orissa, India. *Journal of Apiculture Research*, **51**(1): 10-22.

J. Botan. Soc. Bengal 79(1) : 110-115 (2025), ISSN 0971-2976

FULL LENGTH ARTICLE

Placement of Indian species of *Selaginella* P. Beauv. to their subgeneric rank following the classification of Weststrand and Korall (2016)

Radhanath Mukhopadhyay*
CAS, Department of Botany, The University of Burdwan, Burdwan,713104, India

Received : 17.06.2025 Accepted : 22.06.2025 Published : 30.06.2025

Selaginella is a nomenclaturally problematic genus, particularly at the infrageneric level. Subgeneric names have been changed a number of times by different workers since the late nineteenth century with different combinations of characteristic features. In this perspective, the position of the Indian species of *Selaginella* has been ascertained to their subgeneric categories. Out of the presently recognized seven subgenera of *Selaginella,* Indian members are represented only in four subgenera viz., *Rupestrae, Lepidophyllae, Gymnogynum* and *Stachygynandrum.*

Key words: *Selaginella*, resurrection plant, Indian members, subgeneric classification.

INTRODUCTION

The family Selaginellaceae contains a single living genus *Selaginella*, represented by about 700 - 800 species throughout the world. It is found mainly in the tropical and subtropical areas of both the hemispheres and a few are found in the arctic and alpine regions. *Selaginella* is herbaceous, mostly terrestrial, a few are epiphytic, heterosporous, and bears ligulate microphyllous leaves. In size, it ranges from a few cms to several meters and assumes various growth forms like erect, creeping, rosette, climbing, scandent and mat formation etc. (Jermy, 1990; Zhouand Zhang, 2015, Mukhopadhyay, 2018).

Alston (1945) for the first time enumerated Selaginellas of British India as 58 which includes some species of Burma (*S. griffithii*, *S. pubescens, S. mairei*, *S. minutifolia* and *S. amblyphylla*) and Ceylon (*S. praetermissa*, *S. latifolia*, *S. argentea*, *S. brachystachya* and *S. cochleata*). Dixit (1992) in his book *Selaginellaceae of India* considered 62 species from geographical boundaries of India including some newly discovered species by him and some cultivated species. Fraser-Jenkins *et al.* (2017) considered 55 species of *Selaginella* in India. They did not accept some species (*S. jainii*, *S. nairii*, *S. rajasthanensis*, *S. panchghania*na, *S. nayarii, S. kashmiriana, S. keralensis*) reported by Dixit (1983, 1984) as new species of *Selaginella,* rather considered them as synonyms to other species. Shalimov *et al*. (2019) mentioned in their paper two new Indian species of *Selaginella* (*S. laxistrobila* K.H. Shing & *S. trichophylla* K.H. Shing) from Sikkim, India.

Selaginella has been divided into two, four or five subgenera on the basis of morphological characters (Hieronymous, 1900-1901; Walton and Alston, 1938; Jermy, 1986). Hieronymous (l.c.) broadly divided

**Corresponding author* : rnm.burdwan@gmail.com

Selaginella into two subgenera *Homeophyllum* and *Heterophyllum*, on the basis of isophyllous and heterophyllous nature of vegetative leaves and sporophylls. Walton and Alston (1938) mainly based on Baker's (1887) treatment and divided the genus *Selaginella* into four subgenera viz., *Euselaginella, Stachygynandrum, Homostachys* and *Heterostachys*, based on the size and arrangement of leaves, features of sporophylls and strobili. Jermy (1986) considered five subgenera viz., *Selaginella*, *Ericetorum*, *Tetragonostachys*, *Stachygynandrum* and *Heterostachys*. He created two new subgenera *Ericetorum* Jermy and *Tetragonostachys* Jermy. Actually, Jermy (1986) divided all the homophyllous members of the genus *Selaginella* into three subgenera viz., *Selaginella*, *Tetragonostachys* and *Ericetorum* with different combinations of morpho-anatomical characters and growth forms. Zhou and Zhang (2015) divided *Selaginella* into six subgenera viz., *Selaginella*, *Boreoselaginella*, *Pulvinella*, *Ericetorum*, *Heterostachys* and *Stachygynandrum* on the basis of molecular (chloroplast and nuclear DNA), macromorphological, spore characters and distribution information. The infrageneric divisions identified through molecular data are supported by non-molecular features such as morphological, spore characters and ecological data. Weststrand and Korall (2016b) have divided *Selaginella* into seven subgenera based on morphological characters and supported by DNA sequence data. These subgenera are *Selaginella*, *Rupestrae*, *Lepidophyllae*, *Gymnogynum*, *Exaltatae*, *Ericetorum* and *Stachygynandrum*. Weststrand and Korall (2016b) have replaced the name of the subg. *Tetragonostachys* of Jermy's classification by subg. *Rupestrae.*

With these changing concepts of subgeneric boundaries, the placement of Indian species of *Selaginella* has been made to their subgeneric rank to see how many subgenera of *Selaginella* are present in India.

MATERIALS AND METHODS

The Indian species of *Selaginella* are considered here for subgeneric placement. So, the species occurring in Burma and Ceylon have been excluded from the enumeration of Selaginellas of British India published by Alston (1945). The morpho-anatomical and spore characters were consulted from various published literatures (Panigrahi and Dixit, 1966, 1967, 1968; Mukhopadhyay, 1986, 1997; Sen *et al*., 1989; Dixit 1992; Mukhopadhyay and Das, 2004; Korall and Taylor, 2006; Mukhopadhyay *et al*., 2008; Singh *et al*., 2012; Shalimov *et al*., 2019). Distributional information of Indian species of *Selaginella* was consulted from Fraser-Jenkins (2008) and Fraser-Jenkins *et al*. (2017). The species of *Selaginella* which are cultivated in India (*S. kraussiana*, *S. microdendron*, *S. plana*, *S. uncinata* and *S. vogelli*) have not been considered here. The herbarium sheets of Indian species of *Selaginella* housed at CAL were consulted while pursuing Ph.D. thesis (1984) at Kalyani University.

The Indian members of *Selaginella* are placed in their subgeneric rank in Table1 after comparing the characteristic features of each group with the species, following the classification of Weststrand and Korall (2016b).

OBSERVATIONS

The species of Indian *Selaginella* are placed to their respective subgenera following the subgeneric classification of *Selaginella* by Weststrand and Korall (2016b) in Table 1.

DISCUSSION

The genus *Selaginella* is broadly divided into two groups, the first group of species bears no rhizophore and the leaves and sporophylls are isophyllous and spirally arranged. Such group is represented worldwide by only two species (*Selaginella selaginoides* and *S. deflexa*). These species have been placed under the subgenus *Selaginella* (Jermy, 1986, Weststrand and Korall, 2016a, 2016b). No Indian representative is there in this group with these properties. The other group includes all other rhizophore bearing members of *Selaginella* with tetrastichous arrangement of vegetative leaves and sporophylls. This second group again is subdivided into six subgroups (subgenera). One broad group is the subgenus *Stachygynandrum* in which all the members

Table 1. Placement of Indian species of *Selaginella* in different subgenera of the genus *Selaginella* P. Beauv. according to the concept of Weststrand & Korall (2016b).

Names of subgenera of the genus *Selaginella*	**Characteristic features**	**Indian species of *Selaginella***
Selaginella	Plants perennial, erect, with nonarticulate stems. Stems monostelic. Roots at the base of shoot, from a hypocotular node. Rhizophores lacking. Vegetative leaves and sporophylls monomorphic, helically arranged.	No Indian species
*Rupestrae*Weststrand & Korall	Plants perennial, creeping, mat-forming, or sometimes erect. Stems monostelic, nonarticulate. Rhizophores dorsal. Vegetative leaves monomorphic, helically arranged. Sporophylls monomorphic in tetrastichous strobili.	*S. emodi* Fraser-Jenk. *S. wightii* Hieron.
Lepidophyllae(Li Bing Zhang & X. M. Zhou) Weststrand & Korall	Plants perennial with a rosetted habit. Stems monostelic, nonarticulate, curling inward in dry conditions forming a tight ball. Rhizophores dorsal. Vegetative leaves dimorphic in four rows, the smaller leaves in the two upper (dorsal) rows. Sporophylls monomorphic in tetrastichous strobili.	*S. pulvinata* (Hook. et Grev.) Maxim.
Gymnogynum(P. Beauv.) Weststrand & Korall	Plants perennial, with articulate stems. Stems mono- or bistelic, in rare cases tri- or 4–5-stelic. Rhizophores dorsal. Vegetative leaves dimorphic (at least on distal parts of plant) in four rows, the smaller leaves in the two upper (dorsal) rows. Sporophylls monomorphic in tetrastichous strobili. Strobili with a single (rarely two) basal megasporangium surrounded by enlarged sterile sporophylls. Megaspores with a grid-like exospore pattern in cross section.	*S. remotifolia* Spring
*Exaltatae*Weststrand & Korall	Plants perennial. Stems actinostelic or actino-plectostelic, articulate or not. Rhizophores dorsal. Vegetative leaves dimorphic (at least on distal parts of plants) in four rows, the smaller leaves in the two upper (dorsal) rows. Sporophylls monomorphic in tetrastichous strobili. Strobili with a single (rarely two) basal megasporangium surrounded by enlarged sterile sporophylls.	No Indian species

Table 1 (Cond.)

Table 1 (Contd.)

Ericetorum Jermy	Plants annual or perennial, erect, with nonarticulate stems. Rhizomes (when present) solenostelic with rhizophores. Erect branches commonly polystelic (perennials). Vegetative leaves monomorphic, decussately arranged, at least on proximal parts of plants. Larger species anisophyllous in distal parts of plants. Dimorphic vegetative leaves in four rows, the two upper (dorsal) rows with smaller leaves. Sporophylls monomorphic in tetrastichous strobili. Megaspores with wing-like laesurae and high porosity at the proximal pole, forming a 'complex mass'	No Indian species
Stachygynandrum (P. Beauv. ex Mirb.) Baker	Plants perennial, creeping to erect, sometimes long, scandent, with nonarticulate stems. Stems commonly monostelic, rarely tristelic or actinostelic. Rhizophores mostly ventral (proximally or throughout the plant), dorsal in a few species (*S. sanguinolenta*, *S.nummularifolia* Ching, and possibly close relatives). Vegetative leaves dimorphic (at least on distal parts of plant) in four rows, the smaller leaves in the two upper (dorsal) rows. Sporophylls monomorphic in tetrastichous strobili, or dimorphic in resupinate or nonresupinate strobili (i.e., with the smaller sporophylls in the same plane as the larger vegetative leaves or vice versa).	*S. aitchisonii* Hieron, *S. jacquemontii* Spring, *S. pallidissima* Spring, *S. pennata* (D. Don) Spring, *S. bisulcata* Spring, *S. reticulata* (Hook.et Grev.) Spring, *S. chrysorrhizos* Spring, *S. kurzii* Baker, *S. ciliaris* (Retz.) Spring, *S. crassipes* Spring, *S. proniflora* (Lam.) Baker, *S. watii* Baker, *S. decipiens* Warb., *S. tenera* (Hook.et Grev.) Spring, *S. vaginata* Spring, *S. cataractum* Alston, *S. monospora* Spring, *S. chrysocaulos* (Hook.et Grev.) Spring, *S. subdiaphana* (Wall. ex Hook & Grev.) Spring, *S. tenuifolia* Spring, *S. miniatospora* (Dalzell) Baker, *S. semicordata* (Wall. ex Grev. & Hook.) Spring, *S. ornithopodioides* (L.) spring, *S. radicata* (Hook. et Grev.) Spring, *S. ganguliana* R.D. Dixit, *S. pallida* Spring, *S. mittenii* Baker, *S. Laxistrobila* K.H. Shing, *S. triphylla* K.H. Shing, *S. adunca* A. Braun ex Hieron., *S. biformis* A. Braun ex Kuhn., *S. bryopteris* (L.) Baker, *S. coonooriana* R. D. Dixit, *S. delicatula* (Desv ex Poir.) Alston, *S. frondosa* Warb., *S. helferi* Warb., *S. intermedia* (Blume) Spring, *S*. involvens (Sw.) Spring, *S. helvetica* (L.) Spring, *S. pentagona* Spring, *S. picta* A. Braun ex Baker, *S. repanda* (Desv. ex Poir.) Spring, *S. willdenovii* (Desv. Ex Poir.) Baker, *S. inaequalifolia* (Hook. et Grev.) Spring, *S. wallichii* (Hook. et Grev.) Spring

have ventral rhizophores, developing either at the basal region or along the creeping rhizome /stem and bearing monomorphic or dimorphic sporophylls in strobili. The strobili with dimorphic sporophylls may be resupinate or nonresupinate. In India, out of 49 species mentioned here, 45 belong to this subgenus *Stachygynandrum*. Worldwide, this *Stachygynandrum* subgenus also represents the largest number of species (about 600 spp.) (Jermy, 1986). The rest members of the genus S*elaginella* all have dorsal rhizophores and are divided into five subgenera viz., *Rupestrae, Lepidophyllae, Ericetorum, Exaltatae and Gymnogynum.* In India, *Rupestrae* subgenus is represented by only two species of *Selaginella* (*S. emodi* and *S. wightii*). These species are xeric in nature. *Selaginella pulvinata* is the lone species in India having rosetted habit and having resurrection property, belonging to *Lepidophyllae* group. There is no species in India belonging to the *Ericetorum* subgenus. Patterns of distribution of sporangia in the strobili of *Selaginella* and megaspore ornamentation have been studied earlier (Horner, 1962; Horner and Arnott, 1963; Mukhopadhyay, 1997; Korall and Taylor, 2006) are considered important characters and used effectively in the delineation of subgenera of the genus *Selaginella* like *Ericetorum*, *Exaltatae* and *Gymnogynum*.

Articulatate or nonarticulate nature of stems of *Selaginella* is another aspect which has been taken into consideration for the delineation of species into subgeneric groups. Articulations in *Selaginella* species usually occur just beneath branching, forming a swelling or a dark band, which is best seen on dry specimens (Somers, 1978; Feio and Go'es-Neto, 2018). Articulate stem bearing species of *Selaginella* have been placed both under *Gymnogynum* and *Exaltatae* subgenera with different combinations of stelar types. No Indian species is there in the *Exaltatae* group with actinostelic or plectostelic stele as was found by Mickel and Hellwig (1969) in *Selaginella exaltata.* Only one species of *Selaginella* with articulate stem viz., *S. remotifolia*, with monostelic stem belongs to the subgenus *Gymnogynum*.

ACKNOWLEDGEMENT

I am thankful to Prof. Debabrata Maity, Botany Dept., Calcutta University for providing literature.

DISCLAIMER

The author(s) declares no conflict of interest in the work.

REFERENCE

Alston, A.H.G. 1945. An enumeration of the Indian species of *Selaginella. Proc. Nat. Inst. Sci. India,* **11**: 211-235.

Baker, J.G. 1887. *Handbook of the Fern-allies*.London: George Bell & Sons.

Dixit, R.D. 1983. Four new species of *Selaginella* P. Beauv. from India. *Bull. Bot. Surv. India,* **25**(1-4): 223-227.

Dixit, R.D. 1984.Two new species of *Selaginella* P. Beauv. from India. *Bull. Bot. Surv. India*, **26**(1&2): 104-107. t 1-II, text figs 1-2.

Dixit, R.D. 1992. *Selaginellaceae of India*. Dehra Dun, India: Bishen Singh Mahendra Pal Singh Feio AC and Go'es -Neto LADA. 2018. The Articulated and Non-Articulated Stem in *Selaginella* (Selaginellaceae - Lycopodiopsida). *American Fern Journal*, **108**(4):170-175.

Fraser-Jenkins, C.R. 2008. *Taxonomic Revision of Three Hundred Indian Subcontinental Pteridophytes with a Revised Census List*. Dehra Dun, India: Bishen Singh Mahendra Pal Singh.

Fraser-Jenkins, C.R., Gandhi, K.N., Kholia, B.S. and Benniamin, A. 2017. *An annotated checklist of Indian Pteridophytes*, *Part – I (Lycopodiaceae to Thelypteridace)*. Dehra Dun: Bishen Singh Mahendra Pal Singh.

Hieronymous G.1900-1901. Selaginellaceae *In*: *Engler und Prantl. Die Naturalichen Pflanzenfamilien,* etc. **1**(4): 206, 209-210, 621-775.

Horner, H.T. Jr. 1962. Patterns of sporangial arrangement in North American species of *Selaginella*. *Amer. J. Bot*., **49**: 660.

Horner, H.T. Jr. and Arnott, H.J. 1963. Sporangial arrangement in North American species of *Selaginella*. *Bot. Gaz*., **124**(5): 371-383.

Jermy, A.C. 1986. Subgeneric names in *Selaginella. Fern Gaz*., **13**(2): 117-118.

Jermy, A.C. 1990. Selaginellaceae. *In* Kramer KU and Green PS (Eds.), *The Families and Genera of vascular Plants – Vol I, Pteridophytes and Gymnosperms*. Berlin: Springer Verlag, 39-45.

Korall, P. and Taylor, W.A. 2006.Megaspore morphology in the Selaginellaceae in a phylogenetic context: A study of the megaspore surface and wall structure using scanning electron microscopy. *Grana*, **45**: 22-60, DOI*: 10.1080/00173130500520453*

Mickel, J.T. and Hellwig, R.L. 1969. Actino-plectostely, a complex new stellar pattern in *Selaginella. American Fern Journal,* **59**: 123-134.

Mukhopadhyay, R. 1984. *Studies in Morphology, Anatomy, Palynology and Taxonomy of the Indian Selaginellaceae*. Ph.D. thesis. Kalyani University, Kalyani, India.

Mukhopadhyay, R. 1986. On the anantomy and phylogeny of *Selaginella* Palisot de Beauvois*. Indian Fern J.,* **3**: 60-69.

Mukhopadhyay, R. 1997. Patterns of sporangial arrangement and their evolution in some Indian members of *Selaginella* Beauv. *Sci. & Cult*., **63**(11& 12): 289-290.

Mukhopadhyay, R. 2018. *Selaginella* P. Beauv.– An overview. *In* Verma, S.K., Khullar, S.P., Benniamin, A. *et al*. (eds.), *Pteridology Today*: *Challenges and Opportunities* Kolkata: Botanical Survey of India, 109-130.

Mukhopadhyay, R. and Das, A. 2004. Morpho-Anatomical studies on some Indian species of *Selaginella* P. Beauv. *Phytomorphology*, **54**(1&2): 39-49.

Mukhopadhyay, R., Ganguly, G. and De, P. 2008. Studies in the morpho-anatomy of some Indian species of *Selaginella* Pal. Beauv. *Geophytology,* **37**: 75-80.

Panigrahi, G. and Dixit, R.D. 1966. Studies in the systematics of Indian *Selaginella*-3, *Proc. Natl. Acad. Sci. India*, B**36**(1): 102-108.

Panigrahi, G. and Dixit, R.D. 1967. Studies in the systematics of Indian *Selaginella*-2, *Indian Bot. Soc.*, **46**(2&3): 222-233.

Panigrahi, G. and Dixit, R.D. 1968. Studies in the systematics of Indian *Selaginella* -1, *Proc. Natl. Acad. Sci. India,* **B34**(4): 191-209.

Sen, T., Mukhopadhyay, R. and Sen, U. 1989. On the structure of the sporoderm of some Indian species of *Selaginella* P. Beauv. *Indian Fern J.,* **6**: 184-194.

Shalimov, A.P., Wu, Y.D. and Zhang, X-C. 2019. A taxonomic revision of the genus *Selaginella* (Selaginellaceae) from Nepal. *PhytoKeys*, **133**: 1-76.

Singh, S.K., Srivastava, G.K., Srivastava, M. *et al*. 2012. *Selaginella crassipes* (Selaginellaceae), a New Report from India. *Taiwania*, **57**(3): 283-287.

Somers, P. 1978. A systematic survey of the *Articulatae* series of the genus *Selaginella* and monographic treatment of the *S. sulcata* group (*sensu str*.). Ph.D. Thesis, The University of Tennessee, Knoxville, 370p.

Walton, J. and Alston, A.H.G. 1938. Lycopodiaceae. *InManual of Pteridology*, The Hague: Martinus Nijhoff, 500-508.

Weststrand, S. and Korall, P. 2016a. Phylogeny of Selaginellaceae: there is value in morphology after all! *Amer. J. Bot.*, **103**(12): 2136-2159.

Weststrand, S. and Korall, P. 2016b. A subgeneric classification of *Selaginella* (Selaginellaceae). *Amer J. Bot*., **103**(12): 2160-2169.

Zhou, X.-M. and Zhang, L.-B. 2015. A classification of *Selaginella* (Selaginellaceae) based on molecular (chloroplast and nuclear), macromorphological and spore features. *Taxon,* **64**(6): 1117-1140.

J. Botan. Soc. Bengal 79(1) : 116-120 (2025), ISSN 0971-2976

SHORT COMMUNICATION

Occurrence of *Pecopteris phegopteroides* (Feistm.) Arber from the lower permian bed of Raniganj coalfield, West Bengal, India

A.B. De*

Department of Microbiology, University of Burdwan, Burdwan – 713104, West Bengal, India.

Received : 03.09.2024 Accepted : 04.06.2025 Published : 30.06.2025

Extensive palaeobotanical studies have been carried out in different collieries of Raniganj Coalfield, West Bengal, India such as West Jamuria Colliery (Maheshwari, 1965), Mahabir Colliery (Maithy, 1974), Sonepur-Bazari Colliery (Mandal and Gupta, 2015) etc. but no palaeobotanical study was undertaken in Kalipahari Colliery of Raniganj Coalfield. A number of plant fossils were collected, some of which possess affinity with Pteridophytes. Some of these megafossils were identified as *Pecopteris phegopteroides* (Feistm.) Arber, which are reported here.

The Kalipahari Colliery (23°39'55"N:87°0'58"E) is located in Sripur area of Asansol Subdivision in West Burdwan District of the state of West Bengal (Fig.1). It is an open cast mine situated about 10 km east of Asansol Railway Station, West Bengal. Kalipahari Colliery lies in the Damodar Basin between two rivers, the Damodar and the Ajay, flowing almost parallel to each other. The lithology of the area ranges from coarse to medium-grained, arkosic sandtones, often cross bedded; grey and carbonaceous shales, at times interbanded with coal seams associated with plant fossils (Fig. 2).

The plant megafossils collected from Kalipahari Colliery (Fig. 2.) were studied using ordinary hand lens and were examined under low power binocular microscope and identified as *Pecopteris phegopteroides* (Feistm.) Arber. For identification of the specimens of *Pecopteris phegopteroides* their features have been compared with the authentic descriptions provided by Maheshwari and Prakash, 1965 and Surange, 1966.

All the megafossils are deposited in the Department of Botany, Burdwan Raj College, University of Burdwan, West Bengal, India.

SYSTEMATIC DESCRIPTIONS

Pecopteris phegopteroides (Feistm.) Arber
(Figs. 3,A-E).

Description: Five specimens were collected which represented incomplete sterile fronds, leaves bipinnate, rachis strong, 3-6 mm wide bearing pinnae (Figs. 3A & B) in alternate arrangement (Fig. 3C), pinnules attached to pinnae rachises more or less at 70° (Fig. 3C) to 90° (Fig. 3D), elongated-oblong, apex obtuse (Fig. 3,E), pinnules connate at the base, cleft in between two pinnules distinct, pinnules alternate to subopposite to one another, up to 10 mm long and 4 mm broad, few basal and apical pinnules slightly smaller in size than the rest of the pinnules.

**Corresponding author* : abde.brc@gmail.com

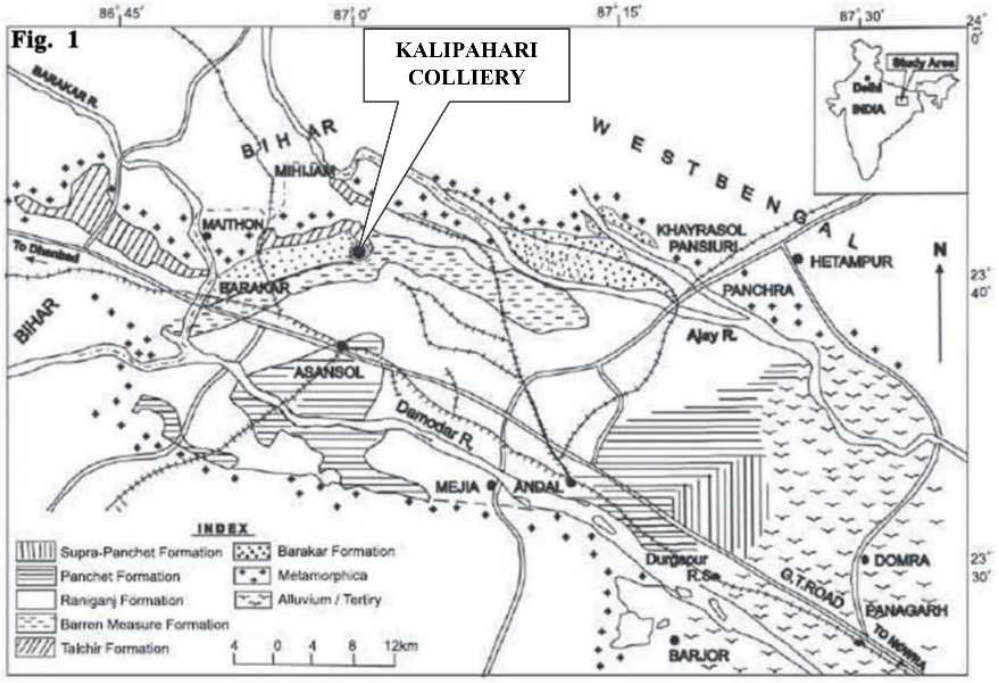


Fig. 1. Geological map of Raniganj Coalfield [after Murthy *et al*. (2010) with some modifications] showing the location of study area.

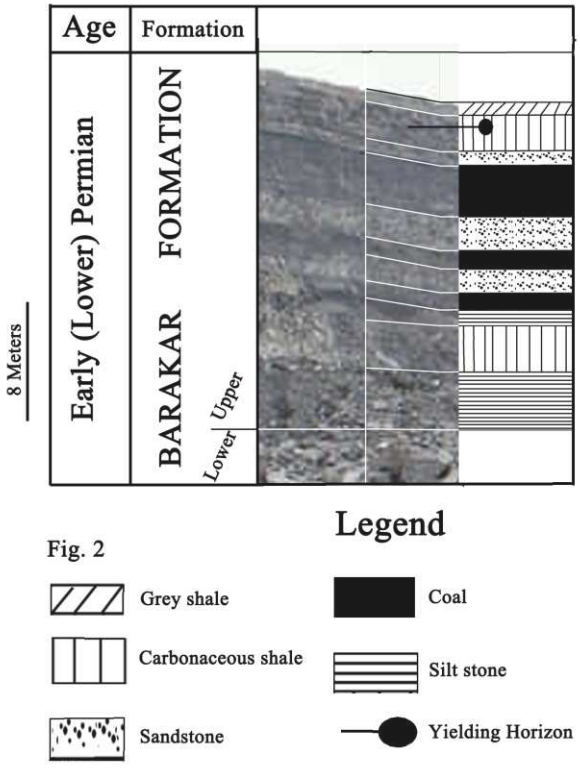


Fig. 2

Fig. 2. Litholog of the studied section showing the location of yielding samples.

Comparison: The fronds resemble closely with the fronds of *Dizeugotheca phegopteroides* (Feistm.) Maithy, Maithy (1975), *Pecopteris phegopteroides* (Feistm.) Arber (Maheswari and Prakash 1965, Surange 1966).

In the present study the fronds of *Pecopteris phegopteroides* (Feistm.) Arber were collected along with different species of *Glossopteris* leaves which indicates that gymnosperms were also present along with pteridophytes in this area during Early Permian. In the present study Maithy's combination *Dizeugotheca phegopteroides* (Feistm.) Maithy is not used because this combination by Maithy (1975) has been criticized by different workers (Herbst, 1978; Wagner *et al.,* 1985). According to Herbst (1978 p.126) Maithy's usage of *Dizeugotheca* does not correspond to the original definition of that genus. Wagner *et al.* (1985) stated that the Indian Gondwana *Pecopteris phegopteroides (Feistm.*) Arber was attributed to *Dizeugotheca* by Maithy (1975) and Lele *et al.* (1981) appeared to be a misidentification since the fructification of *Pecopteris phegopteroides* (Feistm.) Arber consists of small, but mature,

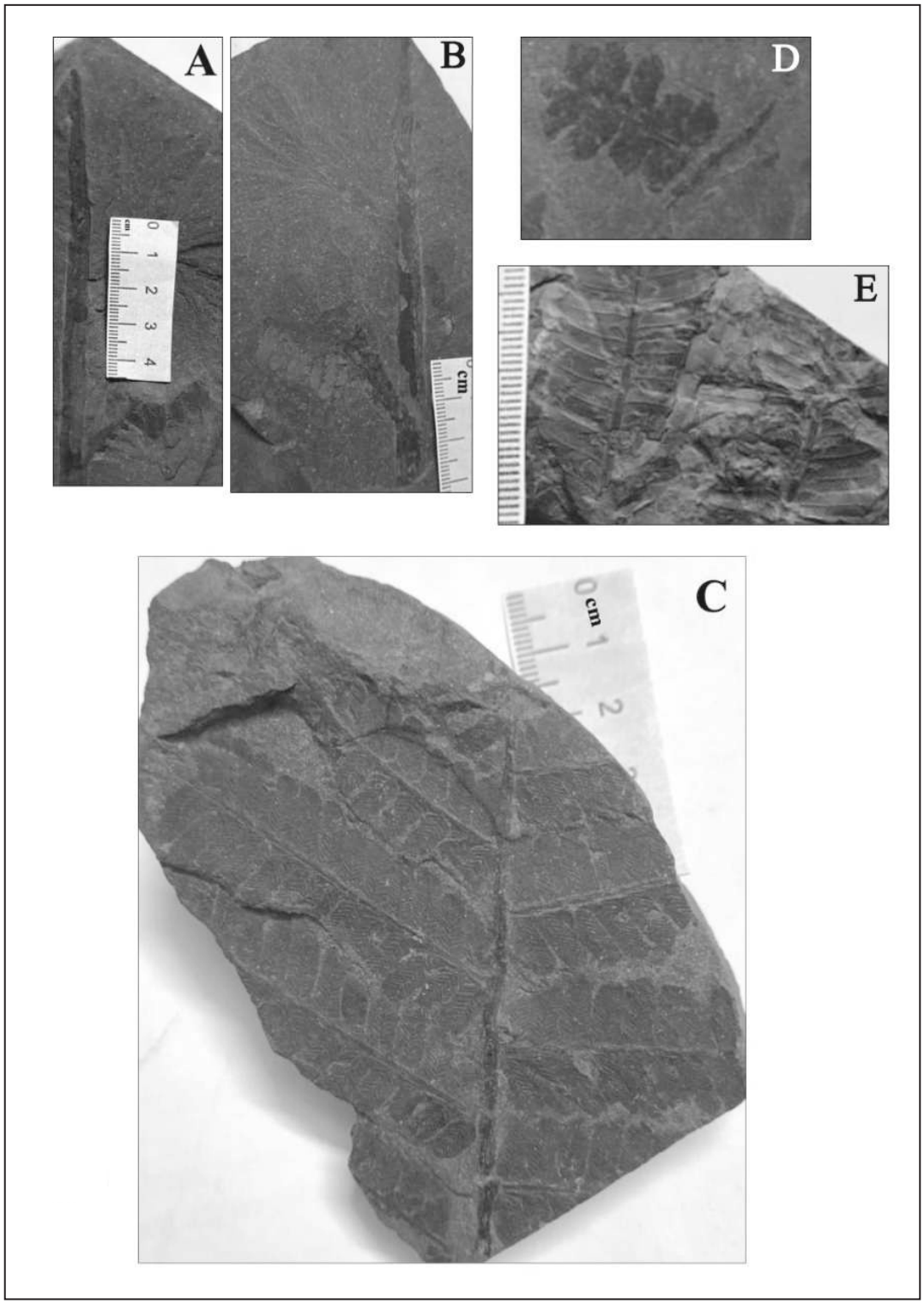


Fig. 3. (**A-E**) *Pecopteris phegopteroides* (Feistm.) Arber.: (**A and B**). Rachis bearing pinna. (**C**) Bipinnate leaf showing alternately arranged pinnae. (**D**) Pinnules attached to pinna rachis at 90°. (**E**) Pinnules with obtuse apex.

Comparison: The fronds resemble closely with the fronds of *Dizeugotheca phegopteroides* (Feistm.) Maithy, Maithy (1975), *Pecopteris phegopteroides* (Feistm.) Arber (Maheswari and Prakash 1965, Surange 1966).

In the present study the fronds of *Pecopteris phegopteroides* (Feistm.) Arber were collected along with different species of *Glossopteris* leaves which indicates that gymnosperms were also present along with pteridophytes in this area during Early Permian. In the present study Maithy's combination *Dizeugotheca phegopteroides* (Feistm.) Maithy is not used because this combination by Maithy (1975) has been criticized by different workers (Herbst, 1978; Wagner *et al.,* 1985). According to Herbst (1978 p.126) Maithy's usage of *Dizeugotheca* does not correspond to the original definition of that genus. Wagner *et al.* (1985) stated that the Indian Gondwana *Pecopteris phegopteroides (Feistm.*) Arber was attributed to *Dizeugotheca* by Maithy (1975) and Lele *et al.* (1981) appeared to be a misidentification since the fructification of *Pecopteris phegopteroides* (Feistm.) Arber consists of small, but mature, tetralocular synangia, placed along the extreme margins of the pinnules (Pant and Misra,1976). It is understood that the natural genus *Dizeugotheca* is reserved for fertile frond. Therefore, the name *Pecopteris phegopteroides* (Feistm.) Arber following Chandra and Rigby (1983) is used for the sterile materials collected from Kalipahari Colliery.

It may be mentioned here that in India *Pecopteris phegopteroides* (Feistm.) Arber [= *Dizeugotheca phegopteroides* (Feistm.) Maithy (Maithy 1975) = *Asansolia phegopteroides* Pant and Misra (Lele *et al.,* 1981)] was previously known to occur in Raniganj Formation (Chandra and Prasad, 1981; Maheshwari and Prakash 1965; Maithy, 1975; Pant and Misra, 1976; Srivastava, 2008; Surange, 1966), Kamthi Formation (Chandra and Rigby, 1983; Goswami *et al*., 2006a, 2006b, 2006c; Tewari, 2007, 2008) and Panchet Formation (Saxena *et al.,* 2019) all of which was assigned to Upper Permian. But the present study reports its occurrence from Upper Barakar Formation of Lower Permian bed of Raniganj Coalfield (Damodar basin) which indicates that this plant evolved much earlier than was previously thought.

ACKNOWLEDGEMENT

The author is greatly indebted to Late Prof. P.K. Pal, Palaeobotany and Palynology Section, Department of Botany, The University of Burdwan, West Bengal, India for his help in the identification of the species of macrofossils presented in this paper and for his suggestions in preparing the manuscript. The author is also indebted to Dr. Amiyanimai Choudhuri and Mr. Kajal Krishna Das of the Department of Botany, Burdwan Raj College, Burdwan, West Bengal, India for their help in photography of the specimens.

DISCLAIMER

The author(s) declare no conflict of interest in the work.

REFERENCES

Chandra, S. and Prasad, M.N.V. 1981. Fossil plants from the Kamthi Formation of Maharashtra and their biostratigraphic significance. *The Palaeobotanist*, **28-29**: 99-121.

Chandra, S. and Rigby, J.F. 1983. The Filicales from the Lower Gondwanas of Handappa, Orissa. *The Palaeobotanist*, **31**(2): 143-147.

Goswami, S., Das, M. and Guru B.C. 2006a. Permian biodiversity of Mahanadi Master Basin, Orissa, India and their environmental countenance. *Acta Palaeobotanica,* **46**(2): 101-118.

Goswami, S., Singh, K.J. and Chandra, S. 2006b. Pteridophytes from Lower Gondwana formations of the Ib River Coalfield, Orissa and their diversity and distribution in the Permian of India. *Journal of Asian Earth Sciences*, **28**: 234-250.

Goswami, S., Singh, K.J. and Chandra, S. 2006c. Palaeobotany of Gondwana basin of Orissa state, India: A bird's eye view. *Journal of Asian Earth Sciences,* **28**: 218-233.

Herbst, R. 1978. *Asterotheca golondrinensis* n. sp. from the Lower Permian Golondrina Series of the Bajo de la Leona, Santa Cruz, Argentina. *Palaeobotanist*, **25** (1976, Silver Jubilee Vol.): 126-130.

Lele, K.M., Maithy, P.K. and Mandal, J. 1981. *In situ* spores from Lower Gondwana ferns- their morphology and variation. *Palaeobotanist*, **28-29**: 128-154.

Maheshwari, H.K. 1965. Studies in the Glossopteris flora of India – 23. On two fructifications from the Raniganj stage of the Raniganj Coalfield, Bengal. *Palaeobotanist*, **13**: 144-147.

Maheshwari, H.K. and Prakas, G. 1965. Studies in the *Glossopteris* Flora of India-21. Plant megafossils from the Lower Gondwana exposures along Bansloi river in Rajmahal hills, Bihar. *Palaeobotanist*, **13**(2): 115-128.

Maithy, P.K. 1974. A revision of the Lower Gondwana *Sphenopteris* from India. *Palaeobotanist,* **21**: 70-80.

Maithy, P.K. 1975. Some contribution to the knowledge of Indian Lower Gondwana ferns. *Palaeobotanist*, **22**: 29-38.

Mandal, A. and Gupta, S. 2015. Palynoassemblage and environment of deposition in the lower Gondwana sediment (Raniganj formation) of Sonepur-Bazari coalfield in Burdwan district, West Bengal. *Journal of Plant Develoment Sciences*, **7**(2): 91-97.

Murthy, S., Chakraborti, B. and Roy, M.D. 2010. Palynodating of subsurface sediments, Raniganj Coalfield, Damodar Basin, West Bengal. *J. Earth Syst. Sci.*, **119**(5): 701-710.

Pant, D.D. and Misra, L. 1976. Compressions of a new type of pteridophyll, *Asansolia* gen. nov. from the Lower Gondwanas of the Raniganj Coalfield, India. *Palaeontographica*, B. **155**: 129-139.

Saxena, A., Singh, K.J., Cleal, C.J., Chandra, S., Goswami, S. and Shabbar, H. 2019. Development of the *Glossopteris* flora and its end Permian demise in the Tatapani-Ramkola Coalfield, Son-Mahanadi Basin, India. *Geological Journal,* **54**(4): 2472-2494.

Srivastava, P.C. 2008. Some aspects of Palaeozoic pteridophytes of India : A critical reappraisal. *The Palaeobotanist,* **57**: 119-139.

Surange, K.R. 1966. *Indian Fossil Pteridophytes. Bot. Monogr. 4*. C.S.I.R. Publ. Delhi.

Tewari, R. 2007. The *Glossopteris* flora from the Kamptee Coalfield, Wardha Basin, Maharashtra, India. *Palaeontographica Abt. B* **277**(1-4): 43-64.

Tewari, R. 2008. The genus *Glossopteris* Brongniart from the Kamthi Formation of camp IV area, Wardha valley Coalfield, Wardha basin, Maharashtra, India. *Journal of the Palaeontological Society of India*, **53**(1): 19-30.

Wagner, R.H., Hill, C.R. and El-Khayal, A.A. 1985. *Gemellitheca* gen. nov. a fertile peco-pterid fern from the upper Permian of the Middle East. *Scripta Geol*., **79**: 51-74.

J. Botan. Soc. Bengal 79(1) : 121-122 (2025), ISSN 0971-2976

Obituary:
Dr. Balaram Majumdar (1931-2025)

Dr. Balaram Majumdar, a former member of the faculty of Bose Institute, Kolkata, one of the illustrious alumnus of Botany Department, Calcutta University and the immediate past President of our Society (2018 -2021) passed away quietly on April 24, 2025, leaving behind his wife, two sons and a daughter. A small, frail man of quiet demeanour, frugal unobtrusive lifestyle, yet bursting with energy – he personified a scholar whose humility outshines fame.

Born in August 10, 1931 to Hubul Chandra Majumdar and Giribala Majumdar in Krishnanagar of Nadia district West Bengal, with early education in a Calcutta Corporation school at Khidirpore, he had to flee Calcutta like many others, during the Second World War Japanese air raids. The family found shelter with Dr. Siddheswar Mukherjee, a reputed physician in Garalgachha village, Chanditala, in district Hooghly. He matriculated from the local high school in 1947, completed the FA examination from erstwhile Ripon College in 1949 and graduated with honours in Botany from City College, Calcutta in 1952. Observing young Balaram's keen interest in studies, Dr. Mukherjee went on to support him in education up to his Masters degree in Botany, at the University of Calcutta. He completed his M.Sc. in 1954. His abiding interest in plant genetics drew him close to Professor Arun Kumar Sharma. He joined Bose Institute as a research fellow to work under Dr. Sunando Bose, earned his doctorate degree and subsequently became a member of its faculty. Later, he left for Italy to work under Professor Barrygozi, at the Institute of Genetics, Milano. He visited different universities in Europe during 1967 to 1969 and made extensive studies on the genetics of maize. On his return to the country, he took up research on high-yielding rice varieties and came up with double-grained rice – one of the multi-faceted approaches to 'green revolution' in India. His interest shifted to developing salt-resistant rice varieties for the Sunderban areas, for which he made extensive trials in the experimental plots of Bose Institute at Taldi, close to Canning. Later, he took up research on gamma-irradiated rice varieties at the Falta field laboratory of Bose Institute – which was Acharya Jagadish Chandra Bose's 'Bagan Bari' – and came up with several improved varieties. His abiding interest in rice genetics took him to international conferences whether in Australia and Hanoi, or closer home at Chittagong and Dhaka. All important rice breeding stations from International Rice Research Institute, Philippines to Cuttack National Rice Research Institute and Chinsurah Rice Research Station have always kept close touch with Dr. Majumdar. He authored more than 150 research articles in reputed journals and in several journals of botanical and agricultural societies.

Following in the footsteps of Satyendranath Bose, he became a prolific writer of scientific articles in Bengali for the student community and the common man. In this role, he was closely associated with the Bangiya Bijnan Parishad created by Satyendranath Bose. He was its most elderly member, Vice-President and a regular contributor to its mouthpiece Jnan O Bigyan. He was closely associated with the Indian Science News

Association since inception, established by Meghnad Saha and Acharya Prafulla Chandra Ray. Dr. Majumdar was also associated with Bangiya Sahitya Parishad and Itihas Sansad. A prolific writer on popular science, he wrote no less than 500 articles in Bengali. He worked on the history of science in the country and the contributions of Ronald Ross in control of malaria. He built up the Garalgachha science club in his native village and helped organise numerous science fairs across the state.

Hailing from a poor middle-class background, Dr. Majumdar lost his father when he was only nine years. He had to struggle against extreme hardship to continue his education. He was drawn to the communist movement in his student days but later in life, Rabindranath and Gandhi became his loadstar. A complete self-made man with Spartan lifestyle, an old guard of science in Bengal in its sunshine years, we fondly remember his brisk walks to his goals, never looking back. We will miss you dearly, Balaramda.

Shyamal K. Chakraborty
Associate Editor

INSTRUCTIONS TO AUTHORS

Manuscript should be typed in 'Times New Roman' font with ~ 25 mm margins, one and a half spaced and mailed to the Editor at editorjbsb@gmail.com in the following formats: Microsoft Word for text and JPG/TIF format for figures and tables, in separate files. Do *not* submit PDFs, DTP files or LaTeX files. All pages should be numbered sequentially. Each line of the text should also be numbered, with the top line of each page being line 1. [Ensure the "Language" is "English (U.K)"]. Use of Large Language Models (LLMs) such as ChatGPT is not permitted.

Preparation of the manuscript: The manuscript should have a brief and informative full title followed by the names of all authors. A running title, not exceeding 75 characters, including spaces, should be provided. Each name of author should have an identifying superscript number ($^{1, 2, 3}$ etc.) associated with the relevant institutional address, to be entered further down the page. The corresponding author's name should have a superscript asterisk*. The institutional address (es) of each author should be listed next, being preceded by the relevant superscript number. The e-mail address of the corresponding author should also be provided

The *second page* will include the ABSTRACT written within 300 words without citation of references, to be followed by three to ten KEY WORDS that include the full botanical name(s) of any relevant plant material. If many species are involved, broad species groups or taxa may be mentioned instead. Core terms used in the title should be included in the key words, to make digital searches more effective. The Title, Abstract and Key words should be self-explanatory.

The *third* and *subsequent pages* will comprise the *text file* written in a lucid language. 'FULL LENGTH ARTICLES' will usually be structured into INTRODUCTION, MATERIALS AND METHODS, RESULTS, DISCUSSION, ACKNOWLEDGEMENTS, DECLARATIONS and REFERENCES. Mention of specialized items of equipment mentioned in MATERIALS AND METHODS section, should be followed by details of the model, manufacturer, city and country of origin. There should be no repetition of data in both tabular and graphical forms, in the RESULTS section. For other descriptive items including literature reviews, other kinds of subdivisions in the text are allowed. For SHORT COMMUNICATIONS, there is no abstract. REVIEW ARTICLES are generally commissioned by the journal. Unsolicited review articles may be considered for publication at the sole discretion of the Editor.

Numbers up to and including ten should be spelt out unless they are measurements. All numbers above ten are to be in numerals except at the beginning of sentences. Appropriate position of tables and figures should be indicated in the text. The journal style prohibits the use of footnotes within articles. If required, such notes may be merged parenthetically in the text, or as a separate paragraph.

Abbreviations should be used sparingly and should be spelt out on first use, with the abbreviation in parenthesis. Measurements should be given in SI or SI-derived units. [See the box item].Standard chemical symbols, names of chemicals (CO_2, ATP), procedures (SEM, UV, PCR, UPGMA), molecular terminology (SDS-PAGE, bp) or statistical terms (s.d., s.e., t-test, ANOVA) are accepted. Tables and figures should be numbered in Indo-Arabic (Figure 1, Table 2 etc).

Mathematical equations are to be used in proper symbolic form; word equations are not accepted. Symbols and equations may be typed directly into MS Word wherever possible. In other cases, use of the MathType software is recommended for display of inline equations, or sometimes the Equation Editor or Microsoft's Insert → Equation function, may be used. However, in running text, do not use MathType, Equation Editor, or the Insert → Equation function to insert single variables, Greek/ other symbols [like Δ, λ or $'$ (prime)], or mathematical operators (e.g. $\pm$, $\geq$, y). Also, these should not be used for only a part of the equation, and equations should not be a mix of different equation tools or appear in “hybrid” forms (part text - part MathType or part MathType - part Equation Editor).

Figures: A list of captions and legends to the figures should be submitted in a separate page. Photographs may be minimized to fit one column. If a plate has more than one photograph, they should all be clear and have the same resolution. If necessary, hand-drawing with India ink, on a Bristol board may be made. An 8-10 pt Microsoft Sans Serif font may be used for text labelling of figures. Use scale bars (instead of magnification factors) and error bars in the figures wherever necessary. For keys to figures, use *verbal cues* (e.g. open circle, closed red circle). Use of an appropriate statistical measure of central tendency (median/average) and error bars is advised, whereever appropriate. Indicate the sample size (*n* number), the test statistic and the *P* value. In calculating the degree of reduction of the figures, authors should keep in mind the column-size (7.5 cm) as well the page-size (16.25 cm x 20 cm) of the journal.

Tables: The tables should come in sequence at the end of the main text (article) file, in JPG/TIF format, and *not* inserted as an image/picture. Tables with statistical analyses should indicate the standard of errors and ranges in the table legend. For tables that feature chemical structures, the native ChemDraw file(s) need to be submitted separately as **.**cdx files. Tables related to experiments should have brief legends describing the experiments, so that data are intelligible.

Nomenclature of plants, genes and sequences: *Plant names* must be written out in full binomial form in the main text for

each organism at first mention, and duly italicized. Later, genus initial may be used followed by species name. The author citation is generally desired. *The International Plant Names Index* and *The Plant Book: a portable dictionary of plants, their classification and uses,* 4th ed., by D.J. Mabberley (Cambridge: Cambridge University Press, 2017) may be used for guidance to correct usage. Any cultivar or variety should also be stated where appropriate, using the ISHS *International Code of Nomenclature for Cultivated Plants* (2004), Eds., C.D. Brickell *et al* (ISBN 3-906166-16-3) for guidance. However, once defined in full, the plants may be named in their common vernacular or quasi-scientific forms without italics or uppercase letters (e.g. arabidopsis, sunflower, tomato), wherever convenient.

For gene nomenclature, authors should adhere to standard rules for nomenclature of genes and their encoded proteins. Species-specific gene nomenclature rules are available for arabidopsis, maize, rice and wheat. The names of genes or gene-families should be spelled out at first mention.

If authors intend to publish novel nucleotide or protein sequences, those should first be submitted in electronic form, at one of the major data repositories -- EMBL Nucleotide Sequence Database, GenBank, or the DNA Data Bank of Japan (for nucleotides) and UniProt or SWISS-Prot (for amino acid sequences). The accession number obtained is to be submitted. The sequence matrices may be presented in the paper only if their alignment is crucial to the core message of the publication. For chemical, biochemical and molecular biological nomenclature, the rules of the *International Union of Pure and Applied Chemistry* (IUPAC) and the *International Union of Biochemistry and Molecular Biology* (IUBMB) need to be followed.

Very large files may be submitted electronically as SUPPLEMENTARY INFORMATION.

Text citation: The author's last name and the year of publication for the source should appear in the text within parentheses - such as (Sharma and Sharma,1994) or (Sharma and Sharma,1994, a,b,c) to distinguish between papers of the same authors published in the same year.

Reference styles with examples.
The references are to be given after the ACKNOWLEDGEMENTS and DECLARATIONS section.
a) Journal articles
- with 1 author:

1] Chakraborty HL. 1954. Morphology of the staminate flowers of Cucurbitaceae with special reference to the evolution of stamens. *Bull Bot Soc Bengal* **8**:186-213.
- with 2 authors:

2] Becker B, Marin B. 2009. Streptophyte algae and the origin of embryophytes. *Ann Bot* 103: 999–004.
- with 7 authors or more: write the names of the first three authors followed by et al.

3] Rhie A, Nurk S, Cechova M *et al.* 023.The complete sequence of a human Y chromosome. *Nature* 121: 344–54.
b) Books and book chapters
i) An authored book
4] Sharma AK, Sharma A. 1994. *Chromosome Techniques: A manual.* Reading, UK: Harwood Academic Publishers.
ii) An edited book
5] Samajpati N, Chattopadhyay SB. 1984. (Eds.) *Interaction of plant pathogens in the host.* Calcutta: Oxford & IBH.
iii) A chapter in an edited book
6] Arora RK. 1981. Native food plants of north-eastern India. *In* Jain SK (Ed.), *Glimpses of Indian ethnobotany* New Delhi: Oxford & IBH, 91-106.
c) Doctoral dissertation
7] Ghosh M 1964. Cytogenetical and embryocultural work in rice (*O. sativa* L.) and related species. PhD thesis. University of Calcutta (unpublished).
d) Dataset with persistent identifier
8] Bennett MD, Leitch IJ. 2012. Plant DNA C-values database. (Release 6.0, Dec 2012) http://data.kew.org/cvalues[accessed 8 July, 2017].
e) Online-only journals

9] Behera PK, Kumar V, Sharma SS, Lenka SK, Panda D. 2023. Genotypic diversity and abiotic stress response profiling of short-grain aromatic landraces of rice (*Oryza sativa* L. *indica*. *Current Plant Biology* 33: 100269 https://doi.org/10.1016/j.cpb.2022.100269.

f) Pre-prints

10] Dolzhenko E,English A, Dashnow H *et al*. 2023. Resolving the unsolved: comprehensive assessment of tandem repeats at scale. Preprint at *Bio Rxiv.* doi: ttps://doi.org/10.1101/2023.05.12.540470.
g) Web links or URLs should come *directly in the main text* and not in the Reference section.

h) Scientific & Technical Reports:
11] Tomato Genome Consortium. 2012. The tomato genome sequence provides insights into fleshy fruit evolution. *Nature* 485: 635-641.
12] Cohen JT, Duggar K, Gray GM, *et al.* 2001. Evaluation of the potential for bovine spongiform encephalopathy in the United States. Boston: Harvard School of Public Health, Center for Risk Analysis, Report No. PB2002-108684, p.116. Supported by the US Department of Agriculture.
i) Software:
13] Felsenstein J. 2022. PHYLIP, v3.698, Dep. Genome Sciences & Dep. Biology, University of Washington https://doi.org/10.12345/ABC000999
A current issue of the journal or the *World List of the Scientific Periodicals* may be consulted, if required.
'Personal Communications' will not feature in the reference list, but it may be cited in the text.

Declarations :

Apart from Acknowledgements, all manuscripts should include the following (where relevant), under the heading 'Declarations': individual authors' contributions, statement on competing interests, statement on funding, consent for publication, information on availability of data and materials, and additional authors' information. Manuscripts reporting studies involving animals must include a statement on *ethics approval*, the name of the committee that approved the study, and its reference number if appropriate.

Proofs of the articles if sent to the authors, must be returned to the Editor within seven days of receiving the proofs. Corrections at the proof stage involving deviations from the text are not allowed and are chargeable to the authors. Only e-reprints will be provided to the corresponding author with effect from January 1, 2020.

Publication : Journal of the Botanical Society of Bengal is a peer reviewed journal and published bi-annually and issued usually during June and December of each year.

Editor-in-Chief : Professor Subir Bera, Department of Botany, University of Calcutta.

Cheques and bank drafts issued should be drawn in favour of **Botanical Society of Bengal**.

Name of Account Holder	:	Botanical Society of Bengal
Name of the Bank	:	UCO Bank
Branch Name	:	Ballygunge Circular Road
Branch Code	:	0837
Bank A/c No.	:	08370100001762
IFSC Code	:	UCBA0000837

Contents : The Editors will be glad to consider all types of the scientific communications and Book Reviews in Botany, Plant Biology and allied subjects.

Processing & printing charges: The rate will be ₹ 600.00 per page (wef. January 1, 2022). Extra charges will be levied on printing of coloured plates @ ₹ 4000.00 per plate.

Membership : Membership of the Botanical Society of Bengal is open to all persons interested in Botany. Membership forms are available from the Office of the Society, Department of Botany, University of Calcutta, Kolkata 700019. It can also be downloaded from the society website. The Botanical Society of Bengal is pleased to provide opportunities to students and research scholars to enrol as Student Members with an amount of ₹ 3000/ for M.Sc. students and ₹ 4000/ for research scholars. The status will be upgraded on a par with other members, after completion of their courses. The life membership will remains unchanged at ₹ 6000/.

Mail all your orders including back volumes and special issues to : Secretary, Botanical Society of Bengal, 35, Ballygunge Circular Road, Kolkata 700 019, West Bengal, India.

Twelve and half per cent discount are allowed to Book Sellers and Purchasers only.

Postage and packing charges extra.

Abbreviations and units of measurement to be used

Abbreviations ought to be spelled out at first mention. Use of the minus index to indicate 'per' (e.g. m^{-2}, $^{L-1}$, h^{-1}) is preferred, except in such cases as 'per plant' , 'per field' etc.

Wherever possible, use the *Systéme international d'unités* (SI units). Use of non-SI unit of protein mass such as Dalton (Da), or kilodaltons (kDa), is permitted.

Units of volume may be expressed in terms of cubic metre (e.g. 8×10^{-9} m^3, 8×10^{-6} m^3 or 8×10^{-3} m^3) or litre (e.g. 8 µL, 8 mL, 8 L), but this use must be consistent in the manuscript. Concentrations may be expressed typically as 8 mmol m^{-3}, 8 µM (for 8 µmol L^{-1}), or 40mg L^{-1}.

Common prefixes		***Units of mass***		***Units of concentration***	
tera (10^{12})	T	kilogram	kg	molar	M
giga (10^{9})	G	gram	g	millimolar	mM
mega (10^{6})	M	milligram	mg	micromolar	µM
kilo (10^{3})	k	microgram	µg	parts per million	ppm
deca (10)	da				
deci (10^{-1})	d	***Units of time***		***Units of temperature***	
centi (10^{-2})	c	hour	h	Kelvin	K
milli (10^{-3})	m	minute	min	Celsius	ºC
micro (10^{-6})	µ	second	s		
nano (10^{-9})	n	day	day		
pico (10^{-12})	p	month	month		
femto (10^{-15})	f	year	yr		
atto (10^{-18})	a				
Units of volume		***Units of length***			
litre	L	metre	m		
millilitre	mL	centimetre	cm		
microlitres	µL	millimeter	mm		
		micrometer	µm		
		nanometer	nm		

Journal Title is covered in *Biological Abstract, Review of Plant Pathology, Agricola Database, BIOSIS, CABS, Cambridge Scientific Abstracts, Current Literature in Plant Science,* and *Indian Science Abstract.*

Journal of the Botanical Society of Bengal for June 2025 (Vol. 79 No. 1) was issued on 30 June, 2025.

Executive Council : 2022-2025

President
Ruma Pal

Vice-Presidents
Probir Kumar Saha
Rita Kundu
Balaram Majumder (Ex-officio)

Secretary
Ashalata D'Rozario

Editor of Publication
Subir Bera

Treasurer
Maumita Bandyopadhyay

Business Manager
Santanu Paul

Librarian
Debabrata Maity

Members of the Council

Saurav Moktan
Soumitra Paul
Bharati Nandi
Radhanath Mukhopadhyay
Usha Chakraborty
Shyamal Kumar Chakraborty
Sushovan Bera
Debleena Roy
Madhab Naskar
Ahinsuk Barua
Animesh Bose
Dipu Samanta
Parimal Nag (Co-opted)

Form IV

(See Rule 8)

1.	Place of Publication	:	Kolkata
2.	Periodicity of its Publication	:	Bi-annual (June and December)
3.	Printer Nationality Address	:	Mrs. Shyamali Das Indian 2, P. C. Das Lane, Serampore, Hooghly, Pin - 712204
4.	Publisher Nationality Address	:	Ashalata D'Rozario Indian Botanical Society of Bengal, Department of Botany, University of Calcutta, Kolkata 700019, India
5.	Editor-in-Chief Nationality Address	:	Subir Bera Indian Botanical Society of Bengal, Department of Botany, University of Calcutta, Kolkata 700019, India
6.	Names and address of individuals who own the newspapers and Partners or Shareholders holding more that one per cent of the total capital	:	Botanical Society of Bengal

I, Ashalata D'Rozario, hereby declare that the particulars given above are true to the best of my knowledge and belief.

June 30, 2025

Sd/ Ashalata D'Rozario
Signature of Publisher

J. Botan. Soc. Bengal 79 (1) June, 2025 Registration No. RN 16610/67

JOURNAL OF THE BOTANICAL SOCIETY OF BENGAL

(UGC-CARE enlisted; NAAS Scores-2021 : 3.02)
VOLUME 79 NUMBER 1, JUNE 2025

CONTENTS

Department of Botany, University of Calcutta,
Taraknath Palit Siksha Prangan,
35, Ballygunge Circular Road, Kolkata 700 019, India
Website: www.botansocbengal.org
E-mail: secbsbengal1935@gmail.com

Published bi-annualy by Ashalata D'Rozario, Secretary, Botanical Society of Bengal, Department of Botany, University of Calcutta, Taraknath Palit Siksha Prangan, 35, Ballygunge Circular Road, Kolkata 700 019, India and Printed by Mrs. Shyamali Das, Anima Printers, 2, P.C. Das Lane, Serampore, Hooghly, Pin 712204.