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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.

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The figure in the cover page showing : *Apis dorsata* visiting flower of *Alangium salvifolium* in the article by Ankush Pal, *J. Botan. Soc. Bengal*, 77(1): 60-65, 2023

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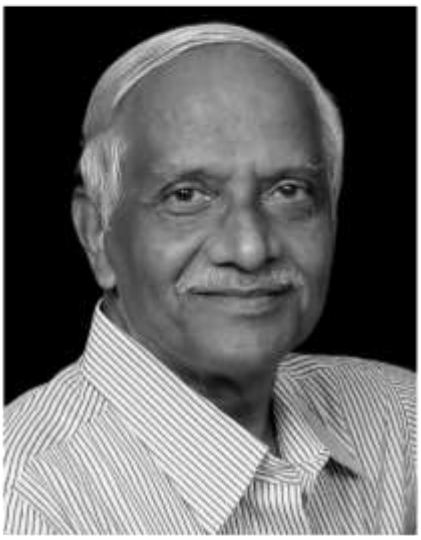
Editorial

Tribute to PROF. NANDADULAL PARIA (01.11.1950–14.01.2023) – a versatile botanist

The study of plant science remains incomplete without taxonomy. The diversity of plants in our planet needs to be understood and organized. Taxonomy deals with naming, describing and classification of plants. Classification is based on behavioural, genetic and biochemical variations. Characterization, identification, and classification are the processes of taxonomy. This classification helps us to easily communicate about plants. Taxonomy identifies and elaborates the components of biological diversity providing basic knowledge underpinning management and implementation of the Convention on Biological Diversity. Not many are there in this branch of botany as the intricacies of nomenclature and naming are not interesting to many. Among the list of a handful of plant taxonomists we lost one of the renowned and distinguished teachers, Prof. Nandadulal Paria of the Department of Botany, University of Calcutta, on 14th January 2023. Death has taken away an outstanding academician, genuine gentleman, a loving husband and father and deprived so many others including us all. Dr. N.D. Paria, Professor and former Head, Department of Botany, University of Calcutta, was actively engaged in teaching and research in Angiosperm Taxonomy, Biodiversity and Conservation, Biosystematics, Palynology, Ethnobotany, Medicinal Plants, Seed and Seedling Morphology.



Born in the district of Midnapore in 1950, Prof. Paria obtained his M.Sc. and Ph.D. degree from the University of Calcutta. He joined the Department of Botany in 1974 as Herbarium Keeper of Calcutta University Herbarium (CUH) and subsequently held the positions of Lecturer, Senior Lecturer, Reader and Professor of Botany in the same Department. He was the Head of the Department of Botany and Chairman of the Board of U.G and P.G. studies in Botany and Dean, Faculty of Fine Arts, Music and Home Science (Calcutta University), Convener, Ph.D. Committee in Botany, Calcutta University. Subsequently he became the Vice Chancellor of Vidyasagar University for about a year in 2011. He also served Netaji Subhas Open University and was also a member of different Selection Committees in colleges and universities.

He was associated with the Botanical Society of Bengal and served in the capacity of President, Secretary, Executive Council member, as well as Chief Editor, Journal of Botanical Society of Bengal. He was also the Associate Editor, Science and Culture, Indian Science News Association.

Prof. Paria's notable contribution to floristics includes the account of the 'Flora of Ballygunge Science College Compound', 'Herbaceous flora of South 24 Parganas', 'Chilkigarh' and 'The Flora of Hazaribagh District, Bihar'

published by the Botanical Survey of India. His interests in medicinal plants led to the publication of 'Medicinal Plant Resources of South West Bengal' published by the Directorate of Forests, Government of West Bengal. He was the contributing author for the Botany part of 'A Treatise of Indian Medicinal Plants' volumes I-VI edited by Prof. Asima Chatterjree and Dr. S.C. Pakrashi, published by the CSIR. He was the member of the editorial board of the book "Biodiversity of Raj Bhaban", Kolkata, West Bengal published in 2015. He introduced and developed the research in seedling morphology in India and is considered as a pioneer in this field of research. He supervised twenty one Ph.D. theses and published more than 104 articles in national and international journals and 10 books.

Prof. Paria was the recipient of the Finnish Government Post-Doctoral Scholarship, Prof. V. V. Sivarajan Gold Medal by Indian Association for Angiosperm Taxonomy (IAAT), Calicut, Kerala, Dr. S.K. Jain Gold Medal from the Association for Plant Taxonomy, Dehradun. He was the Fellow of the Linnaean Society of London, Indian Botanical Society, Indian Association for Angiosperm Taxonomy, Association for Plant Taxonomy and West Bengal Academy of Science and Technology. He was life Member of Botanical Society of Bengal, Indian Science Congress Association and Indian Science News Association. He visited and studied the boreal flora and vegetation of Finnish Lapland and Aland Islands, Botanical Garden of Linnaeus in Uppsala in Sweden, the Palynological Laboratory of Naturhistoriska Riksmuseet (Stockholm), and Institute of Systematic Botany (Uppsala) for exchange of knowledge; V.L. Komarov Botanical Institute, Leningrad, USSR for academic interactions; Jordrell Research Laboratory, Royal Botanic Garden and Herbarium in Kew; British Museum of Natural History, London; Museums, Libraries, Plant Science Laboratory and Herbarium in Department of Botany, University of Reading, U.K. in collaboration with the British Council, Calcutta and London for academic interactions. He also visited Rijks herbarium and several Research Laboratories in Leiden, Netherlands for academic interest.

Life can be fleeting, but a life lived to the fullest leaves behind fond memories. Prof. Paria through his charming personality, decorous behaviour, thoughtfulness, love and concern for others is engraved in our minds. His untimely and sudden demise has been painful for his family as well as to all the Botany fraternity.

Subir Bera
Ashalata D'Rozario

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REVIEW

Old enemy, new tools: Tackling Late blight epidemics in the genomics era

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Epidemics or pandemics is a term we often associate with human diseases, especially after COVID-19. But pandemics aren't just for humans — they also sweep through the plant world. In an era of animal pandemics — tuberculosis, smallpox, COVID-19 — it's easy to overlook the fact that plant epidemics can have an equal or more devastating impact on human society. Even though plants are essential not only for over 98% of the oxygen we breathe and 80% of the food we eat but also for 70% of the drugs we use, besides a myriad of other dependent functions, yet, plant health is not a very familiar term, in contrast to human health. Human global health and plant global health are linked. Food security and livelihoods are linked to agriculture and human health is linked to the food we consume. While it is pretty obvious that plant pathogens causing epidemics on food crops compete with humans for the same food but it still may not be very apparent in our perceptions that they are therefore our enemies. Healthy plants hold the key to the sustainable intensification of agriculture for feeding the growing global population by 2050. Therefore recognition, advocacy, and support for the promotion of plant health are of paramount importance for a food-secure world based on stable and sustainable ecosystems.

Ironically, the UN's 2020 International Year of Plant Health (IYPH), whose goal was to bring about enhanced awareness about plant health, coincided with the peak of COVID-19 infections around the world and had to be extended till 2021 and once again the immediate human health concerns masked the important issue of plant health. However, the Covid experience has made humankind wiser to the fact that animal, human, and environmental health are interconnected, resulting in the 'One Health' concept. One Health has been defined as an approach to the pursuit of public health and well-being that recognizes the interconnections between people, animals, plants, and their shared environment (WHO, 2021). This perspective can help optimize net benefits from plant protection, realizing food security and nutrition gains, while minimizing unintentional negative impacts of plant health practices on people, animals and ecosystems (Hoffmann *et al*., 2022) and perhaps, therefore, is poised to change our understanding regarding the urgency of protecting plant health for human existence.

Plant diseases causing epidemics that have societal impacts are not new (Ristaino *et al*., 2021; Schumann and D'Arcy, 2012), but the approaches for its management have changed which now envisages having improved early detection systems, understanding the attributes of genome biology and population structure of the pathogen related to disease formation, undertaking routine plant disease surveillance in crop fields, and following it up with

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field pathogenomics where feasible; all to help with better grower decisions resulting in preventing the build-up of epidemics through an integrated Decision Support System. Also, as the issues of plant health and biosecurity are borderless and plant pathogens do not respect political boundaries, therefore the approach to tackling them successfully must transcend national boundaries through international collaborative efforts. The strategy must be based on three key principles; (a) risk anticipation (b) risk surveillance and management (c) risk awareness and communication. These require considerable resources and as past experiences have shown, the only way forward is through the formation of global teams and consortia. The overall goal is to detect emerging plant disease outbreaks early on – and contain them – before they begin to spread and cause severe damage. Reducing crop losses from existing and emerging plant health threats is critical for achieving the United Nations Sustainable Development Goal 2 which strives for zero hunger.

Today, we have several plant health threats which are existing, emerging and even remerging, all of which have epidemic potential. However, we also reassuringly have some recent encouraging examples of the use of the above-mentioned new tools as well as active international collaboration which have resulted in epidemics which were prevented or better managed. One such example of an emerging disease is that of the recent 2016 wheat blast (WB) in Bangladesh caused by a South American lineage of a hemibiotrophic filamentous fungus Magnaporthe oryzae Triticum (MoT) pathotype possibly as a result of grain imports into Bangladesh. (Callaway, 2016; Islam *et al.*, 2016). The international coordinated efforts not only allowed detection, strain identification and genome analyses rapidly within a period of about 6 weeks (diseased symptoms first seen in early February 2016, field collection on March 16th and on April 27th 2016 analyses completed) enabling timely management of the epidemic which would have otherwise threatened the food security of Bangladesh and also spread to India. The introduction of WB into Bangladesh had caused great concern for food security in the South Asia region, home to 300 million undernourished people and whose inhabitants consume over 100 million tons of wheat each year. Immediately after the 2016 WB outbreak in Bangladesh, the Government of Bangladesh, the International Maize and Wheat Improvement Center (CIMMYT) and FAO took prompt action and led to the formation of an emergency task force to develop immediate and short-term recommendations for the Ministry of Agriculture to mitigate WB disease in Bangladesh. The Indian Government similarly formed expert committees/task forces and on its part along with other measures ensured a 'wheat holiday' by banning its cultivation near the borderline (~2,200 km) and restricting the movement of wheat grains from the infected area. All these measures have borne fruit and since November 2019, the 'wheat holiday' is finally over in India.

In this instance, genomic surveillance was rapidly applied to monitor plant disease outbreaks and provide valuable information regarding the identity and origin of the infectious agent. Then field pathogenomics (transcriptome sequencing of both symptomatic and asymptomatic wheat leaf samples from fields in different regions of Bangladesh) was used to create RNA-seq libraries using Illumina technology and after trimming, the high-quality reads were aligned to both the *M. oryzae* wheat blast fungus BR32 and wheat genomes. Analyses of the aligned sampled transcriptome indicated that *M. oryzae* was present in symptomatic (infected) wheat samples from Bangladesh. Thus, the identity of the blast fungus which was present in the infected wheat fields of Bangladesh was identified unambiguously and very rapidly. Further, phylogenomic approaches determined how related the fungal pathogen detected in wheat leaf samples from Bangladesh was to *M. oryzae* lineages infecting cereals and grasses and the population genomics analyses allowed insights into the geographic origin of these Bangladeshi isolates. Currently, multiple vigorous approaches are on for identifying wheat blast resistance for rapid varietal development and dissemination (Juliana *et al.*, 2020; ACIAR, 2021; Singh *et al.*, 2021). Also, the EWS or the early warning system (https://beattheblastews.net/) is in place supported by a range of international and national funding agencies as well as research and extension partners [CIMMYT, United States Agency for International Development (USAID), Brazilian Agricultural Research Corporation (EMBRAPA), Climate Services for Resilient Development (CSRD) and the Bill and

Melinda Gates Foundation (BMGF) supported Cereal Systems Initiative for South Asia (CSISA)]. Contrast this with the Cassava Mosaic Disease (CMD) outbreak in Southeast Asia a year earlier in 2015. It could spread because concerns within the region about the economic implications of recognizing the presence of the disease led to further delays in issuing a region-wide alert, allowing CMD to spread into neighbouring regions and countries. (Guha Roy, 2019c).

Another example of an existing disease which benefited from global concerted efforts is the Panama disease in banana, caused now by a strain of the soil-borne fungus, *Fusarium oxysporum* f.sp. cubense tropical race 4 (TR4). The TR4 strain in recent years is spreading globally, with a recent introduction into the Americas. The recent report on the spread of TR4 into the Indian subcontinent is of major concern since India is the largest producer of bananas worldwide. (Thangavelu, *et al.*, 2019). A new initiative under OneCGIAR titled Plant health and rapid response to protect food and livelihood security is being formulated by a team of CGIAR and non-CGIAR partners, led by CIMMYT and Alliance of Biodiversity International and CIAT. The Plant Health Initiative aims to protect agri-food systems of the low- and middle-income countries in Africa, Asia, and Latin America from devastating pests and diseases, including diseases such as Fusarium wilt race TR4 of banana, by leveraging/building strong surveillance, diagnostics, and integrated disease/pest management networks across an array of national, regional, and global institutions.

A classic example of a remerging pathogen (Fry *et al*., 2015) causing epidemics is that of the late blight (LB) of potato (and tomato) caused by the oomycete Phytophthora infestans which has plagued humankind for more than 150 years now. The discovery of *P. infestans* as the LB causal organism initiated the study of the discipline of plant pathology and its detection in 1846 contributed to shaping Louise Pasteur's germ theory which was announced formally almost 20 years later. There are earlier recorded instances of this disease, causing epidemics leading to famines (Bourke, 1964), deciding the outcome of wars (Millar and O'Brien, 1955) and being used as a bio-weapon (Madden and Wheelis, 2003), making it one of those rare pathogens that have shaped human history and continues in current times to not only cause economic losses but also socio-economic disruptions (Guha Roy 2015; Fry, 2016). The pathogen relies on its genome architecture driven by a repeat and transposon driven expansion giving rise to a 'two speed genome' which provides a wide repertoire of ever-changing effectors (Dong *et al*., 2015). It's rapid 'polycyclic life cycle' enables the production of large amounts of inoculum in a short period. These make it an effective and aggressive pathogen and the high mutation rate of its effectors enables it to remain as a reemerging pathogen across the years.

Similar to most other eukaryotic phytopathogens, one mode of propagation is through the sexual cycle, and in this case, they produce oospores mediated by the presence of two opposite mating types A1 and A2 and the other is through a clonally propagated asexual cycle. Sexually propagated lineages carry with them the emergent possibility of having new genotypic and phenotypic variations giving rise to better-adapted pathogens, and thereby a higher risk of epidemics. While sexual reproduction is yet to be detected in India to date, worldwide it is mostly restricted to Eastern European and South American locations. *P. infestans* populations are therefore subjected to mating type determination tests as a part of any routine biosurveillance to understand the possibility of arising of new lineages through sexual reproduction. PCR-based marker mating type detection (Jong *et al*., 2005; Lee and Kim, 2002) needs only a couple of hours to detect the mating type, in contrast to the older culture-based method requiring almost a week. Similarly, mitochondrial haplotyping allows detection of the identity and tracing of the strains, again done with a combination of PCR-based markers and restriction digestion-based assay (Griffith and Shaw, 1998).

The repertoire of genomic resources currently available for the genus Phytophthora in general and *P. infestans* in particular (Haas *et al*., 2009) has allowed us to further understand the nature of the populations causing the epidemics. It has made it possible to include the survey of effector sequences of candidate effectors/Avirulence homologues (Avh) in the biosurveillance routine as effectors have a role in virulence (Bos *et al*., 2010; Whisson *et al*., 2016). A

large no. of them have been found in *P. infestans* [Avr2, Avr4, Avr-blb1, Avr-blb2 (Armstrong *et al*., 2005; van Poppel *et al*., 2008; Vleeshouwers *et al*., 2008; Oh *et al*., 2009) and IPI-O family of effectors (Chen *et al*., 2012)]. Regional variations in isolates have also been found. These effectors affect the pathogenicity and fitness and may be under specific selective pressures after mutation, being disseminated through genetic drift, in response to host populations or other local factors. Local adaptation seems to be playing an important role in the diversification and distribution of the effector alleles/haplotypes in natural and agricultural environments. (Stukenbrock *et al*., 2009). Therefore, knowing about the effectors harboured by the regional *P. infestans* isolates will bring about an informed choice about the type of host cultivars to be sown as the functionality of R genes and effector allele combination will be known, and thereby lessen the chances of crop failure due to late blight. The knowledge will also be informative about the adaptive history of *P. infestans* in this region.

The availability of these genomic resources have further allowed the designing of specific multiplexed SSR markers (Li *et al*., 2013) for genotyping of *P. infestans* populations, capable of detecting the variations at clonal and even sub-clonal levels. Detection of such variation is extremely important to delineate and identify the Multi Locus Genotypes (MLGs)/clonal lineages in epidemic populations. Genotyping efforts worldwide have identified more than 80 genotypically distinct clonal lineages and many 'unknown' genotypes are yet to be formally designated while 'new' clones and sub-clones continue to arise. In India, the aggressive, European-origin 13_A2 clonal lineage continues to dominate for the last couple of years and a similar situation exists in the Indian subcontinent (Dey *et al*., 2018; Guha Roy *et al*., 2021).

These genotypically delineated MLGs/clonal lineages can then be further characterized for their field attributes like fungicide sensitivity, growth temperature, and host specificity, all of which are important parameters for determining the possibility of formation and determining the severity of an epidemic. For example, the European lineage, EU_33_A2 is resistant to the fungicide fluazinam (Schepers *et al*., 2018), similarly, EU_43_A1 lineage is resistant to fungicide mandipropamid (Abuley *et al*., 2023). There are also several host-specific lineages, US lineages like US-8, US-14, and US-24, are predominantly virulent on potatoes, while US-23 infects both tomato and potato (Seville and Ristaino, 2019). Similar variations also exist at even sub-clonal *P. infestans* populations in India (Dey *et al*., 2018). These temporal and spatial variations of phenotypic or field attributes amongst lineages within *P. infestans* makes it essential to characterize the lineages for disease management. Knowledge of the fungicide sensitivity and host specificity of the lineages infecting the field will allow precise spraying schedules and reduced management costs. Similarly, knowledge of growth and sporulation temperatures of the lineages affecting the crop will allow precise prediction about the 'disease window' when correlated with the prevailing or predicted weather conditions, and thereby again, reduced management cost. All these measures in combination would reduce the possibility of the formation of an epidemic. Routine biosurveillance and genotyping are necessary as not only do the populations change both genotypically and phenotypically but also become genotypically complex over time (Guha Roy 2019b; Guha Roy *et al*., 2021). To ensure the correct management options, the identity of the disease causing lineages of *P. infestans* populations in the target region must be accurately known. Genotyping of isolates with a uniform set of these 12-plexed SSR markers not only identifies the lineages but also allows tracing of migration patterns around the globe and standardization of data across genotyping centres/laboratories worldwide, thereby enabling strengthening of quarantine procedures where needed, and also, addressing biosecurity concerns. Furthermore, the linkage of their genotypic and phenotypic attributes, allows management options to be replicated from elsewhere and applied quickly (Guha Roy, 2019a). Once the invading disease-causing specific *P. infestans* clonal lineage/(s) is/are detected by its SSR profile, and since the clonal lineage already has the management option mapped onto it, which had been derived from its linked phenotypic data, the fungicide options and other attributes become immediately available and can be applied directly to the new population, thereby preventing the formation of an epidemic.

Phenotypic or field attributes were traditionally used for management, but the recent advancement which allows the identification of genotypic clonal lineages and mapping their specific attributes have made disease management more precise. The underlying crux of these modern bio-surveillance routines (which integrates genotypic population data as discussed above with phenotypic or field attribute data) is to detect the population changes both spatially and temporally for effective management as pathogen populations cause epidemics rather than a single pathogen. This was a paradigm shift in the management of LB disease. This information has now been mapped onto existing Decision Support Systems (DSS) to provide location-specific precise management options not only for preventing disease-associated economic losses but also for preventing inoculum build-up towards an LB epidemic (Small *et al*., 2015; Fry, 2016).

Translating these outputs from the laboratory (derived from routine bio-surveillance of farmers' fields in potato growing areas) back to the farmer is a major achievement towards preventing crop loss and LB epidemics. However, international cooperation and coordination are necessary which are an integral part of disease management to trace migrating populations and rapidly share epidemic outbreaks by new clones or information about fungicide insensitivity and aggressive clones arising elsewhere. Various regional consortia like the EuroBlight, for Europe (https://agro.au.dk/forskning/internationale-platforme/euroblight/); USABlight, for USA (https://usablight.org/); Tizon Latino, for South America (https://tizonlatino.github.io/quienes-somos/); AfricaBlight (for Africa) and AsiaBlight, for Asia (https://www.asiablight.org/) integrate not only biosurveillance but also all other activities related to control of the Late blight disease and is involved in cross-talk with each other. EuroBlight has been functioning since 1996 and is a very active multi-disciplinary, multi-actor consortium and network launched with initial funding from the EU. USA Blight was created with funding from UDSA's Agriculture and Food Research Initiative (AFRI) grants. A late entrant is the Tizón Latino cooperation network which was established in 2014 within the framework of the XXVI Congress of the Latin American Potato Association in Bogotá, Colombia. The Africa Blight, however, is yet to take off fully.

AsiaBlight too was similarly launched in 2014 and from 2018 is being mentored by International Potato Center (CIP), Lima – Perú (https://cipotato.org/) which is an international legal personality duly registered with the Secretariat of the United Nations under Art 102 of the UN Charter and is one of the 15 CGIAR Research Centers spread worldwide (https://www.cgiar.org/) which are independent, non-profit research organizations, conducting innovative research and is the world's largest global agricultural innovation network. India, represented by the author, (https://www.asiablight.org/the-team/) is a member country of AsiaBlight along with China, Japan, the Republic of Korea, Bangladesh, Pakistan, Nepal, Vietnam, Georgia, Indonesia, Tajikistan, Kyrgyzstan, Uzbekistan, and the Philippines. AsiaBlight is extremely relevant for India as not only is it the 2nd largest potato producer in the world (China is ranked 1st) and eastern India (with West Bengal contributing the major share) but also, Late blight is the most serious limiting factor for potato crop cultivation. Accordingly, of the nine Late blight genotyping centres in the world, two are in Asia. India has one situated in the author's laboratory at West Bengal State University which is also an additional hub for SAARC nations, the other is in China at the CIP–China Center for Asia-Pacific (CCCAP) (https://www.asiablight.org/genotyping-labs/).

Elvin C. Stakman, an internationally renowned pioneering plant pathologist of American origin who worked on stem rust of wheat (another disease with epidemic potential), is credited with improving crop yields both in North America and worldwide as part of the Green Revolution, and was also Norman Borlaug's mentor influencing him to pursue a career in phytopathology. He gave a speech in 1938 entitled "These shifty little enemies that destroy our food crops". Stakman's adage, “Plant diseases are shifty enemies” interestingly holds even today, 85 years after, primarily because pathogens will continue to co-evolve. In the case of *P. infestans*, the high rate of effector evolution is an added concern and is one of the reasons why it has been so successful over centuries. Therefore to 'know thy enemy', surveillance and

detection along with genotypic and phenotypic characterizations must not cease neither should international coordination efforts, if we are to defeat this enemy soon.

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FULL LENGTH ARTICLE

Pharmacognostic study and evaluation of antioxidant property of *Bauhinia acuminata* L. – an important folk medicinal plant

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The bioassay and drug evaluation of medicinal plants of various herbal systems is getting more momentum throughout the world. Now it is very important to validate the data stored in different herbal medicine systems scientifically in respect of their pharmacognostic, phytochemical, and pharmacological attributes. Evaluation of the quality and purity of crude drugs targeting various parameters is the most important aspect of pharmacognosy. The current study focuses on many pharmacognostic features of the leaf and bark of *B. acuminata*, a medicinal plant of the family Fabaceae. The leaves and bark are used in folk medicine to cure malaria, as anti-nociceptive, astringent, cooling agent, in ulcer and asthma. The micromorphological, organoleptic, physical and chemical parameters, and antioxidant activity have been considered for the pharmacognostical evaluation of the leaf and bark parts of this medicinal tree. The plant bears amphistomatic leaves and stomata were mainly of paracytic type with a minimum occurrence of anomocytic type. Palisade ratio was 4.52±0.21 and stomatal index was 29.85±2.31. Chemical tests of both bark and leaf methanol extracts highlighted the presence of alkaloids, flavonoids, reducing sugars, tannins and saponins. Ash value and moisture content of bark part were estimated as 9.1%±0.11 and 10.4%±0.3, respectively which are higher than the values of respective parameters of the leaf. Quantitative chemical analysis of leaf and bark extracts showed the higher amounts of phenolics and flavonoids in leaf that straightly corresponded with its higher antioxidant activity than the activity of bark. Most of these findings could provide the scientific criteria for correct identification and establishment of the standard of both drug parts of *B. acuminata*.

Keywords: *Bauhinia acuminata* L., pharmacognostic characters, phytochemical contents, antioxidant activity.

INTRODUCTION

Plants harbor a great variety of chemical compounds that manifest wide range of structural and physicochemical characteristics, as well as many significant biological activities. In numerous cultures around the world, medicinal plants have been recognized as reliable sources of medications used for treatment of a variety of diseases, including inflammatory problems, auto-immune diseases, diabetes, obesity, cancer, and many more. Many of the commercial pharmaceuticals (e.g., atropine, aescin, digoxin, reserpine, silymarin, teniposide, and others) were developed from plants and other biological sources used in the conventional medicine. Knowledge of the traditional uses of crude plant products plays a large role in drug discovery and

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development (Garzon-Castano *et al*., 2018). Many significant therapeutic plants have already had their pharmacognostic and phytochemical analyses. However, there are still a huge number of traditionally used medicinal plants that are unexplored, necessitating a thorough scientific investigation to assess their pharmacognostic standards, phytochemical characteristics, and diverse biological activities. Accurate knowledge of such crude medications is crucial for the manufacture, safety, and effectiveness of herbal products. In order to authenticate the herbal raw ingredients, it is therefore important to standardize and document the characteristics of raw materials used in production of the herbal medicines. Pharmacognostic criteria essentially give complete details of the unprocessed medications for their accurate identification and quality maintenance (Ray and Rahaman, 2018).

B. acuminata L. belongs to the family Fabaceae of flowering plants. It is native to tropical south- eastern Asia. Different parts of this plant such as bark, roots, leaves and flowers have been used in traditional medicine for curing a wide range of health conditions namely digestive disorders, leprosy, tuberculosis, asthma, fever, stones in urinary bladder and many more. Among the parts used, bark and leaves of B. acuminata are very commonly employed as an effective cure for most of the diseases reported (Kirtikar and Basu, 1935; Khare, 2007). For this reason these two plant parts have been targeted by the scientists for their chemical and pharmacological evaluation.

Previously, scientists have reported that the methanol extract from the leaf part of this plant has significantly displayed certain biological activities such as antioxidant, anti-inflammatory, antimicrobial, and membrane stabilizing functions (Khan *et al*., 2014; Ferdous *et al*., 2014). The aqueous and alcoholic extracts of leaf showed anti-nociceptive activity and an acute toxicity study displayed that the extracts are nontoxic (Padgaonkar *et al*., 2018). The ethanolic extract of leaves had exhibited effectiveness against diarrhea (Islam and Fahad, 2014).

Despite its above mentioned studies on phytochemistry and bioactivity, a few pharmacognostic studies of only the leaf part of this plant have been conducted (Gupta *et al*., 2018; Sebastian and Nirmal, 2020). However, there are some gaps in the detailed pharmacognostic evaluation of leaf part, and also no studies on pharmacognostic parameters of the bark have been performed earlier. Consequently, the main objective of this study was to investigate the pharmacognostic, phytochemical, and antioxidant profiles of both leaf and bark parts of *B. acuminata* as these two parts of the plant are very commonly used as crude drugs for various healing purposes.

MATERIALS AND METHODS

Material

Scientific name: *Bauhinia acuminata* L.
Synonyms: *Alvesia bauhinioides* Welw., *Bauhinia candida* Roxb., *B. grandiflora* Juss., *B. linnaei* Ali.
Common English name: Mountain ebony, Dwarf white orchid tree.
Bengali vernacular name: Swet Kanchan.
Parts used: Leaf, root and stem bark.
Botanical characters: A small tree, 2.5-3.5 m tall. Stem smooth, upright, with messy slender branches. Leaves bi-lobed, obovate, 3.5-9 cm x 0.8-1.2 cm; petiole long, pubescent, 2-3 cm; stipule 6-7mm. Flowers large, cream or white, in axillary racemes; bracts lanceolate, 6-7 mm. Calyx spathaceous, connate below, cleft into 5 subulate teeth at tip. Petals 5, unequal, elliptic to oblanceolate. Stamens 10, anthers 4-7 mm long with puberulent connective. Fruit legume, linear and flat, glabrous, dark brown. Seeds 5-13, small, flat, suborbicular and smooth (Fig.1A).
Flowering and Fruiting time: February - May.
Distribution: It is widely distributed in Southeast Asia such as Indonesia, Philippines, Malaysia. In India, the plant is found in Maharashtra, Pune, Karnataka, Kerala, Assam, Meghalaya, Uttar Pradesh, West Bengal and some other states.

Medicinal uses

Bark: Bark serves as a cooling and astringent agent. Decoction of it is used to treat dysentery. Additionally, it works well for strangury, leukoderma, biliousness, tuberculosis, asthma, cough, ulcers, vaginal discharges, and as an anthelmintic (Kirtikar and Basu,

1935; Sanjeev and Raphael, 2017).

Leaves: Decoction of leaves is used to cure malaria. In folk medicine, the paste of leaves and calcium hydroxide is applied to abscesses for quick opening and clearing of pus. Leaf paste is applied to sores, wounds, itch, bone fractures, and skin diseases. The leaf is used in curing urinary bladder stones, fever, throat infection, chicken pox, venereal diseases, leprosy, asthma, and digestive diseases (Khare, 2007; Sanjeev and Raphael, 2017).

Root: The root parts work wonders for curing ulcers and inflammation, as well as cough, cold, and sore throat. Roots are also applied to cure burns (Sebastian and Nirmal, 2020).

METHODS

Collection and sample preparation: The fresh leaves and bark of *B. acuminata* were collected from the plants grown in Mrinalini Girls' Hostel area, Santiniketan (23.6803ºN, 87.6888ºE) in the month of January, 2021. The plant materials were properly washed with tap water and dried in the shade. Then the dried materials were coarsely powdered and further utilized for preparation of extracts. The nomenclature of the identified plant species has been updated following the standard website like Plants of the World Online (https://powo.science.kew.org/).As future reference, a herbarium was prepared using the collected plant specimens and stored in the Department of Botany at Visva-Bharati, Santiniketan, India. [Voucher specimen number: INDIA, West Bengal, Birbhum district, Santiniketan, 14.04.2021, CHRZA 100 (VBH)].

Study of foliar micromorphology: For mounting a drop of 10% glycerine and 1% aqueous safranin was added to the cleared leaf samples on a slide. Then this slide was examined under a compound light microscope. Leaf-clearing was done by Bokhari's method (1970). In this method leaf pieces (apical, middle & basal part regions) were treated with KOH (10 – 20 %) solution for 10–12 h. in a petridish. Pieces were washed with distilled water. Few drops of hydrogen peroxide were added to the distilled water taken in a petridish and pieces were kept in it for 3 – 4 h. Again, the pieces were washed with distilled water and transferred to the petridish containing Eau de Javelle solution (50g $CaCl_2$ +100g Na_2CO_3 + 1000mL Distilled water) till the removal of chlorophyll.

Vegetative anatomy (stem bark and leaf): Anatomical study was done by free hand sections of the leaf lamina, and stem bark of the selected plant, stained suitably following safranin-light green staining schedule (Johansen, 1940) and studied under a compound light microscope (ZEISS, AXIOSTAR plus, 176045).

Bark maceration study: The small pieces of barks (1cm long) were macerated following the standard method (WHO, 1998). The macerated sample was washed several times with distilled water, teased with needles, stained in 1% safranin solution and mounted on the slide with 10% glycerine and observed under microscope. The measurements were taken with standardized ocular micrometer.

Organoleptic study: Organoleptic study of the powdered samples of bark and leaf has been performed using sensory organs following the standard method which includes colour, odour, taste, exterior morphology and other properties (Alam and Saqib, 2015).

Physicochemical evaluation: Following the WHO norms, various physicochemical characteristics, including moisture content, ash value (total ash, acid insoluble ash, and water soluble ash), and extractive value of the powdered plant samples were determined.(Majid *et al*., 2021).

Determination of extractive value: 10 g of powdered samples were extracted separately with four different solvents (Methanol, Chloroform, Ethyl acetate, and Hexane). of 100 mL for 48 h. in a continuous shaking condition at room temperature. The extract was filtered and then it was dried in a vacuum using a rotary evaporator at a temperature of 45°C. After drying, weight of each solvent extract was noted and the extractive value was determined by the following formula (Borhade *et al.*, 2014):

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

Histochemical study: One to two drops of specific reagents (Wagner's, Dragendorff's, Mayer's, Lugol's, Millon's, Ferric chloride, Phloroglucinol, 1% lead acetate, etc.) were added to the transverse sections of

the bark and leaf petiole. Then it was kept for a few minutes to allow the specific reaction between reagents and phytochemicals present in the cells of the sections. Samples were then observed under the compound light microscope to detect different phytochemical groups localized in various tissue zones of the sections (Harborne and Williams, 1994; Trease and Evans, 2008).

Phytochemistry

Preliminary phytochemical screening: In accordance with accepted practices, methanolic extracts of powdered bark and leaf samples were utilized in various chemical colour reaction experiments with the aid of various reagents to identify various phytochemical groups present in the powdered samples (Pal *et al.*, 2014).

Estimation of total phenolic content: 0.5 g of powdered plant material was mixed with 5 mL of 80% methanol in a centrifuge tube. The mixture was then homogenized at 10,000 rpm for 20 minutes at room temperature. Supernatant was collected in a petriplate and it was allowed to dry at room temperature (24 to 26°C) for 5-6 h. After drying, the residue was taken in a test tube and dissolved in 5 mL of distilled water. Then 0.5 mL of folin-ciocalteau reagent was added to the test tube and volume was made up 10mL with distilled water. After 3 minutes, 2mL of 20% sodium carbonate was added to the test tube and mixed it thoroughly. The mixture was then boiled for one minute in a water bath and cooled it at room temperature. Then absorbance was measured at 650 nm wavelength against a blank containing the mixture of folin- ciocalteau reagent, distilled water and sodium carbonate reagent except the plant extract (Ainsworth and Gillespie, 2007).

Estimation of total flavonoid content: It was estimated by employing the aluminum chloride method. 100 mg of extract from each plant part was dissolved in 5 mL of methanol to make a stock solution, and 10 mL of methanol was added to make the volume. A test tube containing 0.5 mL of the sample extract was then filled with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride solution, 2.8 mL of distilled water and 0.1 mL of 1M potassium acetate solution. All the contents were then properly mixed by shaking the test tube. Using a Shimadzu UV-Vis1800 double beam spectrophotometer, absorbance was measured at 415 nm against the appropriate blank (Ray *et al.*, 2018).

Estimation of total tannin content: The powdered plant sample of 500 mg and 75 mL of distilled water was taken in a conical flask. It was then boiled for 30 minutes. After cooling, the boiled plant sample was centrifuged at 2000 rpm for 20 minutes. The residue was discarded and the volume of supernatant was adjusted to 100 mL with distilled water. The extract was then used for estimation of the tannins. One mL of the plant extract was taken in a volumetric flask containing 75mL distilled water. Then 5mL of Folin-Denis reagent and 10mL of sodium carbonate solution were added to the flask and volume was adjusted to 100 mL with distilled water. Content in the flasks was thoroughly mixed, kept 30 minutes and absorbance was measured at 700 nm on Shimadzu UV-Vis1800 double beam spectrophotometer. A blank was prepared with distilled water instead of the sample. Tannins were estimated after a calculation made with the help of standard curve of tannic acid (0.1mg/mL) and expressed as mg of TAE/g (Selvakumar *et al.*, 2019).

Estimation of total alkaloid content: The 1,10-phenanthroline method reported by Singh *et al.* (2004) was employed to measure the total alkaloid content of the plant samples with a few minor changes. 10mL of 80% ethanol was used to extract 100mg of powder materials. After filtering the mixture via filter paper, this liquid sample was centrifuged at 5000 rpm for 10 minutes. The recovered supernatant was utilized to estimate the total alkaloids in more detail. 1mL of plant extract, 1mL of 0.025M $FeCl_3$ in 0.5M HCl, and 1mL of 0.05M 1,10-phenanthroline in ethanol made up the reaction mixture. The mixture was incubated in a hot water bath for 30 minutes at a continuous temperature of 72°C. At 510 nm, the absorbance of the red-coloured compound was measured in comparison to a reagent blank. With the aid of the pilocarpine standard curve (10 mg dissolved in 10 mL ethanol, then 100 mL of distilled water added to make 0.1 mg/mL), the alkaloid contents were assessed and computed. Alkaloid were estimated after a calculation made with the help of the standard curve of Pilocarpine (0.1mg/mL) and expressed as mg of PE/g

Antioxidant activity study.

DPPH radical scavenging activity: The conventional approach was used to determine DPPH radical scavenging activity (Karadag *et al.*, 2009). To make the stock solution, 24 mg of DPPH was dissolved in 100 mL of methanol. Using a UV-Vis spectrophotometer, the working solution was created by combining 10 mL of the stock solution with 45 mL of methanol to produce an absorbance of 1.1± 0.02 at 515 nm. 0.015 mLplant extract mixed with .85mL of DPPH solution was kept in dark for 24 hours. At 515 nm the absorbance was measured. Ascorbic acid was used as a standard compound and the standard curve of it was generated. The results were expressed as a percentage of scavenging activity. The same experiment was performed in three sets. Percentage of DPPH radical scavenging activity was estimated by the following equation: DPPH radical scavenging activity (%) = $(A_0 - A_t / A_0) \times 100$

where, A_t is the absorbance of the plant extracts or standard and A_0 is the absorbance of the control.

ABTS Radical Scavenging Assay: ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity was determined following the standard method (Thaipong *et al*., 2006). The stock solution was prepared by 7.4 mM ABTS+ dissolved in 2.6 mM potassium persulfate solution. The working solution was made by mixing 10 mL stock solution with 40mL methanol to obtain an absorbance of 1.1± 0.02 at 734 nm using the UV-Vis spectrophotometer (UV1800). Plant extracts of 0.15 mL volume were then allowed to react with 2.85 mL of the ABTS solution for 2 hrs in a dark condition. Then absorbance was taken at 734 nm using the UV-Vis spectrophotometer (UV1800). The standard curve was prepared with ascorbic acid. Results were expressed in percentage of scavenging activity. Each experiment was carried out in triplicate. Using the formula given below, the percentage of ABTS radical scavenging activity was determined.

ABTS radical scavenging activity (%) =

$$(A_0 - A_t / A_0) \times 100$$

where, A_t is the absorbance of the plant extracts or standard and A_0 is the absorbance of the control.

Statistical Analysis

One-way analysis of variance was performed on all data (ANOVA) given as the mean±standard deviation of three copies. Difference among the average values of all the resulting data was compared using Tukey's HSD test (honestly significant difference; level of significance $p \leq 0.5$). The best fit method and regression equation were used for calculating IC_{50} values.

RESULTS

Foliar micromorphology: It gives a general description of the epidermal cells, trichomes and stomata with their measurements (Fig.1B-D).

Epidermis cells: Both leaf surfaces contain irregular-shaped epidermal cells. Cell wall of the epidermal cells was wavy. On the upper leaf surface, frequency of the epidermal cells was 834.55/mm^2 and it was 721.97/mm^2 in case of lower leaf surface. The size of the epidermal cells on lower surface was 93.13±3.78 µm × 47.90±2.65 µm and it was 56±1.76 µm × 34.21±3.9 µm on the upper surface of the leaf. Palisade ratio was 4.52±0.21 (Fig.1B and C).

Stomatal complex: Leaf is amphistomatic type. Stomata are mainly of paracytic type distributed with few anomocytic stomata. The value of stomatal frequency of upper epidermis and lower epidermis were 198.77±1.2/mm^2 and 234.65±2.4/mm^2 respectively. In the upper leaf surface, length and breadth of the stomata were 43.89±0.22 µm and 20.71±0.12 µm, respectively where as in case of lower leaf surface, size of the stomata was 56±0.21µm × 32.54±0.17µm. Stomatal index was 9.85%±0.31 on the upper surface and value of stomatal index was 13.05%±0.1 for the lower surface of leaf (Fig.1B and C).

Trichomes: Trichomes are non-glandular, unicellular, horn shaped with pointed tips and present in both the surfaces of the leaf. The size of trichomes of the adaxial surface was 120.65±3.44 µm x 18.67±0.11 µm. It was 89.81±3.91 µm x 19.29±0.48 µm in case of abaxial leaf surface. The trichome frequencies were 6.97±0.47/mm^2 and 3.77±0.31 /mm^2 for adaxial and abaxial surfaces, respectively (Fig.1D).

Petiole anatomy: In T.S., outline of petiole is circular. The epidermis is single-layered, consisting of small rhomboidal cells. The cuticle is relatively thick. Beneath the epidermis a two-cell layered hypodermis

is present. Below the hypodermis 5-6 layers of parenchymatous cells are present. A large circular ring-like vascular bundle is present at the centre and it is opened at one point with strongly incurved ends. It is composed of xylem tissue present in inner side and phloem on the outer side. Above the phloem, a distinct collenchyma zone is observed encircling the vascular bundle. Pith is scanty, comprised of 2-3 layers of parenchyma cells (Fig.1E).

Leaf anatomy: Upper and lower epidermises of the leaf are uniseriate in nature with thick cuticles on outer walls of the epidermal cells. Mesophyll showed upper palisade cells, single layered, elongated and compactly arranged; and lower spongy parenchyma cells, more or less spherical in shape. The vascular bundle is composed of xylem tissue present on the lower side of it and phloem on the upper side.

Bark anatomy: The stem bark surface is fairly smooth and whitish grey in colour. The bark consists of periderm and secondary phloem. The sections of the bark cut in the transverse, tangential longitudinal and radial longitudinal planes (T.S., T.L.S. & R.L.S.) exhibited the following anatomical features (Fig.1I-K).

T.S.: The transverse section of bark showed several outer most layers of typical cork or phellem cells. The cells are thick walled, polygonal, closely packed, dead at maturity, arranged in regular radial rows. Cork cells are tangentially elongated due to the secondary growth of the plant body. A multilayered zone of phellogen was situated between the outer phellem and inner phelloderm. Phellogen cells are rectangular in shape and compactly arranged. The phelloderm was of two to many layers of rectangular cells. Scattered patches of the stone cells were found in the secondary phloem region.

T.L.S.: Ray structures are uniseriate, long, and elliptical in shape. The frequency of the ray structure was 2.54±0.4/mm^2. The height and width of the ray structure were 311±9.32 µm and 7.9±0.56µm, respectively. Number of the cells per ray structure was varied from 14-17. Fibers are lignified with very thick wall, packed closely on either side of ray structure. Rays are mostly uniseriate and are less frequently biseriate. The biseriate rays were 310±1.3 µm in height and 20±7.85 µm in width

R.L.S.: Radial longitudinal section exhibited the internal structure of ray. It was homogeneous and had thin-walled, rectangular, ray parenchyma cells. Fibers were elongated with thick, lignified walls, longitudinally arranged.

Bark element study: Different cell types were observed in the bark. The phloem fibres were long, thin-walled with broad lumen, tapered at both ends, with blunt end. Sometimes septate fibres were observed. The size of phloem fibre was 836.87±13.89 µm × 26.78±1.69 µm. The frequency of it was 6.54±1.07/mm^2. Sclereids are of brachysclereid type, with highly thick lignified cell walls. Length of the sclereid was 27.43±0.32 µm and breadth was 24.2±0.15 µm. Cork cells are polygonal in shape with moderately thick wall. Length of the cork cell was 30.16±0.28 µm and breadth was 10.56±0.11 µm (Fig. 1F-H).

Foliar micromorphological studies of the *B. acuminata* revealed certain characteristics which will be very useful in taxonomic identification of the leaf drug obtained from this medicinal plant. Palisade ratio of *B. acuminata* was 4.52 ± 0.21 and it is distinct among the values of palisade ratio estimated earlier in some other species of *Bauhinia* by different workers. Stomatal indices of upper and lower epidermises in leaf part of our investigated plant were 9.85±0.31% and 13.05±0.1%, respectively which are very different from the stomatal indices recorded in earlier research done on many *Bauhinia* species. In the leaf of *B. acuminata* only non-glandular unicellular type of trichome was noticed and this attribute of trichome makes this species unique among the different species of *Bauhinia*, except *B. tomentosa* studied previously where non-glandular, uniseriate and multicellular trichomes were recorded. Presence of uniseriate palisade layer in the leaf makes our plant species distinguished from the *B. variegata* which exhibited biseriate palisade layer. A typical circular vascular bundle opened at one point of its ventral side with distinctly two incurved ends was noticed in the petiole of our plant which is not found in other genera of Leguminosae including other species of *Bauhinia* studied so far.

Organoleptic features of the powdered plant samples: The colour, odour, taste and texture of the two parts of the investigated plant have been presented in the table below (Table 1).

Table 1. Organoleptic features of leaf and bark powder of *B. acuminata*

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

Physicochemical evaluation: Moisture content and ash value of the leaf and bark powder drugs are given in tabular form (Table 2). Percentage of moisture contents of leaf and bark powder were 8.89%±0.4 and 9.1%±0.11, respectively. Ash values in leaf and bark were 7.5%±0.12 and 10.4%±0.3, respectively. In leaf, percentage of acid insoluble and water-soluble ash was 2.2%±0.16 and 2.5%±0.02, respectively. Percentage of acid insoluble and water-soluble ash in bark was 3.5%±0.22and 1.8%±0.15, respectively.

Determination of extractive value: The type of solvents basically determines the extractive value of the crude drug. The extractive value of the leaf and bark parts was maximum in methanol among other solvents such as ethyl acetate, chloroform, and hexane (Table 3).

Histochemical study: A histochemical analysis was done to identify different phytochemical groups that were concentrated in specific tissue zones of bark. Numerous phytochemical groups, including tannins, proteins, alkaloids, lignin, and saponins, have been located in various bark tissue zones. Additionally, it has been noted that in the leaf petiole, cortical and vascular bundle zones serve as the active sites for the localization of various phytochemical groups (Table 4, Fig. 2A-D).

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

Powdered plant Samples	Moisture content (%)	Total ash (%)	Water Soluble ash (%)	Acid insoluble ash (%)
Leaf powder	8.89±0.4	7.5±0.12	2.5±0.02	2.2±0.16
Bark powder	9.1±0.11	10.4±0.3	1.8±0.15	3.5±0.22

Table 3. Extractive value of leaf and bark of the studied plant

Plant parts	Extractive values (%)			
	Methanol	Ethyl acetate	Chloroform	Hexane
Leaf	2.65±0.2	1.76±0.08	1.44±0.07	0.53±0.05
Bark	1.90±0.17	1.26±0.1	1.77±0.19	0.30±0.02

Preliminary phytochemical screening

In order to find new sources of chemicals that are important both therapeutically and commercially, medicinal plants must undergo a phytochemical examination first. Initial chemical analysis of the methanol extracts of bark and leaf revealed the presence of primary and secondary metabolites including sugar, carbohydrate, amino acid, lipid, quinone, steroid, phenol, saponin and alkaloid (Table 5, Fig. 3A and B).

Table 4. Histochemical localization tests for leaf and bark portions

Test for	Test/ reagent	Leaf petiole	Bark
Alkaloids	Wagner's reagent Dragendorff;s reagent	Xylem tissue and cortex zone Sclerenchyma zone	Cork cells Secondary phloem
Lignin	Phloroglucinol +HCl	Sclerenchyma zone	Phelloderm
Tannins	1% lead acetate solution $FeCl_3$ test	Vascular bundle Cortex zone	Few pheloem and cork cells Few phloem fibre cells

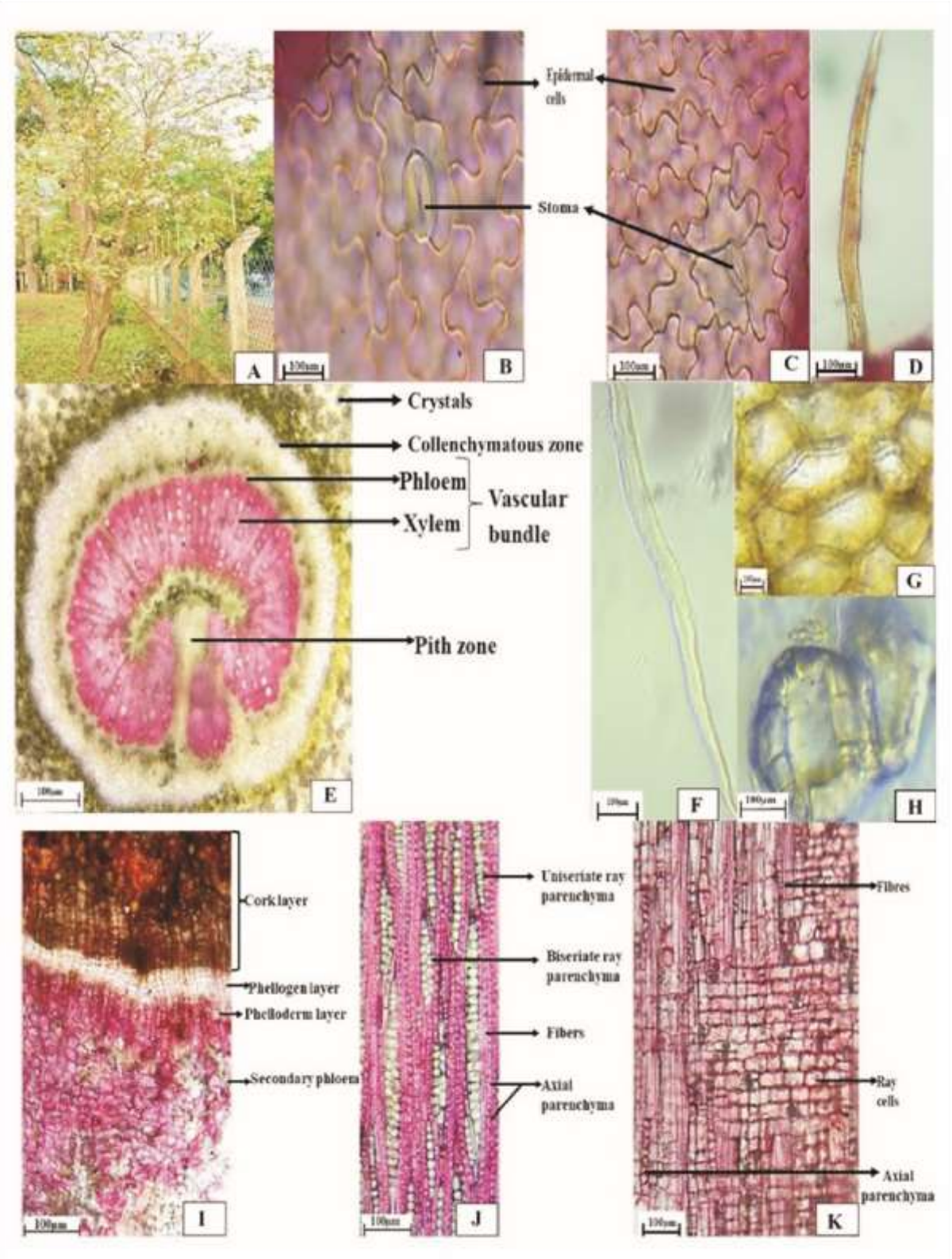

Fig. 1. *Bauhinia acuminata* L. **A.** Plant habit, **B.** Lower epidermis with paracytic type stomata, **C.** Upper epidermis with anomocytic type stomata, **D.** A foliar trichome, **E.** T.S. of petiole: Vascular bundle (enlarged view), **F.** A portion of bark fibre, **G.** Cork cells, **H.** Brachysclereids of bark, **I.** T.S. of bark, **J.** T.L.S. of bark, **K.** R.L.S. of bark.

Table 5. Microchemical tests of leaf and stem bark methanol extracts of *B. acuminata*

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

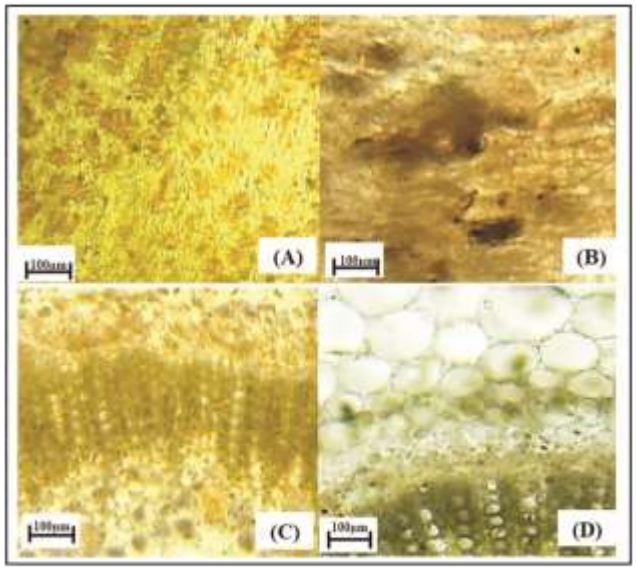

Fig. 2. Histochemical localization test. T.S. of bark: **(A)** Dragendorff's test, **(B)** $FeCl_3$ test, and T.S. of petiole: **(C)** Wagner's test, **(D)** $FeCl_3$ test.

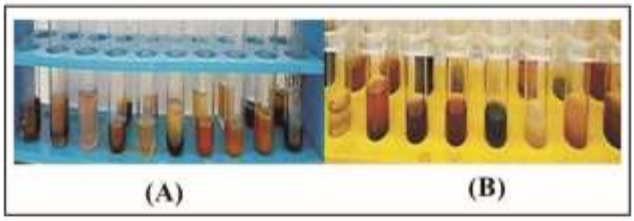

Fig. 3. Microchemical colour reaction test of methanol extracts: **(A)** Leaf, and **(B)** Bark.

Estimation of total phenolic content: The amounts of total phenolic compounds estimated in the leaf and bark extracts were 143.67±1.3 mg of GAE/g tissue and 101.34±1.5 mg of GAE/g tissue, respectively (Fig.4.).

Estimation of total flavonoid content: Methanol extracts of leaf and bark have total flavonoid levels of 33.24±0.3 mg of CE/g tissue and 32.56±0.2 mg of

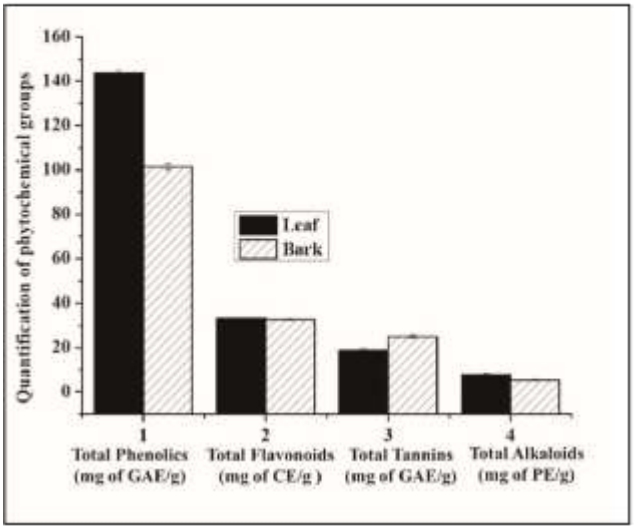

Fig. 4. Phytochemical profiles of the methanolic extracts of leaf and bark.

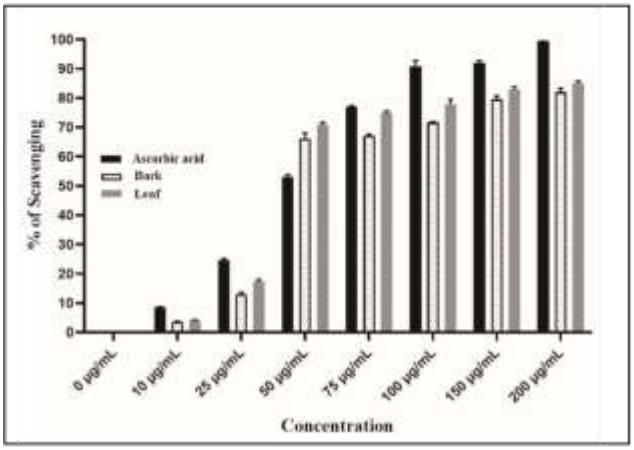

Fig. 5. DPPH free radical scavenging activity of the leaf and bark extracts.

CE/g tissue, respectively. Leaf showed slightly higher total flavonoid content than bark (Fig. 4).

Estimation of total tannin content: The tannin content in the bark extract was estimated to be greater (24.870±0.12 mg of TAE/g) than the tannin content of the extract of leaf (18.760±0.21 mg of TAE/g) (Fig. 4).
Estimation of total alkaloid content: Here leaf part showed slightly higher content of alkaloids (7.8±0.02 mg of PE/g) than the alkaloid content of bark (5.3±0.01 mg of PE/g) (Fig. 4).

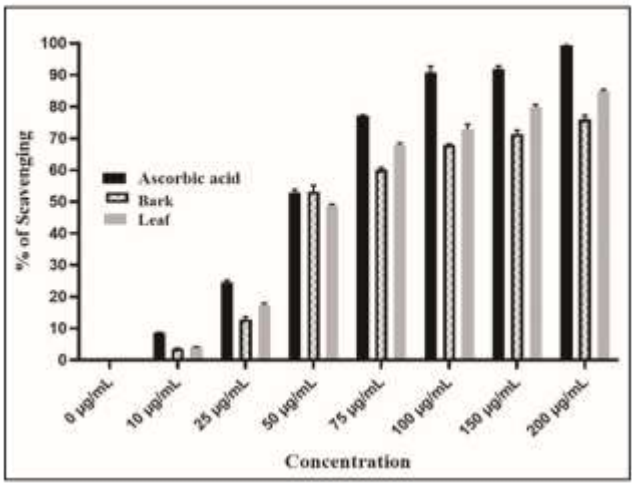

Fig. 6. ABTS radical scavenging activity of leaf and bark extracts.

Antioxidant activity

DPPH radical scavenging activity: Reduced absorbance, which indicates quenching of the free radicals by the plant extracts, was used to measure the reduction of DPPH radicals. The leaf extract was found to have a lower IC_{50} value of 72.40±0.45 µg/mL than the IC_{50} value of bark i.e., 81.25±0.78 µg/mL. At 100 µg/mL concentration, the scavenging activity of the leaf was found to be 77.88±1.6% which is higher than the scavenging activity of bark i.e. 71.65±1.98% at the same concentration, when compared to the reference compound ascorbic acid (Fig. 5).

ABTS radical scavenging activity: The scavenging activity of the leaf was found to be 72.88±0.21% at 100 µg/mL concentration, which is higher than the scavenging activity of the bark (67.99±0.33%) measured at the same concentration. The IC_{50} values of the bark and leaf extracts were estimated as 93.35±0.41 µg/mL and 82.97±0.19 µg/mL, respectively. ABTS radical scavenging activity was significantly high for leaf methanol extract of the studied plant species. Here also ascorbic acid was used as standard (Fig.6).

DISCUSSION

Pharmacognostic characters elucidate the specific information necessary for identification and authentication of crude drugs obtained from plants, which include macromorphology, micromorphology, physicochemical parameters, and qualitative estimation of phytochemical groups.

Some of the characteristics of the present studied plant conform the features identified in other members of the family Fabaceae by earlier workers (Metcalf and Chalk, 1950). Studies in leaf micromorphology can have great taxonomic as well as pharmacognostic value in identification of different plant taxa, including medicinal plants (Ray *et al*., 2018).

In our present experiment, it has been observed that epidermal cell's shape is irregular and cell wall outline is nearly wavy for both the epidermal layers of leaf. Palisade ratio is 4.52±0.21, which is also a very distinctive character for this medicinal plant species because it varies from species to species but it is specific to a particular taxon. The palisade ratio remains constant as it is the ratio of the palisade and epidermal cells present in leaf. It is used as one of the reliable taxonomic character for identification of leaf drug as well as corresponding plant species. For example, the palisade ratio of *B. variegata* is 7.6 (Gupta *et al*., 2009) and it differs from the palisade ratio of our investigated plant *B. acuminata* (4.52±0.21) which is a species of the same genus *Bauhinia*. Stomatal characters have immense importance while identifying the plant taxa and evaluating the leaf drugs. Although paracytic, and anomocytic types of stomata are found in the members of Fabaceae, but anomocytic type is most frequently observed in this family (Albert and Sharma, 2013). In *B. acuminata* both paracytic and anomocytic types of stomata were found in upper and lower surfaces of leaf. This finding conforms the observation made in the previous works on stomatal types of other species of *Bauhinia* (Albert and Sharma, 2013). The stomatal index is 9.85%±0.31 in the upper leaf surface and the value of stomatal index is 13.05%±0.1 in the lower surface, such differences in quantitative values of stomatal index for both epidermal surfaces of leaf make this taxon very distinct among other studied species of *Bauhinia*. Trichome features also serve as marker parameter for identification of leaf drugs. A wide range of structural variation in both glandular and non-glandular types of trichome is observed in different species of *Bauhinia* (Albert and Sharma, 2013). Here, in *B. acuminata* only non-glandular type of trichome was noticed.

Physicochemical parameters are also vital for standardization and quality control of herbal drugs, which include loss on drying and ash content. Loss on drying is a commonly used test procedure for determination of moisture content in a powdered drug sample. Moisture content of drugs should be at minimal level to discourage the growth of bacteria and fungi during its storage. In present work, the higher percentage of moisture in bark than the leaf indicates that self-life of bark drug would be short as high moisture content of bark degrades its quality assurance more quickly than leaf.

Ash value, one of the physicochemical criteria is employed to check quality and purity of crude drug. It provides a distinct quantitative value which is specific to a particular crude drug. Ash value bears an official standard articulated for a crude drug material. Total ash value indicates the presence of various impurities like dirt, mud in the form of silicate matter mainly and few other mineral groups such as carbonate, phosphates, etc. In present study, the total ash value of the leaf part in our studied plant species is 7.5 ±0.12 % which is very much distinct from the other two species, such as *B. variegata* (8.15%) (Khare *et al*., 2017) and *B. purpurea* (11%) (Pahwa *et al*., 2014). The total ash value of bark powder (10.4±0.3%) was higher than the leaf part (7.5±0.12%) of the studied plant. Here, leaf and bark parts studied for their ash value bear two different quantitative values (for total ash, water soluble and acid insoluble ash) that make these two crude drug samples distinct from each other.

The water soluble ash, one of the portions of total ash, highlights the amount of inorganic salts such as phosphate and carbonate present in 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 part (2.5±0.02%) is higher than the bark part (1.8±0.15%) which gives an impression about presence of greater amount of therapeutically important inorganic salts like phosphate and carbonate in leaf sample of this studied plant. So, in respect of bioactivity of minerals, the crude drug of leaf is in more advantageous position than bark drug. Although therapeutic performance of the crude drug is not only associated with certain mineral salts, but with many other groups of bioactive chemicals present in it.

The acid insoluble ash consists of mainly silica in the form of sand material and other type of silicate matter which may present in the cell wall and as infillings of the cell lumen, and/or may adhere in trace amount to the plant sample even after its thorough washing. There are two possible contributory sources of acid insoluble ash content estimated in the crude plant sample - one is the cellular source and another contribution may come from the adherent matter. Like total ash, content of acid insoluble ash also provides a very confirmatory character that helps in authentication and quality control of the plant-based crude drug. Our result showed that the acid insoluble ash value of the leaf part is very low i.e., 2.2±0.16% in comparison to the foliar acid insoluble ash value (5.5%) of *B. variegata* (Khare *et al*., 2017). Quantitative values for foliar acid insoluble ash of two species of *Bauhinia* are found very distinct.

In our study, the value of acid insoluble ash of leaf (2.2±0.16%) was found lower than the acid insoluble ash value of bark powder (3.5±0.22%). This result indicates a high content of acid insoluble ash estimated in bark sample which means this plant sample contains more residue of extraneous matter (such as sand, dust, etc.) adhering to it even after the thorough washing performed before its drying. There may also be a possibility regarding contribution of silica matter to acid insoluble ash by the cell walls of bark tissue as presence of silica has been evidenced in the cell walls as well as in the in fillings of cell lumen of many plant families namely Poaceae, Cyperaceae, Urticaceae, Cucurbitaceae and others (Currie *et al*., 2007; Prychid *et al*., 2003; Hodson *et al*., 2005).There is a difference in quantitative values at all three levels of ash content of the two plant parts studied and such difference in ash value will be very helpful in identification of leaf and bark drugs of the investigated medicinal plant.

Estimation of extractive values determines the amount of the active constituents in a given amount of plant material when extracted with a particular solvent. The extraction of any crude drug with a particular solvent yields a content of different phytoconstituents. The compositions of these chemical constituents depend upon the nature of the drug and the solvent used. It also provides an indication whether the crude drug is exhausted or not. Here in our study, methanol solvent showed the greatest extractive value for both leaf and bark parts among the other three solvents used. This result illustrates that among four solvents methanol is the best one for extraction of maximum amounts of different phytochemical groups soluble in this solvent. Such trends in extractive values are noticed in many previous works where highest extractive values of crude drugs in methanol solvent had been recorded (Ray *et al.*, 2018; Tatiya *et al*., 2012). Quantitative scores of extractive value in different solvents are also considered one of the diagnostic features for raw drug sample identification. Here leaf exhibited greater extractive value than the bark sample in case of all used solvents except chloroform, where bark showed the extractive value (1.77±0.19 %) higher than the leaf (1.44±0.07%). Such difference in extractive value makes the leaf and bark samples of this medicinal plant very distinct and it will be very useful in identification of these two drug samples of the studied plant.

From the histochemical localization test, it is observed that alkaloids, saponins, proteins, tannins, lignin, reducing sugar, amino acids are present in detectable amount in different tissue zones of the leaf and bark (Kadam *et al.*, 2013). In case of bark, this test highlights the secondary phloem zone as the main site for storage of different phytochemicals. Various phytochemical groups have also been detected mostly in the cortical zone of leaf petiole.

Preliminary phytochemical screening is useful in prediction of chemical nature of crude drug and it is also valuable for detection of chemical constituents present in it (Pal and Rahaman, 2015). In present study, the important phytochemical groups identified

both in leaf and bark samples were alkaloids, tannins, steroids, glycosides, flavonoids, etc. Though the earlier studies performed by other workers had recorded therapeutically significant only one chemical group, i.e., saponin and some primary metabolite groups namely carbohydrates, proteins and amino acids from this plant (Anjukrishna *et al*., 2015). In this case, our study reveals the presence of medicinally important several chemical groups in different parts of this medicinal tree and it clearly elucidates the therapeutic attributes of the studied plant. Detection of some important bioactive chemical groups in the bark and leaf samples of this investigated plant also addresses the rationale of its wide range of ethnomedicinal uses.

Among all secondary metabolites present in plants, phenolics are recognized as one of the most therapeutically important phytochemical groups. Phenolic compounds in plants are further classified into different groups such as simple phenolics, anthocyanins, isoflavones, hydroxycinnamic acid derivatives and flavonoids. All these classes of phenolics have gained extensive attention because of their wide range of bioactivities including free radical scavenging, anti-inflammatory, and anti-carcinogenic effects (Tungmunnithum *et al*., 2018). It has been reported that the antioxidant activity of phenolic compounds is mainly due to their redox potential, and hydrogen donating properties (Chen *et al*., 2020). From this study, it is evident that both parts of B. acuminata contain quite good amounts of phenolics (101.34±1.5 to 143.67±1.3 mg GAE/g), and flavonoids (32.56±0.2 to 33.24±0.3 mg CE/ g), which highlight the medicinal properties of this plant used as an antioxidant, anti-inflammatory agent. Phenolic contents of both 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) (Brat *et al*., 2006; Wu *et al*., 2004). It is also well established that phenolics, flavonoids and tannins are very much effective against various types of inflammation, diabetic effects, wounds and body pain (Mutha *et al*., 2021; Das *et al*., 2020). Presence of good amount of these therapeutically active compounds also highlights the prospect of this plant to be an effective anti-oxidant and anti-inflammatory drug source.

Here antioxidant activities of the methanol extracts of selected plant parts were assessed using the DPPH and ABTS methods. Plant extracts rich in phenolics and other antioxidant phytochemicals exhibited significantly high scavenging value. In this research, we determined the scavenging properties of leaf and bark extracts using DPPH and ABTS radical scavenging assays. The scavenging activity of test samples is expressed by its estimated IC_{50} values, which highlight the ability of test samples to scavenge 50% of the free radicals existing in the reaction mixture at a specific concentration of it. High IC_{50} values indicate the low antioxidant activity of the plant sample. The IC_{50} values were 72.40±0.45 µg/mL for leaf and 81.25±0.78 µg/mL for bark in the DPPH method. In the ABTS assay, the estimated IC_{50} values for bark and leaf part were 93.35±0.41 and 82.97±0.19 µg/ mL, respectively. In both antioxidant assays, the leaf was estimated to have a lower IC_{50} value than the bark part of the investigated plant. It may be due to presence of more amount of phenolics in the leaf (143.67 ±1.3 mg of GAE/g tissue) than bark part (101.34 ±1.5 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 quenchers.

Both the parts of the investigated plant manifested variable scavenging values which are due to unequal distribution of antioxidant molecules such as phenolics, flavonoids, etc. identified in those different parts of this medicinal plant. Based on the results obtained here in this study, it was found that the methanol extracts of leaf showed greater antioxidant activity in the both methods employed, than the antioxidant activity recorded in bark part of the plant. The higher antioxidant activity of leaf is correlated with its higher contents of phenolics and flavonoids estimated. The compounds of different phenolic groups have various functional groups including hydroxyls that are responsible for their radical scavenging activity (Chen *et al*., 2022). This result confirms the importance of different groups of phenolics as the potential antioxidant agents.

Antioxidant activity of both parts of *B. acuminata* is nicely correlated with the total phenolic and total flavonoid contents of leaf and stem bark which illustrate the species as a potent source of antioxidant substances and simultaneously encourages the scientific world to investigate novel antioxidants as well as therapeutically active natural products. Among the leaf and stem bark parts, it was found that the leaf is more potent in respect of its phytochemical content and antioxidant activity. Hence, this plant could be used as an easily accessible source of natural antioxidants in the pharmaceutical industries. For this reason, further scientific studies of these two parts of the investigated medicinal plant are highly recommended to standardize noble antioxidant phytochemicals. The present study provides valuable preliminary data through a demonstration of its efficient antioxidant capacity. Some of the pharmacognostic characters obtained through present study will be used as marker in proper identification of the crude drugs obtained from the leaf and stem bark of *B. acuminata*.

CONCLUSION

The diagnostic features derived from this pharmacognostic investigation are found effective in proper identification and quality control of unprocessed crude drugs of leaf and bark obtained from *B. acuminata* L. Phytochemical analysis revealed that the studied plant contains a good amount of therapeutically important chemical groups such as phenolics, flavonoids, tannins, and alkaloids in its stem bark and leaf. The presence of such phytochemicals in significant amounts clearly indicates the healing potential of this medicinal tree used traditionally in a varied range of health conditions and elucidates the rationale for investigating its various medicinal uses. Presence of a good amount of phenolics and flavonoids in the investigated two plant parts co-relates their satisfactory antioxidant activity. Finally, present study highlights some promising areas for further investigations of its chemical and pharmacological profiles.

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FULL LENGTH ARTICLE

Morphological and Physicochemical Alteration in *Arabidopsis* in Response to Sulphur Dioxide Stress

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This study was designed to observe the effect of exposure to different concentration of sulphur dioxide on the model plant *Arabidopsis thaliana*. Morphological and physiological parameters of seedlings and mature plants were studied to evaluate the effect of regular exposure to low and high concentration of sulphur dioxide i.e. 0.25 ppm and 0.50 ppm over a time course. In young seedlings, root and shoot growth were observed to be impacted more in the higher concentration whereas seedlings were observed to tolerate the lower concentration of 0.25 ppm. Retardation in root growth ultimately disrupted the root:shoot ratio inhibiting healthy growth. In mature plants also, relative water content was significantly reduced under sulphur dioxide stress in both concentrations but relatively lesser in the lower concentration. The same trend was observed in relative electrolyte leakage. Comparative analysis of the morphological and physiological parameters indicate that 0.25 ppm of SO_2 is efficiently tolerated by the Arabidopsis plant system whereas 0.50 ppm concentration is above its tolerance limit at which plant cannot sustain its growth and maintain water or ion balance thus affecting its survival.

Keywords: Sulphur dioxide, Abiotic stress, Root: shoot ratio, Relative water content, Relative electrolyte leakage, *Arabidopsis thaliana.*

INTRODUCTION

Sulphur dioxide is one of the major air pollutants in recent era and is more concerning around urban and industrial belts especially around thermal power plants and metal industries (Jain *et al*., 2016, Likus-Cieślik *et al*., 2020). Apart from anthropogenic sources, volcanic eruptions are also key contributors of this gas into the environment (Carn *et al*., 2017). Naturally, vegetation in SO_2 contaminated environment is challenged with this pollutant. Impact on vegetation is by far associated with leaf damage, soil acidification, imbalance in nutrient availability (Singh *et al.*, 2012; Prakash *et al.*, 2022; Han *et al*., 2022).

So_2 associated damages are mostly visualized on aerial parts of plants as they are naturally exposed to the gaseous pollutant. Most of the studies on effect of sulphur dioxide on plants were focused on growth retardation, photosynthesis inhibition, chlorophyll degradation etc. on full grown plant systems (Liu *et al*., 2017; Han *et al*., 2022; Li *et al*., 2022), while the effects on seedling stage is largely ignored. Screening

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based on seedling parameters is an effective way to assess the tolerable level of plants to various abiotic stresses like temperature, drought and salinity as published in earlier reports (Khaeim *et al*., 2022).

During environmental stresses, the relative water content is observed to be adversely affected which greatly contributes to plant mortality (Sapes *et al.*, 2021). Electrolyte leakage is also a reliable parameter which is directly related with cell viability in multiple stresses like UV radiation, heat, cold and salinity (Hatsugai *et al*., 2018).

Thus far most available literatures on sulphur dioxide, deal with high dosage on mature plants (Li *et al*., 2021). Here we have used seedlings as well as older Arabidopsis plants exposed to two different concentrations of SO_2 for comparison and better understanding of the effect of sulphur dioxide on plant health.

MATERIALS AND METHODS

Growing of *Arabidopsis thaliana* seedlings

Arabidopsis thaliana Col-0 ecotype seedlings were vernalized at 4ºC for 48hrs and sown separately on commercial soilrite mixture (Keltech Industries, USA) consisting of peat moss, vermiculite and perlite in (9:3:1 ratio) and grown within plant conviron at 23ºC at 12h LD photoperiod. To observe stress effect on seedling root and shoot, seedlings were grown on wet tissue paper. Seedlings with cotyledonary leaves and grown plants of 4 weeks old were subsequently exposed to SO_2 stress.

Application of SO_2 stress

Standardized protocol of Wang *et al.* (2017) was followed with some modifications. Two leaved seedlings of *A. thaliana* were exposed to SO_2 by fumigation at a concentration of 0.25 and 0.50 ppm for 3 days. Concentration was measured by portable SO_2 gas detector (Bosean Electronic Technology, China: Model-BH-90A).

Measurement of root length shoot length, root: shoot ratio of seedling

Individual seedlings of each experimental sets were measured for root and shoot length and were recorded accordingly. Estimation of root: shoot ratio in unstressed as well as SO_2 exposed seedlings were calculated following the protocol of Rogers et al. (2019).

Calculation of Relative Water Content

Relative water content was estimated by following the protocol of Chowdhury *et al.* (2018). Fresh weight, dry weight and turgid weight of similar sized leaves from each of the experimental sets were measured and RWC was measured using the formula *(fresh weight – dry weight)/(turgid weight – dry weight) × 100*

Estimation electrolyte leakage (EL)

Electrolyte leakage was measured using conductivity meter (Systronics, India) following the protocol of Hatsugai *et al.* (2018). Leaves were cut and incubated in 10ml distilled water at 28ºC for 8 hours and initial conductance (C1) was measured. Electrolyte conductivity (C2) was estimated following the boiling of the sample in water bath for 10 minutes. EL was measured using the expression: *(C1/C2) ×100*

Statistical analysis

Statistical analysis was done following to our previous publications (Koley *et al.*, 2022). Each experiment was performed in completely randomized design (CRD) with three independent experiments each with at least three replicates. Each of the data are represented as mean ± SEM. The data was analyzed by one-way ANOVA.

Lettering above bars indicate significant differences between different datasets at $p < 0.05$, according to Duncan's multiple range test (DMRT), using SPSS version 16, 2007 software package.

RESULT

Significant inhibition of seedling growth occurred under higher concentration of SO_2

Seedling growth was observed to be inhibited by 0.50 ppm of SO_2 while 0.25 ppm SO_2 caused insignificant retardation (Fig. 1.A). In control plant, gradual increase of root: shoot ratio from 1.60 at 0 dpe to 2.23 at 3dpe, indicates healthy seedling growth. Interestingly, 0.25ppm concentration was well tolerated by the seedlings as the root and shoot growth were similar with that of control. In 0.50 ppm exposure, significant decrease in root: shoot ratio and visible stunting of seedling length had been observed in comparison to control sets as well as 0.25 ppm sets (Fig. 1.A-D).

Sulphur dioxide exposure leads to relative water content reduction and electrolyte leakage

Relative water content (RWC) has been observed to be impacted by sulphur dioxide exposure. From external observation, the plants exposed to higher SO_2 concentration were visibly shriveled and dehydrated whereas plants exposed to lower concentration showed considerably less dehydration in comparison to control sets (Fig. 2.A). Quantification of RWC revealed that in unstressed plant, water content remained almost constant at around 92%. In both concentrations of SO_2, RWC had been observed to be reduced over the time course. In 0.25 ppm of SO_2, no significant changes in RWC were observed on 1 dpe whilst almost 15% reduction was observed in 0.50ppm exposed plants at the same time point. Up to the end of time course of study, RWC in lower concentration was reduced to 65% and in the case of higher SO_2 concentration, RWC of experimental plants was reduced further lower to almost 50% (Fig. 2.B).

In the control plants, electrolyte leakage remained at a constant basal level of around 10% whereas, with SO_2 exposure, electrolyte leakage was observed to be increased each day of the experiment. Interestingly, under 0.25 ppm exposure, at 1 dpe, 30% electrolyte leakage was observed which nearly stabilized in the following days to reach at only 37% at the end of 3 dpe. In contrast, 0.50 ppm concentration not only caused more electrolyte leakage at the initial time points than low concentration, it also resulted in a spike in electrolyte leakage to 55% at the end of 3dpe (Fig. 3).

DISCUSSION

From the results, it is evident that sulphur dioxide disrupts the physicochemical balance of *Arabidopsis* plants leading to seedling growth disruption, relative water content reduction and electrolyte leakage.

In earlier published reports, UV stress was shown to inhibit Brassica seed germination and seedling growth (Begum *et al.*, 2021). Also, role of ozone exposure were elucidated in reduction on larch seedling (Sugai *et al.*, 2019). Acidification of cellular environment which is a common effect in sulphur dioxide toxicity (Chauhan *et al.*, 2015). It has been previously reported that acidification of cellular content leads to root and shoot growth in seedling (Bláha *et al.*, 2019). This can explain our result where higher SO_2 exposure inhibited root, shoot length and overall root:shoot ratio.

Relative water content (RWC) is an important parameter in assessing abiotic stress severity which had been well elucidated in case of drought stress in soy bean and tomato (Chowdhury *et al.*, 2018; Patanè *et al.*, 2022). In those studies, higher RWC retention was associated with better tolerance to plant. In the present study, plants exposed to 0.25 ppm SO_2, retain more RWC than that of 0.50 ppm indicating better tolerance. Cell membrane damage is a direct effect on plant in response to air pollutants (Gall *et al.*, 2015). Membrane damage subsequently causes water deficit thereby reducing RWC (Azizi-Chakherchaman *et al.*, 2008) which corroborates with our result.

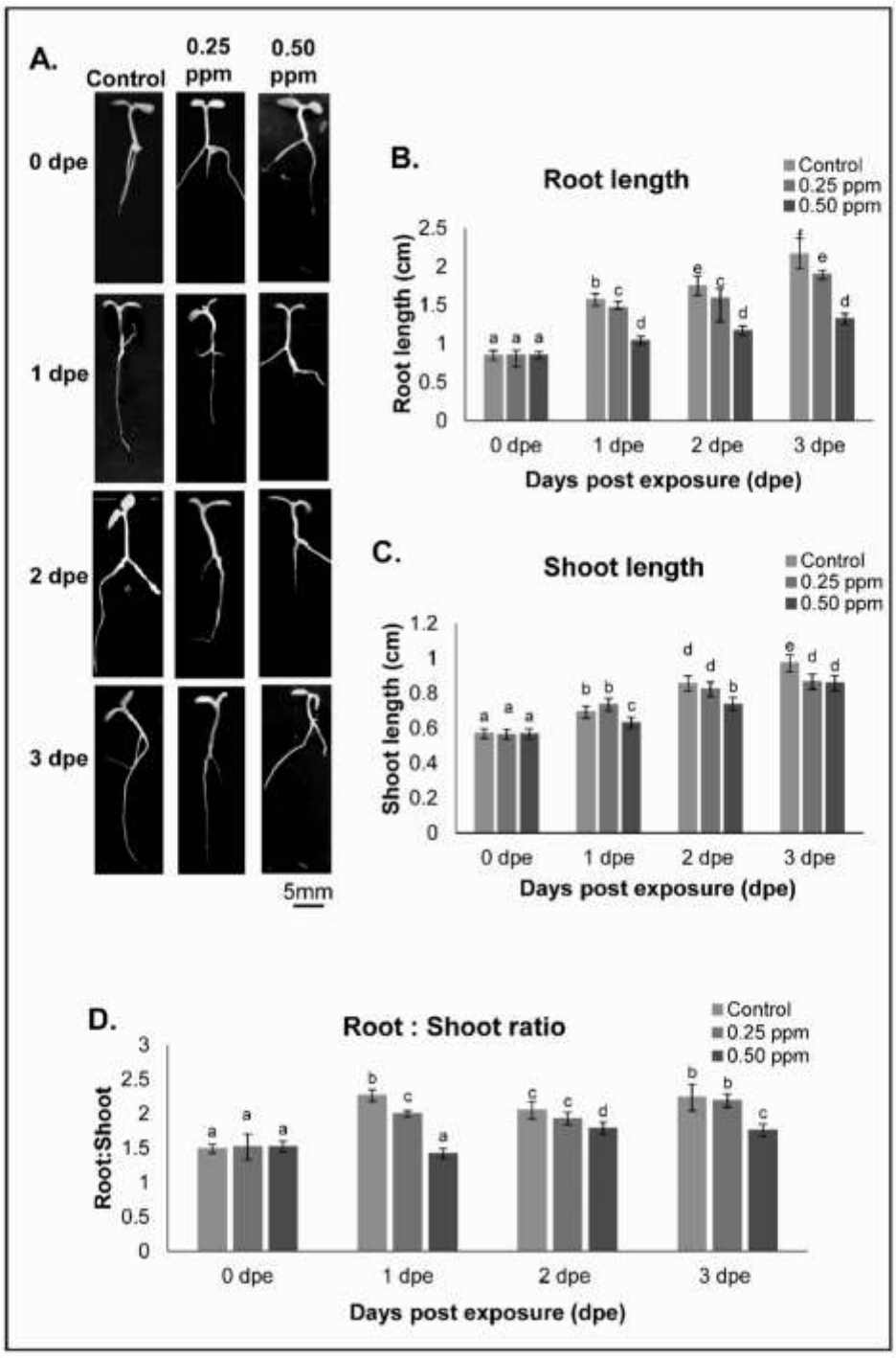

Figure 1. Effect of exposure to SO_2 at different concentrations on germinated seedlings. (**A**) Seedlings showed significant stunting of growth in response to 0.50ppm of SO_2 whereas showed very less stunting in 0.25 ppm SO_2 and is comparable with control until 3 days post exposure (dpe). Bar = 5mm (**B, C, D**) Comparative analysis of root, shoot length and root: shoot ratio respectively showing significant disruption of growth in higher SO_2 concentration and minimal effect in response to lower SO_2 concentration, with the root : shoot ratio being lowered with increasing concentration of SO_2. Bars represent standard error (SE) of the mean (n = 3). Different letters indicate significant differences among treatments at $p < 0.05$, according to Duncan's multiple range test.

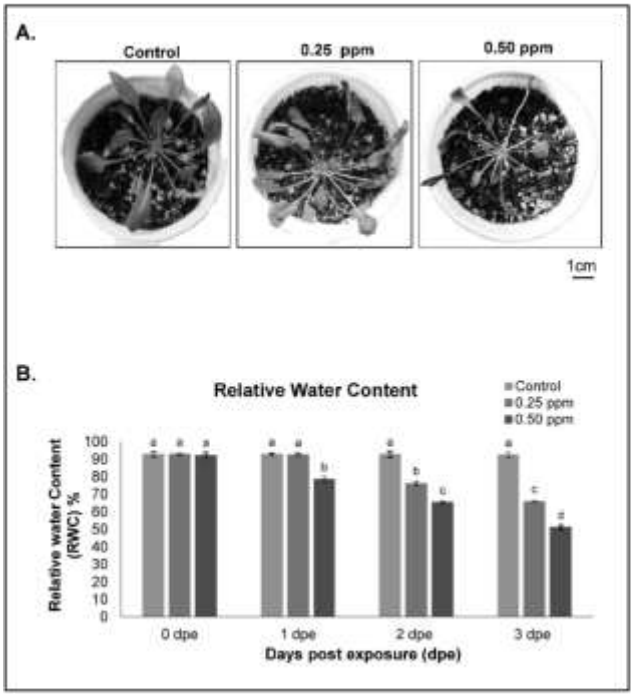

Figure 2. Comparison of relative water content (RWC) between plants exposed to SO_2 at two different concentrations and control sets. (A) Arabidopsis plants after 3 days post exposure of SO_2 showing severe dehydration in 0.50 ppm exposure while moderate dehydration in comparison to control plant (B) Comparison of percentage of relative water content in SO_2 exposed *A. thaliana*. Bars represent standard error (SE) of the mean (n = 3). Different letters indicate significant differences among treatments at $p < 0.05$, according to Duncan's multiple range test.

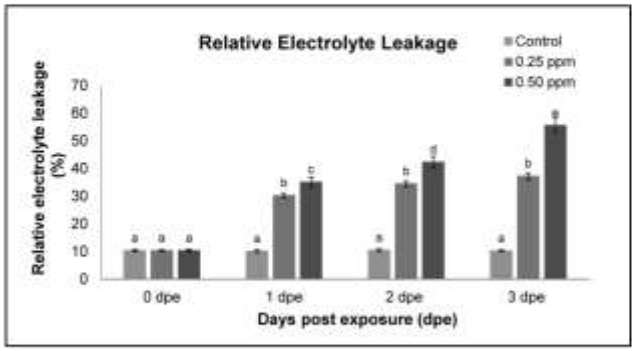

Figure 3. Comparison of relative electrolyte leakage amongst plants exposed to SO_2 at two different concentrations and control sets. Bars represent standard error (SE) of the mean (n = 3) Different letters indicate significant differences among treatments at $p < 0.05$, according to Duncan's multiple range test.

Electrolyte leakage is mainly related to loss of K+ ions from plant cells and is accompanied with oxidative stress and programmed cell death (Hniličková *et al*., 2019). This correlates with the tolerance level of plant in response to several environmental stresses like hypoxia, salinity and water stress (Jayawardhane et al., 2022). This corroborates our result where less electrolyte leakage was recorded at 0.25 ppm suggesting effective tolerance mechanism. Whereas higher concentration incapacitated the plant and hindered effective tolerance which is evident from the wilted appearance of the plant.

Analyzing all the results, it is evident that sulphur dioxide impacts normal growth parameters, physiological and chemical balance of both seedling and older Arabidopsis plants. Interestingly, at lower concentration of 0.25ppm, the seedlings and plants are able to tolerate the stress and are able to maintain physicochemical parameters whereas when exceeding that level, the plants failed to maintain critical physicochemical parameters leading to mortality at later stages.

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FULL LENGTH ARTICLE

A Checklist of the genus *Impatiens* L. (Balsaminaceae A. Rich.) for Darjeeling Himalaya

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The genus *Impatiens L.* (Balsaminaceae A. Rich.) comprises of more than 1,000 species that are present in the tropical and subtropical regions of Africa, Europe and Asia, as well as in the northern temperate regions. In India, it is represented by about 280 species, most of which are either endemic to the Himalaya or Western Ghats. The present work enumerates a list of 43 species of *Impatiens* L. of Darjeeling Himalaya along with their accepted name, original citation, synonyms (if any) and their phenology.

Keywords: *Impatiens* L., Darjeeling Himalaya, Original Citation, Phenology.

INTRODUCTION

The genus *Impatiens* L. (Balsaminaceae) is characterized by annual or perennial herbaceous habit with semi-succulent stems and leaves and zygomorphic flowers. Due to their varied sepal and petal organization, they are difficult to classify and identify (Grey-Wilson 1980). As many as 1000 species of Impatiens (Janssens *et al*., 2006, 2009a,b; Morgan, 2007) are distributed widely in the tropical and subtropical regions of Africa, Europe and Asia, as well as in the northern temperate regions. Impatiens is primarily concentrated in five different diversity hotspots, viz. tropical Africa (Guinean forests of West Africa), Madagascar, Western Ghats and Sri Lanka, the Himalayas, and Southeast Asia. (Yuan *et al*., 2004). It shows maximum diversity in tropical and subtropical montane forests of Southeast Asia (Stevens, 2001). There are about 280 species of Impatiens on record in India and they display a disjunctive distribution in the Himalayas and the Western Ghats with more than 130 species in each hotspot. The present work prepares an account of *Impatiens* L. from different places Darjeeling Himalaya along with the correct or accepted name of the taxa, original citation and other available references, synonyms (if any) and phenology.

MATERIAL AND METHODS

The present work enumerates 43 species of *Impatiens* L. of Balsaminaceae A. Rich. from different parts of Darjeeling Himalayan region. This work is the result of a detailed study in the field, herbarium and library. Several field surveys were conducted from 2014-2016 to record the genus *Impatiens* L. of the family Balsaminaceae A. Rich. from different parts of

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Darjeeling district. The collected specimens were identified consulting literature as well as the Central National Herbarium, Howrah and the Herbarium of Llyod Botanic Garden, Darjeeling. The literature consulted includes "Flora of British India", Vol.-1 by J.D. Hooker (1874), "The Flowering Plants of Darjeeling" by A. Mukherjee (1988), "Flowers of the Himalaya" by O. Polunin and A. Stainton (1997), "Flora of Darjeeling Himalaya and foothills (Angiosperm)", by Deepak Kr. Ghosh and Jayanta Kr. Mallick (2014), "Wild Balsams of Darjeeling and Sikkim Himalaya: A Pictorial Handbook" by R. Gogoi, N. Sherpa and S. Rai (2021), along with other publications of Bhuijel (1996), K.P. Biswas (1967), A.P. Das (2004), Mallick (2017).

The sequence of presentation of data of the investigated taxa is given below.

- Correct or accepted name of the taxon (following the WFO plant list).
- Original citation and other available references.
- Synonyms, if any.
- Phenology.

ENUMERATION

1. *Impatiens amplexicaulis* Edgeworth, Trans. Linn. Soc. London. 20: 37. 1846.
   Flowering: July-August; Fruiting: August-September.
2. *Impatiens arguta* J. D. Hooker & Thomson, J. Proc. Linn. Soc., Bot. 4: 137. 1860. *Impatiens arguta* var. bulleyana J.D. Hooker; I. gagei J.D. Hooker; *I. namchabarwensis* R. Morgan *et al.*; *I. taliensis* Lingelsheim & Borza. N.
   Flowering: July-September; Fruiting: July-October.
3. *Impatiens balsamina* L., Sp. Pl. 938. 1753; Hook.f., Fl. Brit. India 1: 453. 1874. *Impatiens coccinea* Sims. Bot. Mag. 31: t. 1250 1809. *Impatiens eriocarpa* Launert, Bol. Soc. Brot., sér. 2, 36: 59, t. 7 1962. *Impatiens stapfiana* Gilg, Bot. Jahrb. Syst. 43: 111 1909. *Balsamina angustifolia* Blume, Bijdr. Fl. Ned. Ind. 5: 239. 1825. *Balsamina cornuta* (L.) DC., Prodr. 1: 686 1824. *Balsamina foeminea* Gaertn. Fruct. Sem. Pl. 2: 113 1790. *Balsamina hortensis* Desp. Dict. Sci. Nat. 3: 485 1805. *Balsamina lacca* Medik Malvenfam, 71 1787. *Balsamina minutiflora* Span., Linnaea 15: 185 1841. *Balsamina mollis* G. Don, Gen. Hist. 1: 749 1831. *Balsamina odorata* Buch.-Ham. ex D. Don, Prodr. Fl. Nepal. 203 1825. *Balsamina racemosa* Buch.-Ham. ex D. Don, Prodr. Fl. Nepal. 203 1825. *Balsamina salicifolia* Bojer ex Baker, J. Linn. Soc., Bot. 20: 115 1884.
   Flowering: July-October; Fruiting: Mar- October.
4. *Impatiens bicornuta* Wallich in Roxb., Fl. Ind. 2: 460. 1824; Hook. f. & Thomson in J. Linn. Soc., Bot. 4: 147. 1860; Hook. f., Fl. Brit. India 1: 475. 1875 & in Rec. Bot. Surv. India 4: 8. 1904; Biswas, Pl. Darj. Sikkim Himal. 1: 204. 1966; H. Hara in Fl. E. Himal. 1: 195. 1966, 2: 75. 1971 & 3: 78. 1975. *I. pradhanii* H. Hara in J. Jap. Bot. 40: 99. 1965 & in Fl. E. Himal. 1: 196. 1966; Akiyama in J. Jap. Bot. 62(12): 368. 1987.
   Flowering: June-August; Fruiting: July- August.
5. *Impatiens bracteata* Colebr. ex Roxb., Fl. Ind. ed. Carey ii. 459. 1824.
   Flowering: June-August; Fruiting: August-October.
6. *Impatiens cathcartii* Hook. f., Fl. Brit. India 1: 473. 1875 & in Rec. Bot. Surv. India 4: 14. 1905; Biswas, Pl. Darj. Sikkim Himal. 1: 209. 1966.
   Flowering: July-December; Fruiting: July-December.
7. *Impatiens chinensis* L., Sp. Pl. 2: 937. 1753; Hook. f., Fl. Brit. India 1: 444. 1874 & in Rec. Bot. Surv. India 4: 28. 1905; Biswas, Pl. Darj. Sikkim Himal. 1: 203. 1966; Akiyama *et al.* in Bull. Natn. Sci. Mus. ser. B. 21(4): 151-168. 1995. *Impatiens cosmia* Hook.f., Hooker's Icon. Pl. 30: t. 2915 1910. *Impatiens heterophylla* Wall., Fl. Ind. 2: 458 1824.
   Flowering: June-August; Fruiting: July-December.
8. *Impatiens cymbifera* J. D. Hooker, Fl. Brit. India. 1: 474. 1875.
   Flowering: August-September; Fruiting: August-October.
9. *Impatiens decipiens* Hook. f. in Rec. Bot. Surv. India 4: 17. 1905.
   Flowering: June-October; Fruiting: June- October.

10. *Impatiens discolor* DC., Prodr. 1: 687. 1824; H. Hara in Fl. E. Himal. 1: 195. 1966.
Flowering: Jul- October; Fruiting: August-October.

11. *Impatiens exilis* Hook. f. in Rec. Bot. Surv. India 4: 13. 1905; H. Hara in Fl. E. Himal. Impatiens exilis Hook. f. in Rec. Bot. Surv. India 4: 13. 1905; H. Hara in Fl. E. Himal. 1:195.1966 & 3:79.1975.
Flowering: June–October;Fruiting:July-October.

12. *Impatiens falcifer* J. D. Hooker, Bot. Mag. 129: t. 7923. 1903.
Flowering: August-September; Fruiting: October- November.

13. *Impatiens florigera* C.B.Clarke ex Hook.f., Rec. Bot. Surv. India iv. 13. 1905.
Flowering: June-August; Fruiting: July- August.

14. *Impatiens gamblei* Hook. f. in Rec. Bot. Surv. India 4: 15. 1905; H. Hara in Fl. E. Himal. 3: 79. 1975.
Flowering: July – September; Fruiting: August-October.

15. *Impatiens gammiei* Hook. f. in Rec. Bot. Surv. India 4: 16. 1905.
Flowering: Jul- October; Fruiting: August-November.

16. *Impatiens graciliflora* Hook. f. in Ree. Bot. Surv. India 4: 15. 1905; H. Hara in Fl. E. Himal.1:195.1966.
Flowering: September- October; Fruiting: September- October.

17. *Impatiens infundibularis* Hook. f. in Rec. Bot. Surv. India 4: 13. 1905.
Flowering: July- September; Fruiting: August-October.

18. *Impatiens insignis* DC., Prodr. 1: 688. 1824; Hook. f., Fl. Brit. India 1: 477.1875 & in Rec. Bot. Surv. India 4: 14. 1905; H. Hara in Fl. E. Himal. 1: 195. 1966.
Flowering: September- October; Fruiting: October- November.

19. *Impatiens jurpia* Buch. -Ham. ex Hook. f. & Thomson, Numer. List 4761 1831; Hook. f., Fl. Brit. India 1: 471. 1875 & in Rec. Bot. Surv. India 4: 14. 1905; Biswas, Pl. Darj. Sikkim Himal. 1: 207. 1966; H. Hara in Fl. E. Himal. 1: 196. 1966 & 2: 75. 1971.
Flowering: June-December; Fruiting: July-December

20. *Impatiens kingii* Hook.f., Rec. Bot. Surv. India iv. 14. 1905.
Flowering: June-August; Fruiting: July- August.

21. *Impatiens laevigata* Wallich ex Hook. f. & Thomson in J. Linn. Soc., Bot. 4: 146. 1860; Hook. f., Ft. Brit. India 1: 473. 1875 & in Rec. Bot. Surv. India 4: 30. 1905; H. Hara in H. Hara & Williams, Enum. Fl. Pl. Nepal 2: 79. 1979.
Flowering: July-December; Fruiting: July-December.

22. *Impatiens laxiflora* Edgeworth, Trans. Linn. Soc. London. 20: 40. 1846.
Flowering: June- December, Fruiting: July-December.

23. *Impatiens leptocarpa* Hook. f. in Rec. Bot. Surv. India 4: 17. 1905.
Flowering: August- September; Fruiting: September- October.

24. *Impatiens longipes* J. D. Hooker & Thomson, J. Proc. Linn. Soc., Bot. 4: 151. 1860.
Flowering: Jul-September; Fruiting: September-October.

25. *Impatiens occultans* Hook.f., Rec. Bot. Surv. India iv. 17. 1905.
Flowering: August-September; Fruiting: August-September.

26. *Impatiens porrecta* Wall. ex Hook. f. & Thomson in J. Proc. Linn. Soc., Bot. 4: 138. 1859.
Flowering: August-October; Fruiting: August-October.

27. *Impatiens puberula* DC., Prodr. 1: 687. 1824; Hook. f., Fl. Brit. India 1: 470. 1875 & in Rec. Bot. Surv. India 4: 17. 1905; Biswas, Pl. Darj. Sikkim Himal. 1: 206. 1966; H. Hara in Fl. E. Himal. 1: 196. 1966 & 2: 75. 1975; *Balsamina puberula* (DC.) G. Don, Gen. Hist. 1: 749,1831.
Flowering: Jun-September; Fruiting: September-October.

28. *Impatiens pulchra* Hook. f. & Thomson in J. Linn. Soc., Bot. 4: 139. 1860; Hook. f., Fl. Brit. India 1: 459. 1874 & in Rec. Bot. Surv. India 4: 12. 1905; Biswas, Pl. Darj. Sikkim Himal. 1. 205. 1966.
Flowering: September- November; Fruiting: September- November.

29. *Impatiens racemosa* DC., Prodr. 1: 688. 1824; Hook. f. & Thomson in J. Linn. Soc., Bot. 4: 147. 1860; Hook. f., Fl. Brit. India 1: 479. 1875 & in Rec: Bot. Surv. India 4: 6. 1904; Biswas, Pl. Darj. Sikkim Himal. 1: 210. 1966; H. Hara, Fl. E. Himal. 1: 196. 1966. *Impatiens microsciadia* Hook. f., Rec. Bot. Surv. India 4: 16 1905. *I. microsciadia* Hook. f. in Rec. Bot. Surv. India 4: 16. 16. 1905; *Petalonema racemosum* (DC.) Peter, Abh. Königl. Ges. Wiss. Göttingen, Math.-Phys. Kl. n.f., 13(2): 84 1928.
Flowering: June- August; Fruiting: Jul-September.

30. *Impatiens radiata* Hook. f., Fl. Brit. India 1: 476. 1875 & in Rec. Bot. Surv. India 4: 15. 1905; Biswas, Pl. Darj. Sikkim Himal. 1: 210. 1966; H. Hara in Fl. E. Himal. 1: 196. 1966 & 2: 75. 1971 et H. Hara in H. Hara & Williams, Enum. Fl. Pl. Nepal 2: 80. 1979; Akiyama *et al*. in Bull. Natn. Sci. Mus. ser. B. 21(4): 151 - 168. 1995. *Impatiens centiflora* H. Lév. Cat. Pl.Yun-Nan120,1916.
Flowering: June- August; Fruiting: July-September.

31. *Impatiens scabrida* DC., Prodr. 1: 687. 1824; Hook. f., Fl. Brit. India 1: 472. 1875 & in Rec. Bot. Surv. India 4: 7. 1904; Biswas, Pl. Darj. Sikkim Himal. 1: 208. 1966; H. Hara in Fl. E. Himal. 1: 196. 1966 & 2: 75. 1971. *I. practermissa* Hook. f. in J. Linn. Soc., Bot. 37: 29. 1904 & in Rec. Bot. Surv. India 4: 18. 1905; I. cristata Roxb., Fl. Ind. 2: 456. 1824; Hook. f. in Rec. Bot. Surv. India 4: 10. 1904. *Impatiens tricornis* Lindl., Bot. Mag., pl. 9 1840.
Flowering: July-September; Fruiting: August-September.

32. *Impatiens scitula* Hook. f. in Rec. Bot. Surv. India 4: 14, 20. 1905.
Flowering: August-October; Fruiting: August-October.

33. *Impatiens spirifer* Hook. f. & Thomson in J. Linn. Soc., Bot. 4: 135. 1860; Hook. f., Fl. Brit. India 1: 471. 1875 & in Rec. Bot. Surv. India 4: 17. 1905; Biswas, Pl. Darj. Sikkim Himal.1:208.1966.
Flowering: September- December; Fruiting: September- December.

34. *Impatiens stenantha* Hook. f., Fl. Brit. India 1: 478. 1875 & in Rec. Bot. Surv. India 4: 17. 1905; Biswas, Pl. Darj. Sikkim Himal. 1: 213. 1966; H. Hara in Fl. E. Himal. 1: 197. 1966 & 2: 75. 1971.
Flowering: July – November; Fruiting: August-December.

35. *Impatiens sulcata* Wallich in Roxb., Fl. Ind. 2: 458. 1824; Hook. f. & Thomson in J. Linn. Soc., Bot. 4: 129. 1860; Hook. f., Fl. Brit. India 1: 469. 1875 incl. var. minor & in Rec. Bot. Surv. India 4: 5. 1904; Blatter, Beaut. Fl. Kashmir 1: 71. 1927; Biswas, Pl. Darj. Sikkim Himal. 1: 206. 1966; H. Hara in Fl. E. Himal. 3: 179. 1975. *I. gigantea* Edgew. in Trans. Linn. Soc. London 20: 38.1846.
Flowering: Jul-October; Fruiting: August-December.

36. *Impatiens trigonopteris* Hook. f. in J. & Proc. Asiat. Soc. Bengal n. ser. 4: 189. 1908.
Flowering: October; Fruiting: November.

37. *Impatiens trilobata* Colebr. in Hook., Exot. Fl. 2: t. 141. 1825; Hook. f., Fl. Brit. India 1: 451. 1874 & in Rec. Bot. Surv. India 4: 13. 1905; Biswas, Pl. Darj. Sikkim Himal. 1: 203. 1966. I. flavida Hook. f. & Thomson in J. Linn. Soc., Bot. 4: 127. 1860.
Flowering: Jul-October; Fruiting: August-December.

38. *Impatiens tripetala* Roxb. ex DC., Prodr. 1: 687. 1824; Roxb., Fl. Ind. 2: 453. 1832; Hook. f., Fl. Brit. India 1: 470. 1875 & in Rec. Bot. Surv. India 4: 13. 1905; Toppin in Bull. Misc. Inform. 1920: 358. 1920; Biswas, Pl. Darj. Sikkim Himal. 1: 206. 1966; H. Hara in Fl. H. Himal. 1: 197. 1966 & 2: 76. 1971. *I. tripetala* Roxb. ex DC. var. microscypha Hook. f. in Bull. Misc. Inform. 1910: 298. 1910. *I. multiflora* Wallich ex Hook. f. & Thomson in J. Linn. Soc., Bot. 4:126.1860.
Flowering: November-December; Fruiting: November- December.

39. *Impatiens tuberculata* J. D. Hooker & Thomson, J. Proc. Linn. Soc., Bot. 4: 155. 1860.
Flowering: August-September; Fruiting: August-October.

40. *Impatiens tubifer* B.Heyne ex Roxb., Fl. Ind. 2: 464 1824; Hook. f. in Rec. Bot. Surv. India 4:14.1905.
Flowering: July- October; Fruiting: October-November.

41. *Impatiens uncipetala* C.B. Clarke ex Hook. f.,

Rec. Bot. Surv. India iv. 18. 1905.
Flowering: June-August; Fruiting: July-August.

42. *Impatiens urticifolia* Wallich in Roxburgh, Fl. Ind.2:457.1824.
Flowering: July-October; Fruiting: August-December.

43. *Impatiens wallichii* Hook. f. in Rec. Bot. Surv. India 4: 20. 1905; H. Hara in Fl. E. Himal. 3: 79.1975.
Flowering: July- October; Fruiting: August-November.

CONCLUSION

The present work aims to record the species of *Impatiens* L. found only in Darjeeling Himalayan region. Among the 45 species of this genus growing in Darjeeling-Sikkim Himalaya (Gogoi et al., 2007), 43 are present in Darjeeling Himalayan regions of West Bengal. This enumeration highlights the presence of *Impatiens* L. specifically in West Bengal. Moreover, this work is anticipated to aid the finding of distributional pattern and conservational status of different species of *Impatiens* L.

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FULL LENGTH ARTICLE

Allelopathic response of certain Cyanoprokaryotes to *Oscillatoria subbrevis* Schmidle (Oscillatoriales)

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Allelopathic response of seven species of filamentous and non-heterocystous cyanoprokaryotes e.g. *Arthrospira khannae, Limnothrix planctonica, Lyngbya martensiana, Oscillatoria subbrevis, Phormidium defloxides, Planktothricoides raciborskii* and *Spirulina subsalsa* to the culture filtrate of *Oscillatoria subbrevis* was studied. The allellochemicals released by *O. subbrevis* has demonstrated autostimulatory effect and in addition, also increased chl-a content of *S. subsalsa*. However, in the other 5 cyanoprokaryotes showed inhibitory effect; maximum inhibition was found in *L. plactonica* (54.37%) and minimum in *A. khannae* (7.8%).

Keywords: Allelopathy, Cyanoprokaryotes, Chlorophyll-a.

INTRODUCTION

The nature is a common niche providing shelter to several groups of living organisms including plants, algae, fungi and animals / grazers, and most of them grow in the form of mixed population. Many biologically active secondary metabolites are released by living organisms including microalgae in that particular ecosystem where they usually grow and that usually act as defense tools to control the growth of other competing phytoplanktons and grazers (Michael, 2003). The bioactive compounds affect other organisms of the same biotope positively or negatively, that ultimately influence the structure of ecosystem are known as allelochemicals ((Molisch, 1937). The allelopathic interaction is a widespread common event among freshwater primary producers and influences the aquatic ecosystem (Indrajit and Dakshini, 1994). Algae are primary colonizers (Tiwari *et al*., 2007), found growing as mixed population in the same biotopes and release a large number of secondary metabolites like fischerellin (Gross *et al*., 1991), isonitrile and calothrixin (Doan *et al*., 2000), microcystin (Carmichael, 1997) and Nostoclamides (Todorova and Jüttner, 1996). Allelopathy contribute significantly in blooms formation in stagnant water bodies and play important role in algal succession in the ecosystem (Vardi *et al*., 2002). But allelopathy may be less influential for planktonic communities in flowing water streams, because exudates released by the algae carried away quickly by the water current. The bioactive compounds cause allelopathic interaction include alkaloids, cyclic peptides, terpens and volatile organic compounds (Leflaive and Ten-Hage, 2007; Zuo *et al*., 2015; Vieira *et al*., 2016; Walton and Berry; 2016). The allellochemicals

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released as secondary metabolites act as antibiotic, antifungal, anti-predator and anti-algal or anti-cyanobacterial activity (Smith and Doan, 1999; Gross, 2003, Legrand *et al*., 2003). The production of allelochemicals may be highly strain specific and some of them were reported to be toxic (Griffiths and Saker, 2003). The growth of mixed population of bloom forming cyanoprokaryotes along with *Oscillatoria subbrevis* Schmidle, a non-heterocystous filamentous cyanoprokaryote, was observed in aquatic biotopes.

In the present investigation effect of allelochemicals of *O. subbrevis* on the growth of seven species of non-heterocystous filamentous cyanoprokaryotes of other genera, *Arthrospira khannae, Limnothrix planctonica, Lyngbya martensiana, Oscillatoria subbrevis, Phormidium defloxides, Planktothricoides raciborskii* and *Spirulina subsalsa* was studied.

MATERIALS AND METHODS

The experimental organisms used in the study were seven non-heterocystous filamentous cyanoprokaryotes e.g. *Arthrospira khannae, Limnothrix planctonica, Lyngbya martensiana, Oscillatoria subbrevis, Phormidium defloxides, Planktothricoides raciborskii* and *Spirulina subsalsa*. These were isolated from mixed algal population in different polluted biotopes of Meerut, India and their unialgal cultures were raised by repeated culturing and sub-culturing in BG-11 medium (Stanier *et al*., 1971) as described by Kant *et al*. (2005). The cultures were maintained under photo-illumination of 4K lux at 28±2°C, 14:10 h light : dark cycle. For the study of allelopathic effect, the culture filtrates of *O. subbrevis* was obtained by growing the culture of *O. subbrevis* in conical flasks containing 100 ml BG-11 medium with nitrogen, cultured for 30 days and then filtered. For experiments 10 ml of culture filtrate of *O. subbrevis* was added to 90 ml of sterilized BG-11 medium and then 5ml of exponentially grown homogenized unialgal cultures of all the seven species were inoculated separately in triplicates and cultured for 10 days. The sets without culture filtrates of *O. subbrevis* were used as control. The allelopathic effect of *O. subbrevis* extract was measured on the basis of changes in Chl-a content of the organisms. Chlorophyll-a was extracted using 80% ice cold acetone and measured in a UV-VIS spectrophotometer (Systronics 118) following Lichtenthaler and Welbum (1983). All the experiments were performed in triplicate. Standard deviation and error of the values was calculated and presented.

RESULTS AND DISCUSSION

Results showed stimulatory effect of the extract of *O. subbrevis* on the chlorophyll-a content of the same species as well as of *S. subsalsa*. Maximum increase in chl-a content was observed in *O. subbrevis* (55.79%) followed by *S. subsalsa* (21.67%). But the culture filtrate of *O. subbrevis* showed inhibitory effect on the other five species of cyanoprokaryotes under different genera. Maximum inhibition was observed in *L. plactonica* (54.37%) while minimum in *A. khannae* (7.8%) (Fig. 1).

In nature, the cyanoprokaryotes occur as primary colonizer (Tiwari *et al*., 2007) in every aquatic biotope as mixed population with other algal forms belonging to bacillariophytes, dianoflagellates, green algae and other phytoplankton groups. They produce a wide range of bioactive chemicals (Legrand *et al*., 2003; Singh *et al*., 2021), which directly or indirectly affect the growth of all the organism occurring in the same aquatic biotopes.

Singh *et al.* (2001) studied the anti-algal activity of *M. aeruginosa* against certain green algae and cyanobacteria, heterotrophic bacteria and fungi, and revealed that microcyst in toxin produced by *M. aeruginosa* inhibited the growth of green algae and cyanobacteria. Further, they also observed complete inhibition in growth, CO_2 uptake, O_2 evolution and nitrogenase activity in *Nostoc muscorum* and *Anabaena* BT1 (Singh *et al*., 2001). Yamasaki *et al*. (2007) observed allelopathic effect between the bacillariophyte *Skeletonema costatum* and the raphidophyte *Heterosigma akashiwo*. Singh *et al*. (2007) studied the allelopathic interaction between the members of the Nostocales and Stigonematales and reported autostimulatory and inhibitory effect on each other. Gantar *et al*. (2008) studied the allelopathic effect of *Fischerella* sp. 52-1 and *Chlamydomonas* sp. in co-cultivation and reported growth and

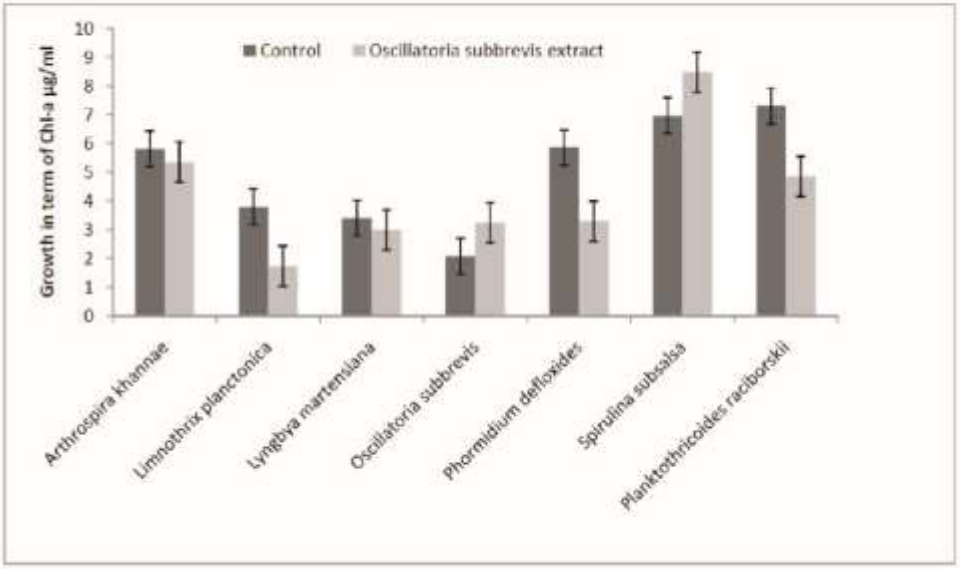

Fig. 1. Effect of culture filtrate of *O. subbrevis* on growth of *A. khannae, L. planctonica, L. martensiana, P. defloxides, P. raciborskii* and *S. subsalsa*

photosynthesis inhibition in *Chlamydomonas* sp. Singh *et al*. (2021) reported allelopathic interaction in three species of bloom forming coccoid cyanoprokaryotes i.e. *Chroococcus mimimus, Merismopedia glauca* and *Microcystis aeruginosa* which showed *M. glauca* is auto-inhibitory for itself and for *M. aeruginosa* but promote the growth of *C. minimus*. In present study, it is shown that the allellochemicals released by *O. subbrevis* has autostimulatory effect on itself. In addition, it promoted increased chlorophyll content of the same species and *S. subsalsa* but it inhibited chlorophyll-a synthesis in rest species of different genera of cyanoprokaryotes including *L. plactonica, A. khannae, L. martensiana, O. subbrevis, P. defloxides, P. raciborskii* and *S. subsalsa.*

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FULL LENGTH ARTICLE

Brine shrimp cytotoxicity assay of leaf extracts of *Lantana camara* L. obtained from different solvents

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Brine Shrimp cytotoxicity assay is a common method to check cytotoxic activity of a bioactive compound. The present work entails a preliminary screening of leaf extracts of *Lantana camara* L. in different solvents for antitumor activity. In this study benzene, chloroform, methanol and 50 % aqueous ethanol – leaf extracts of *Lantana camara* were subjected to Brine shrimp cytotoxic assay to observe antitumor activity. The results showed that *Lantana camara* may be a source for obtaining antitumor principles.

Keywords: Brine Shrimp, cytotoxicity, solvents, antitumor.

INTRODUCTION

Now-a-days interest towards active phytochemicals is gaining a worldwide acceptance to formulate nontoxic, antihazardous and cost effective management for different therapeutic approaches. *Lantana camara* a flowering species of the family Verbenaceae is an evergreen, tall shrub many parts of which are used as traditional medicines (Dharmagada *et al*., 2005). Leaves of *L. camara* is a rich source of bioactive compounds, viz., flavones, isoflavones, flavonoids, anthocyanins, coumarins, lignans, catechins, isocatechins, alkaloids, tannin, saponins, and triterpenoids (Sharma *et al*., 2013). The leaves are used as a potent source of antitumoral, antibacterial and antihypertensive agent (Taoubi *et al.*, 1997).

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Different parts of *Lantana camara* have been reported to have several tri-terpenoids, napthaquinones, flavonoids, alkaloids and glycosides and known to exert diverse biological activities including cytotoxic and anticancer properties (Ghisalberti *et al*., 2000). It was reported that leaf extract of *Lantana camara* contained phenolics, anthocyanins, and proanthocyanidins which are responsible for the antibacterial properties (Ganjewala *et al.*, 2009). The active principle of the plant extracted from leaves disrupt the permeability of cell membrane structure and thus inhibits the bacterial growth (Priya *et al.*, 2007). Leaf extract from benzene, chloroform, and methanol fractions of *L. camara* were tested against *Escherichia coli* (ATCC 10536), *Salmonella typhi* (ATCC 686), *Streptococcus aureus* (ATCC 6538), and *Pseudomonas aeruginosa* (ATCC 25619) and found that chloroform and methanol extracts showed activity against all the bacteria tested, while benzene fraction

only against *P. aeruginosa* and benzene fraction only against *S. Typhi* (Sukul *et al*., 2001). It was reported that leaf extract of *L. camara* reversibly paralyzed *M. incognita* provided a new mechanism by which the green manure reduced the nematodes population (Ahmad *et al*., 2010). Anti-leukemia activity of methanolic extract of *Lantana camara* leaf was found against Jurkat leukemia cell line (Pour et al., 2009).

Hence the present work was carried with benzene, chloroform, methanol and 50% aqueous ethanolic leaf extract of *Lantana camara* L. to asses antitumor activity by Brine Shrimp assay (Meyer et al., 1982) – an Internationally accepted , less expensive, simple protocol for assaying antitumor action.

MATERIALS AND METHODS

Preparation of plant extracts

Healthy *Lantana camara* plant leaves were collected from the campus of Barrackpore Rastraguru Surendranath College in March, 2022. The collected plant leaves were dried in the room temperature for 10 days. 50 gm of the powered plant material was taken and soaked respectively in 500 ml. of Benzene, methanol, chloroform and 50% aqueous ethanol for 3 days and then filtered. Residue was repeatedly washed against each solvent and filtered until the extract became colourless. The filtrate was evaporated under reduced pressure in a vacuum evaporator to a deep brown sticky substance and finally dried. 1gm of each dried residual mass thus obtained was dissolved in propylene glycol and stock solutions 100mg/ml were prepared by adding distilled water. Brine shrimp cytotoxic assay was done by using different concentrations of the samples.

Preparation of reagents

Clean test tubes were taken. Stock solution of 100 mg/ml was prepared. Then 70 mg/ml, 60 mg/ml, 50 mg/ml, 40 mg/ml, 30 mg/ml, 25 mg/ml, 20 mg/ml, 15 mg/ml, 10 mg/ml, 5 mg/ml and 1 mg/ml were prepared by serial dilution from the stock solution. A control set was prepared with salt water.

Brine Shrimp Lethality Assay

Brine Shrimp cytotoxicity assay was done following method of Meyer *et al.* (1982). About 1 gm of *Artemia salina* (Linnaeus) cysts (Sanders Great Salt Lake, Brine Shrimp Company L.C., U.S.A.) was properly aerated in 1L (Litre) capacity rectangular glass container (separating funnel) containing filtered seawater (30 ppt NaCl solution, pH about 8.2. Incubation was done at room temperature (25-29°C). After 48 hours of incubation newly hatched free-swimming pink-coloured nauplii were harvested from the bottom under continuous illumination of type T2 LUX fluorescence lamp. When the nauplii floated on the surface, they were collected and these freshly hatched free-swimming nauplii were used for the bioassay. The assay system was prepared with 10 ml of filtered seawater containing chosen number of nauplii andconcentration of extract and 1% yeast extract (for feeding) in watch glass. Proper aeration to the solution of watch glass was ensured. In each watch glass, some nauplii were transferred and the set up was allowed to remain for 24 h, under constant illumination of florescent lamp (LUX T2 type). Numbers of survived nauplii were counted in 3 hours interval. Three replicates were prepared for each dose level and after 24 hours LC_{50} (Lethal concentration at which 50% nauplii died) values were determined, based on the per cent mortality, by using statistical software IBM SPSS 21.

RESULTS

All the experimental observations are given in the following Tables 1-4.

Table 1. Brine Shrimp Lethality assay of the sample extract of leaf of *Lantana camara* prepared from Benzene

Sample concentration (mg/ml)		No. of survivals after 0 hours	No. of survivals after 4 hours	No. of survivals after 8 hours	No. of survivals after 12 hours	No. of survivals after 16 hours	No. of survivals after 20 hours	No. of survivals after 24 hours	LC_{50}
0	Mean	28	28	28	28	27.33	27	26.33	
	Std.Dev.	0.0	0.00	0.00	0.00	0.57	0.00	0.57	
1	Mean	28	27.33	25.33	23	21.33	19.33	17.33	
	Std.Dev.	0.0	0.57	0.57	0.00	0.57	0.57	0.57	
5	Mean	28	26.33	25.33	23	21.33	19.33	17.33*	
	Std.Dev.	0.0	0.57	0.57	0.00	0.57	0.57	0.57	
10	Mean	28	25.33	23	20.33	18.33	17	15.33*	
	Std.Dev.	0.0	0.57	0.00	0.57	0.57	1.00	0.57	
15	Mean	28	24	21.66	18.33	16.33	13.66*	12*	**15mg/ml**
	Std.Dev.	0.0	0.00	0.57	0.57	0.57	0.57	0.00	
20	Mean	28	22.66	19.33	15.66*	13.66*	10.66*	8.66*	
	Std.Dev.	0.0	0.57	1.15	1.52	0.57	0.57	0.57	
25	Mean	28	21	18.66	15.33*	12.66*	9*	6.66*	
	Std.Dev.	0.0	0.00	0.57	1.15	0.57	0.00	0.57	
30	Mean	28	20.66	16.66	13.66*	10.66*	6.66*	4.66*	
	Std.Dev.	0.0	0.57	0.57	1.15	0.57	0.57	0.57	
40	Mean	28	21.33	13.33*	10.66*	7.66*	4.66*	2.66*	
	Std.Dev.	0.0	1.15	1.15	0.57	0.57	0.57	0.57	
50	Mean	28	19	12.66*	8.66*	6*	2.66*	1.66*	
	Std.Dev.	0.0	0.00	0.57	0.57	1.00	0.57	0.57	
60	Mean	28	18.66*	10.66*	6*	3.66*	1.66*	0.33*	
	Std.Dev.	0.0	0.57	0.57	1.00	0.57	0.57	0.57	
70	Mean	28	16.33	9*	2.66*	1*	0	0	
	Std.Dev.	0.0	1.15	1.00	1.52	1.00	0.00	0.00	

*Indicates significance at ($P<0.05$) in respect of control. LC_{50} seems to be 15 mg/ml of the extract

Table 2. Brine Shrimp Lethality assay of the sample extract of leaf of *Lantana camara* prepared from Chloroform

Sample concentration (mg/ml)		No. of survivals after 0 hours	No. of survivals after 4 hours	No. of survivals after 8 hours	No. of survivals after 12 hours	No. of survivals after 16 hours	No. of survivals after 20 hours	No. of survivals after 24 hours	LC_{50}
0	Mean	26	26	26	26	25.66	25.33	24.33	
	Std.Dev.	0.00	0.00	0.00	0.00	0.57	0.00	0.57	
1	Mean	26	25.66	25.33	23	21.66	19.66	17.33	
	Std.Dev.	0.00	0.57	0.57	0.00	0.57	0.57	1.15	
5	Mean	26	25	22.66	20.66	18.33	16	14.66*	
	Std.Dev.	0.00	1.00	0.57	0.57	0.57	1.00	1.15	
10	Mean	26	22.66	20	17.66	15.66*	13.33*	12.33*	**10mg/ml**
	Std.Dev.	0.00	1.15	1.00	0.57	0.57	0.57	0.57	
15	Mean	26	23.33	18.66	15.33*	13.66*	11.66*	9.66*	
	Std.Dev.	0.00	0.57	1.15	0.57	0.57	0.57	0.57	
20	Mean	26	20.66	17*	13.66*	10.66*	7.33*	5.66*	
	Std.Dev.	0.00	0.57	1.00	1.15	1.15	1.52	0.57	
25	Mean	26	19.66	15.66*	10.66**	7.66*	5.66*	3.33*	
	Std.Dev.	0.00	1.15	0.57	1.15	0.57	0.57	0.57	
30	Mean	26	19	14*	9.66*	5.66*	3.33*	2.33*	
	Std.Dev.	0.00	1.00	1.73	0.57	0.57	0.57	0.57	
40	Mean	26	18	13.33*	8*	3.66*	1.66*	1.33*	
	Std.Dev.	0.00	1.00	1.52	1.00	0.57	0.57	0.57	
50	Mean	26	16.66*	12*	6.66*	1.33*	0.33*	0.00	
	Std.Dev.	0.00	1.15	1.73	1.15	0.57	0.57	0.00	
60	Mean	26	17*	10.66*	3.66*	1*	0.00	0.00	
	Std.Dev.	0.00	1.00	1.15	1.15	1.00	0.00	0.00	
70	Mean	26	12.33*	6.33*	1.66*	0.33*	0.00	0.00	
	Std.Dev.	0.00	1.52	0.57	1.15	0.57	0.00	0.00	

*Indicates significance at ($P<0.05$) in respect of control. LC_{50} seems to be 10 mg/ml of the extract

Table 3. Brine Shrimp Lethality assay of the sample extract of leaf of *Lantana camara* prepared from Methanol

Sample concentration (mg/ml)		No. of survivals after 0 hours	No. of survivals after 4 hours	No. of survivals after 8 hours	No. of survivals after 12 hours	No. of survivals after 16 hours	No. of survivals after 20 hours	No. of survivals after 24 hours	LC_{50}
0	Mean	30	30	30	30	29.66	29.33	29	
	Std.Dev.	0.00	0.00	0.00	0.00	0.57	0.00	1.00	
1	Mean	30	28.33	26.66	22.66	20	17.66	16.33	
	Std.Dev.	0.00	0.57	0.57	1.15	1.00	0.57	1.52	
5	Mean	30	25.66	20.33	17	15.33	14.33*	13*	**5mg/ml**
	Std.Dev.	0.00	1.15	1.52	1.00	0.57	0.57	1.00	
10	Mean	30	24	19.33	14.66*	12.66*	10*	7.66*	
	Std.Dev.	0.00	1.73	1.15	0.57	1.15	1.00	0.57	
15	Mean	30	20.66	19	13*	9.66*	7*	5.66*	
	Std.Dev.	0.00	1.52	0.00	1.00	0.57	0.00	0.57	
20	Mean	30	19.66	16.33*	11*	6.66*	5*	4.33*	
	Std.Dev.	0.00	1.52	1.52	0.00	1.15	0.00	0.57	
25	Mean	30	18.33	14.33*	9.33*	5.66*	3.66*	3.33*	
	Std.Dev.	0.00	0.57	1.52	0.57	1.15	0.57	0.57	
30	Mean	30	16.66	13.66*	8.33*	5*	3*	2.66*	
	Std.Dev.	0.00	0.57	0.57	1.52	1.00	1.00	0.57	
40	Mean	30	15.33*	12*	7.66*	4.33*	3*	2*	
	Std.Dev.	0.00	1.52	0.00	1.15	0.57	0.00	1.00	
50	Mean	30	14*	10*	8*	4*	2.66*	1.33*	
	Std.Dev.	0.00	1.73	1.00	0.00	0.00	0.57	0.57	
60	Mean	30	9.66*	6.33*	4.66*	1.33*	0.66*	0.33*	
	Std.Dev.	0.00	0.57	0.57	0.57	0.57	0.57	0.57	
70	Mean	30	9.33*	4.66*	2.33*	0.66*	0	0	
	Std.Dev.	0.00	0.57	0.57	0.57	0.57	0.00	0.00	

*Indicates significance at ($P<0.05$) in respect of control. LC_{50} seems to be 5 mg/ml of the extract

Table 4. Brine Shrimp Lethality assay of the sample extract of leaf of *Lantana camara* prepared from 50% aqueous ethanol

Sample concentration (mg/ml)		No. of survivals after 0 hours	No. of survivals after 4 hours	No. of survivals after 8 hours	No. of survivals after 12 hours	No. of survivals after 16 hours	No. of survivals after 20 hours	No. of survivals after 24 hours	LC_{50}
0	Mean	22	22	22	22	22	21.66	21.33	
	Std.Dev.	0.00	0.00	0.00	0.00	0.00	0.57	0.57	
1	Mean	22	21.33	20.66	19	18.33	16.33	15.33	
	Std.Dev.	0.00	0.57	0.57	0.00	0.57	0.57	0.57	
5	Mean	22	20.33	20	17.66	16.33	15	12.66*	
	Std.Dev.	0.00	0.57	0.00	1.15	1.52	1.00	1.15	
10	Mean	22	17.66	16.66	16	14	11.33*	11*	**10mg/ml**
	Std.Dev.	0.00	1.15	1.15	0.00	1.00	0.57	1.00	
15	Mean	22	16.33	15.33	13.66	10.66*	10*	7.33*	
	Std.Dev.	0.00	1.15	0.57	1.15	0.57	0.00	0.57	
20	Mean	22	19.66	16.33*	11*	6.66*	5*	4.33*	
	Std.Dev.	0.00	1.52	1.52	0.00	1.15	0.00	0.57	
25	Mean	22	14.33	10.66*	9*	7*	5.33*	4.33*	
	Std.Dev.	0.00	1.52	1.15	0.00	1.73	1.52	0.57	
30	Mean	22	13.66	9.66*	7.66*	6*	4.33*	3.33*	
	Std.Dev.	0.00	1.15	1.52	0.57	0.00	0.57	0.57	
40	Mean	22	12.66*	9*	6.33*	3.66*	2.66*	2*	
	Std.Dev.	0.00	1.15	1.73	0.57	0.57	0.57	0.00	
50	Mean	22	11*	8.33*	5.33*	3.66*	2*	1.33*	
	Std.Dev.	0.00	0.00	0.57	1.52	1.15	0.00	0.57	
60	Mean	22	10.33*	7*	4*	2.66*	1.33*	0.66*	
	Std.Dev.	0.00	0.57	0.00	0.00	0.57	0.57	0.57	
70	Mean	22	10*	5.33*	2.33*	1.33*	0.66*	0.33*	
	Std.Dev.	0.00	0.00	0.57	0.57	0.57	0.57	0.57	

*Indicates significance at ($P<0.05$) in respect of control. LC_{50} seems to be 10 mg/ml of the extract

DISCUSSION

Brine Shrimp cytotoxicity assay is an easy, simple and important bioassay to detect bioactivity of plant extract (Quazi *et al*., 2017). This procedure has been used in this study to establish cytotoxic activity of leaf extract of Lantana camara from benzene, chloroform, methanol and 50% aqueous ethanol. In the present study lethality of the nauplii were counted by comparing the mean surviving larvae of the test and control set. All the experimental sets in this study responded positively in Brine Shrimp cytotoxic assay as reported in some study (Olowa *et al*., 2013; Pour *et al*., 2011). All the results obtained from different concentrations of benzene, chloroform, methanol and 50% aqueous ethanolic extracts of leaves are statistically significant in respect of control set. By ANOVA it is clear that this lethality rate always crossed 95% confidence level (significant at 0.05 level). From all the tables it is clear that active principles obtained from methanol extract showed highest cytotoxic activity (Pour *et al*., 2011) over all the other treatments having LC_{50} at 5 mg/ml, followed by active principles obtained from chloroform and 50% aqueous ethanolic extract both having LC_{50} at 10 mg/ml and then benzene extract showed 15 mg/ml LC_{50}. The significant lethality of methanol, benzene, chloroform and 50% aqueous ethanol extracted samples to brine shrimp lethality suggests the presence of potent cytotoxic phytocompounds in *Lantana camara*.

CONCLUSION

Leaf extract of *Lantana camara* from benzene, chloroform, methanol and 50% aqueous ethanol responded positively in Brine Shrimp cytotoxic assay. Hence *Lantana camara* may be designated as a specimen that may be indexed as a source for obtaining antitumor principles. Further research work should be done to find out if these active principles extracted from benzene, chloroform, methanol and 50% aqueous ethanol have any antibacterial and antifungal activity, so that they may be designed as a source to formulate antihazardous, cost effective, ecofriendly preparation to manage some diseases in crop plants.

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FULL LENGTH ARTICLE

Evaluation of alpha-amylase inhibition activity of three taxa of Sundarbans, West Bengal

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Plant extracts provide an alternative source to manage various human pathophysiological conditions due to the presence of diverse metabolites in different parts of the plants. People of Sundarbans use the crude extracts of mangrove and other plants to treat conditions like rheumatism, painful arthritis, inflammation, asthma, hyperglycemia etc. The aim of this study was to identify the plant(s) possessing antihyperglycemic potential in their capacity to inhibit alpha amylase enzyme activity. In the present study seven mangrove plants, namely *Ceriops decandra, Ceriops tagal, Bruguiera gymnorrhiza, Heritiera fomes, Aegialitis rotundifolia, Avicennia marina, Avicennia alba* and three non-mangrove plants *Sida cordifolia* (Malvaceae), *Nerium indicum* (Apocynaceae), *Euphorbia dracunculoides* were primarily chosen from PatharPratima, Sundarbans, and screened for anti-alpha-amylase activity. Aqueous extract of two mangroves, *Avicennia marina* and *Ceriops tagal* and one non-mangrove *Euphorbia dracunculoides* were identified as alpha-amylase blockers. Those three plants were further screened for their phytochemicals present and cytotoxicity in SH-SY5Y cell line. The plants were found rich in secondary metabolites, like, polyphenols, tannins, flavonoids etc. The cytotoxic effects of the three plants, namely, *Avicennia marina, Ceriops tagal* and *Euphorbia dracunculoides* when tested on SH-SY5Y(neuro blastoma cell line) found to have no toxicity.In addition to that, high free radical scavenging activities was seen in *Euphorbia dracunculoides*. Identification of the compounds in these three extracts will lead to the establishment of new alpha amylase blocker(s).

Keywords: Alpha amylase-blockers, Mangrove plants, Secondary metabolites, Cytotoxicity.

INTRODUCTION

Sundarbans, the home of more than 300 species of plants, is one of the biodiversity hotspots in India. People of Sundarbans are not only dependent on the plants for their livelihood but also use crude extracts of whole plants, leaves, barks to treat different pathophysiological conditions like arthritis, inflammation, diabetes etc. (Bandaranayake, 1998).We focussed on the antidiabetic property of the plants and screened them based on their anti-amylase activity. Amylases, both salivary and gut are involved in the breakdown of starch to glucose in our alimentary canal and thus increase blood glucose concentration. Hence, amylase blockers are used as a measure to control blood sugar level in diabetes type II and there is always search for new blockers of natural sources.

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Hence, objective of this study was to identify the plants which are used by local people of Sundarbans for anti-glycemic property.

Site of sample collection

Sundarbans in Patharpratima, West Bengal area were chosen for sample collection. The area was visited several times and conversations with local tribal people were noted before choosing the plants.

MATERIALS AND METHODS

Sample collection and preparation

Young and fresh twigs of seven mangrove plants, *Herittiera fomes, Aegialitis rotundifolia, Avicennia alba, Avicennia marina, Ceriops decandra, Ceriops tagal, Bruguiera gymnorrhiza* and three non mangroves *Sida cordifolia, Nerium indicum, Euphorbia dracunculoides* were collected from Sundarban area, West Bengal with prior permission from forest department in the month of March (Table 1). Twigs of each sample were preserved for herbarium sample and identified with the help of preserved herbaria in National Herbaria in Botanical Garden and four of them, namely, *Aegialitis rotundifolia* (Specimen Id : XC/SB-01), *Avicennia marina* (Specimen Id : XC/SB-04), *Ceriops decandra* (Specimen Id : XC/SB-02, XC/SB-03) and *Ceriops tagal* (Specimen Id : XC/SB-05) were authenticated by experts from Indian Botanical Garden, Shibpur, Kolkata. Leaves from twigs of each sample were collected, washed, sun-dried and 10 grams of dry weight of each sample were kept in clean sterilised glass container in powdered form at 25ºC.

All the chemicals were of analytical grade. All the enzymes (fungal diastase, Human salivary α-amylase, porcine pancreatic α-amylase) were purchased from Merck. Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS) and antibiotic-antimycotic solution were purchased from Gibco-BRL, DMSO purchased from Sigma Aldrich, USA. (3-(4,5-dimethylthiazol-2yl-)-2,5-dihenyl tetrazolium bromide (MTT)was purchased from Sigma.

Preparation of crude extract

The crude extracts of all the three plant leaves for each experiment was prepared by using powdered sample of same dry weight with double distilled de-ionised water in same volumes. In each case, 5 mg of dried leaf powder was soaked in 10 ml of water overnight, centrifuged at 1000 rpm (REMI R-303, max. RCF-

Table 1. Plant samples studied

Sample	Common name	Scientific name	Family	Availability
S1	Sundari	*Heritiera fomes*	Sterculiaceae	Nearly threatend
S2	Tora	*Aegialitis rotundifolia*	Plumbaginaceae	Abundant
S3	Kali Baani	*Avicennia marina*	Avicenniaceae	Abundant
S4	Peyara Baani	*Avicennia alba*	Avicenniaceae	Abundant
S5	Maath Goraan	*Ceriops decandra*	Rhizophoraceae	Nearly threatened
S6	Chaand Goraan	*Ceriops tagal*	Rhizophoraceae	Abundant
S7	Maankraa	*Bruguiera gymnorrhiza*	Rhizophoraceae	Abundant
S8	Berela	*Sida cordifolia*	Malvaceae	Abundant
S9	Karabi	*Nerium indicum*	Apocynaceae	Abundant
S10	Dragon Spurge	*Euphorbia dracunculoides*	Euphorbiaceae	Abundant

S1– S7 : mangrove and S8– S10 : non-mangrove plants

1450 g) for 10 minutes. The supernatant was filtered with the help of Whatman no. 1 filter paper and used as the crude sample (Saravanan, 2016).

Alpha-amylase inhibition assay

The experiment was done according to the DNSA method originally of Sumner and Sisler and modified by Miller (Miller, 1959), viz, 1 ml of enzyme was mixed with 1 ml of inhibitor and incubated at 25°C for 10 minutes, then added1 ml of starch in it and incubated for another 10 minutes at same temperature. After that 2 ml of DNSA was added to it and incubated in water bath having the temperature between 90° to 100°C for 5 minutes. The reaction was stopped by the addition of 2 ml of sodium potassium tartrate solution in it. After cooling, OD was measured in 540 nm. Acarbose was taken as standard, or the positive control and 1 ml of double distilled water was added in place of inhibitor in the negative control. The percentage of inhibition was measured by the following formula:

$$\text{Percentage inhibition} = \frac{(\text{Absorbance of negative control} - \text{Absorbance of experimental set})}{\text{Absorbance of negative control}}$$

IC50 determination

Six different concentrations (0.5mg/ml, 1mg/ml, 2mg/ml, 3mg/ml, 4mg/ml, 5mg/ml) of inhibitors were taken to perform the same DNSA method of inhibition assay and IC50 of each inhibitor was determined in all three enzymes, fungal amylase, human salivary amylase and porcein pancreatic alpha amylase, and compared with the positive control (Acarbose) in each case. Conc. of inhibitors used in the experiment were 0.5 mg/ml to 5 mg/ml and conc. of starch and enzymes were 0.5 mg/ml.

Anthocyanin

100 mg of fresh leaves were extracted in 10 ml of acidified methanol (1:99::HCl: Methanol, viz. 0.1 ml HCl mixed with 9.9 ml Methanol). After 24 hours of incubation in 0°C, volume was made up to 10 ml. The absorbance was read at 530 nm and the content was expressed as mg/g Gallic acid equivalent (Wu *et al*., 2006).

Total phenols

1 g of dry sample was ground with a mortar and pestle in 5 ml of 80% ethanol. The supernatant was evaporated and dissolved in 5ml of distilled water. 1 ml of this was treated with 0.5 ml of Folin-Ciocalteau reagent (reagent diluted at 1:1 ratio), then added 20% sodium carbonate solution and boiled for 1 minute. The colour intensity was measured at the absorbance at 765 nm against a reagent blank and content was expressed as mg/g Gallic acid equivalent.

Total flavonoids

The flavonoids were extracted from 100 mg of samples in 80% acidified methanol (methanol: water: HCl :: 80:20:1) for 12 hours at 4°C in dark. Absorbances were taken at 315 nm and the content of flavonoids were expressed as optical density units (Mirecki and Teramura, 1984).

Total flavonols

Total flavonols were estimated according to the method of Kumaran and Karunakaran, 2006. The leaves were taken in 1 g quantity and crushed in 10ml of methanol, centrifuged and the volume of the supernatants were made upto 10 ml. 1 ml of extracts 1 ml of 2% $AlCl_3$ ethanol and 3 ml sodium acetate solution (50g/L) were mixed and incubated for 2.5 hours at 20°C. Absorbance was taken in 440 nm and the content of flavonols were expressed as mg equivalent quercetin /g (Kumaran and Karunakaran, 2006).

Tannin

0.5 g of dried powdered materials were boiled for 30 minutes in a 250 ml conical flask with 75 ml water and then made up to 75 ml., again 1 ml of extractions were taken and diluted up to the volume 75 ml by adding more water. 5 ml of Folin-Denis reagent and 10 ml of sodium carbonate solution were added to the diluted extractions and incubated for 30 minutes in 25°C. Colour intensity of the reaction mixture were read at 700 nm and expressed as mg equivalent of tannic acid (Hagerman, 1978).

Cellular toxicity test

To evaluate the cytotoxicity effects of *Avicennia marina, Ceriops tagal, Euphorbia dracunculoides in-vitro* using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), SH-SY5Y, a neuroblastoma cell line was used. The cells were cultured in DMEM high glucose, supplemented with 10% FBS and antibiotic-antimycotic solution and maintained at 37°C in humified incubator maintaining 5% CO_2. About 1X104 cells were seeded in each well (200µl/well) of 96 wells plate. The 5gm/ml stock was diluted and added to the wells at a serial dilution of 200µg/ml, 100µg/ml, 50µg/ml and so on up to 6.25µg/ml. Control wells were kept with water. MTT assay was performed after 48 hours upon addition of the samples. Briefly 10µl of MTT solution (5mg/ml) were added in each well and incubated for 4 hours at 37°C. Following incubation, the plates were centrifuged and the media containing MTT was removed and equal volume DMSO was added to solubilize formazan crystals. The absorbance was taken at 570 nm in Synergy H1 microplate reader. The IC50 was calculated using inhibitor versus normalised response in non-linear regression analysis (Plumb, 2004).

Free Radical Scavenging Activity

The free radical scavenging activity of ethanolic extracts of all the plant extracts were determined using a stable radical DPPH(1,1-diphenyl-2-picrylhydrazyl). 3.9 ml of freshly prepared DPPH (0.0025% in 95% ethanol) was mixed with 100 µl of plant extract and incubated for 30 mins in room temperature. Absorbance was measured at 517 nm. The capacity to scavenge the DPPH radical was calculated, using the following equation:

$$\text{DPPH scavenged (\%)} = \{(Ac - At)/Ac\} \times 100$$

Where Ac is the absorbance of the control reaction and At is the absorbance of the sample reactions. An antioxidant value of 100% indicates the strongest antioxidant activity. 95% ethanol was used as blank and the DPPH – ethanol mixture as control (Ravikumar, 2010).

Statistical analysis

The data were processed using GraphPad prism version 7 with the concentration (in micromolar) of compounds in abscissa and percentage viability of cells in ordinate. No. of replicates were six (n=6) and error bars in all experiments represent mean± SD and p values < 0.05 were considered significant.

RESULT

Percentage inhibition

The plant extracts were tested for their anti-amylase activity against three alpha amylases, fungal diastase (FD), porcein pancreatic alpha amylase (PPA) and human salivary alpha amylase (HSA).The percentage of inhibition values are presented in Table 2. Among the mangrove plants (S1-S7) percentage of inhibition (%) of FD was maximum in *Ceriops tagal* (88.9 ± 0.05) followed by *Avicennia marina* (44.5±0.05), *Ceriops decandra* (37.8 ± 0.05) and *Avicennia alba* (11.10 ±0.05. In non-mangrove plants (S8-S10) only *Euphorbia dracunculoides* exhibited inhibition of FD (45.7±0.05). The increase in inhibition percent of HSA was in the order of *Avicennia marina* > *Ceriops tagal* > *Avicennia alba* > *Ceriops decandra*. Inhibition of PPA was highest in *Ceriops tagal* (61.2 ± 0.05) followed by *Avicennia marina, Avicennia alba* and *Ceriops decandra* while the percent inhibition of PPA was (39.9.7 ± 0.05) in *Euphorbia dracunculoides*.

Selection of sample

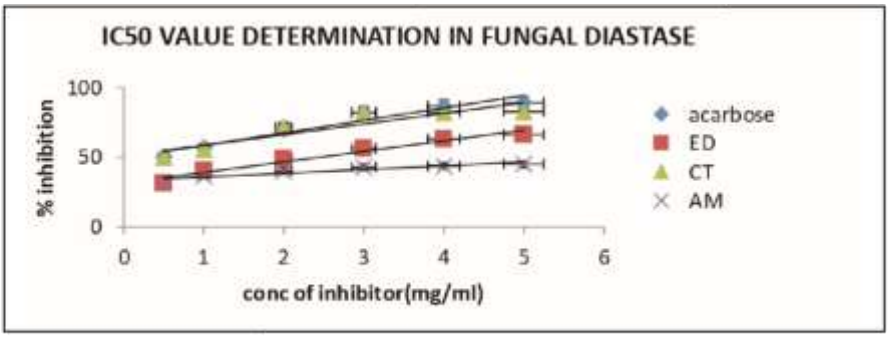
Three plants *Avicennia marina, Ceriops tagal* and *Euphorbia dracunculoides* were selected based on the percent inhibition and preceded for further studies (Fig. 1).

Determination of IC_{50} values

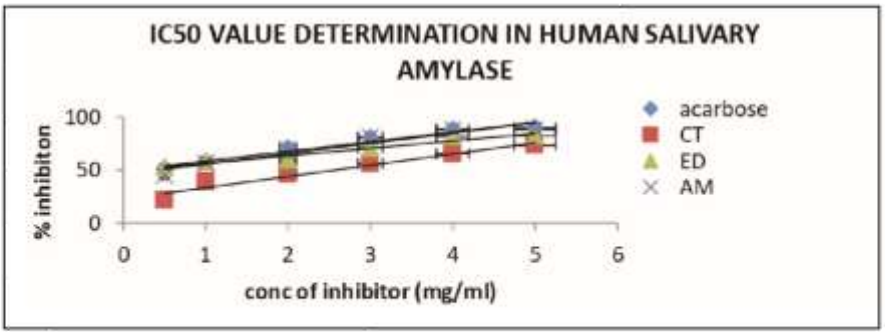
Ic_{50} values of the three plant extracts were determined against FD, HSA and PPA andcompared with standard drugs(Table 3 and Fig. 2).

The IC_{50} value of *Ceriops tagal* was (0.6 ± 0.05 mg/ml) minimum among the three against FD and the value

Fig. 1. Twig of *Euphorbia dracunculoides* showing trilocular fruits in it found in Sundarban terrestrial belt **(A)**, Plant *Avicennia marina* showing fruits and leaves in it found in summer time at the working place **(B)**, Plant *Ceriops tagal* showing germinated seedlings when the fruits are still attached with plant (viviparous germination) found within Sndarban forest **(C)**.



(A)



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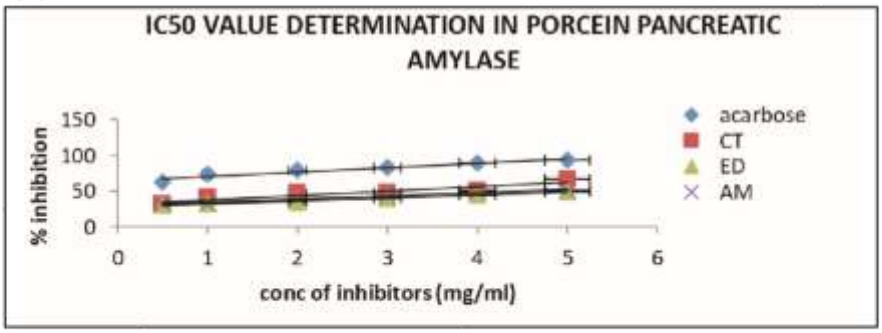

(C)

Fig. 2. IC_{50} values of *C. tagal, A. marina* and *E. dracunculoides* against FD **(A)**, HSA **(B)** and HPA **(C)** have been shown.

Table 2. The percentage of inhibition of three alpha-amylases; fungal diastase (FD), porcine pancreatic amylase (PPA) and human salivary amylase (HSA) by plant leaf extracts.

Sample	Inhibition Percentage in FD	Inhibition Percentage in HSA	Inhibition Percentage in PPA
Herittiera fomes	No Inhibition	No Inhibition	13.3 ± 0.05
Aegialitis rotundifolia	No Inhibition	No Inhibition	No Inhibition
Avicennia marina	44.5 ± 0.05	90.5 ± 0.05	59.1 ± 0.05
Avicennia alba	11.1 ± 0.05	15.6 ± 0.05	11.2 ± 0.05
Ceriops decandra	37.8 ± 0.05	10.8 ± 0.05	5.6 ± 0.05
Ceriops tagal	88.9 ± 0.05	73 ± 0.05	61.2 ± 0.05
Bruguiera gymnorrhiza	No Inhibition	No Inhibition	No Inhibition
Sida cordifolia	No Inhibition	No Inhibition	No Inhibition
Nerium indicum	No Inhibition	No Inhibition	No Inhibition
Euphorbia dracunculoides	45.7 ± 0.05	87.5 ± 0.05	39.9 ± 0.05

The values are represented as mean±SD. n=6

Table 3. IC_{50} values of *Avicennia marina, Ceriops tagal, Euphorbia dracunculoides and* Acarbose against FD, PPA and HSA.

Sample (standard blockers and plant samples)	IC_{50} values in Fungal Diastase (mg/ml)	IC_{50} values on salivary amylase (mg/ml)	IC_{50} values on salivary amylase (mg/ml)
Acarbose (Positive control compound)	0.05 ± 0.05	0.26 ± 0.05	0.07 ± 0.05
Avicennia marina	6.20 ± 0.05	0.61 ± 0.05	4.30 ± 0.05
Ceriops tagal	0.60 ± 0.05	2.58 ± 0.05	3.90 ± 0.05
Euphorbia dracunculoides	2.45 ± 0.05	0.47 ± 0.05	6.70 ± 0.05

The values are represented as mean±SD. n=6

was close to the IC_{50} value of Acarbose (0.5 ± 0.05 mg/ml). The IC_{50} values of *A. marina* and *E. dracunculoides* were 6.2 ± 0.05 mg/ml and 2.451 ± 0.05 mg/ml respectively.

The IC_{50} values of *A. marina* and *E. dracunculoides* were 0.61 ± 0.05 mg/ml and 0.47 ± 0.05 mg/ml respectively, close to the IC_{50} of acarbose (0.26 ± 0.05 mg/ml). The IC_{50} value of *Ceriops tagal* was (2.576 ± 0.05 mg/ml) high against HSA.

The IC_{50} value of *Ceriops tagal* was (3.9 ± 0.05 mg/ml) minimum among the three against PPA though quite high compared to acarbose (0.07 ± 0.05 mg/ml). The IC_{50} values of *A. marina* and *E. dracunculoides* were 4.3 ± 0.05 mg/ml and 6.7 ± 0.05 mg/ml respectively.

Assessment of secondary metabolites

All the three plants were rich in secondary metabolites as shown in the Table 4. The amount of total polyphenols was almost comparable in all three species, *Avicennia marina, Ceriops tagal* and *Euphorbia dracunculoides*. *Avicennia marina* exhibited the highest amount of tannin, followed by *Euphorbia dracunculoides*. Flavonoid content of *Ceriops tagal* was remarkably low. Anthocyanin and Flavonol contents were again almost similar in all three, though *Avicennia marina* exhibited slightly higher values in both the assessments.

Free radical scavenging activity

The leaf extract of *E. dracunculoides* exhibited high, 87% of free radical scavenging activity. *C. tagal, A. marina*, leaf extracts showed 53% and 52% activity respectively (Fig. 4).

DISCUSSION

Plant based secondary metabolites serve as an excellent source of various therapeutic agents (Sahoo *et al.*, 2012; Okla *et al.*, 2019). Many such secondary metabolites like quercetin 3-(6-malonylglucoside),

Table 4. Amount of secondary metabolites, specifically, polyphenol, tannin, flavonoid, anthocyanin and flavonol in the leaves of *Avicennia marina, Ceriops tagal* and *Euphorbia dracunculoides*

Specimen	Polyphenol content (mg/gm)	Tannin content (mg tannic acid/gm)	Flavonoid content (mg quercetin/gm)	Anthocyanin content (mg gallic acid/gm)	Flavonol content (mg quercetin/gm)
Avicennia marina	102.42 ± 0.05	87.38 ± 0.05	78 ± 0.05	0.51 ± 0.05	16 ± 0.05
Ceriops tagal	107.63 ± 0.05	58.37 ± 0.05	25 ± 0.05	0.49 ± 0.05	10 ± 0.05
Euphorbia dracunculoides	98.53 ± 0.05	60.65 ± 0.05	62 ± 0.05	0.42 ± 0.05	13 ± 0.05

The values are represented as mean±SD. n=6

Table 5. IC_{50} values of *Avicennia marina, Ceriops tagal* and *Euphorbia dracunculoides* against SH-SY5Y cell line, represting cytotoxicity of these plants on SH-SY5Y cell line.

PLANTS	IC_{50}
Avicennia marina	>100 µg/ml
Ceriops tagal	>100 µg/ml
Euphorbia dracunculoides	>100 µg/ml

Cellular Toxicity

No significant toxicity was found against SH-SY5Y, neuroblastoma cell line, for the extracts of *C. tagal, A. marina* and *E. dracunculoides*, as shown in Fig. 3 and Table 5, rendering the extracts physiologically safe.

rutin, isoquercitrin, epigallo catechin gallate, caffeic acid, p-coumaric acid, curcumin derivatives, diosgenin are well known anti hyperglycemic agents (Hamdan, 2004; Iniyan *et al*., 2010; Karthic, 2008). People of Sundarbans use plant extracts to treat diabetes for a long time. It has been found that methanolic stem extract of *Excoecaria indica* (Rahman *et al*., 2010), methanol extract of *Heritiera fomes* (Ali *et al*., 2011), bark part of *Bruguiera gymnorrhiza* extracted with ethanol solvent (Karimulla, 2011) have anti-diabetic property. However, the exact mechanism is not known.

In this study, we screened ten plants from Sundarbans based on their anti α-amylase property. Aqueous extracts of the leaves of two mangrove plants, *Avicennia marina* and *Ceriops tagal* and one non mangrove plant, *Euphorbia dracunculoides* showed

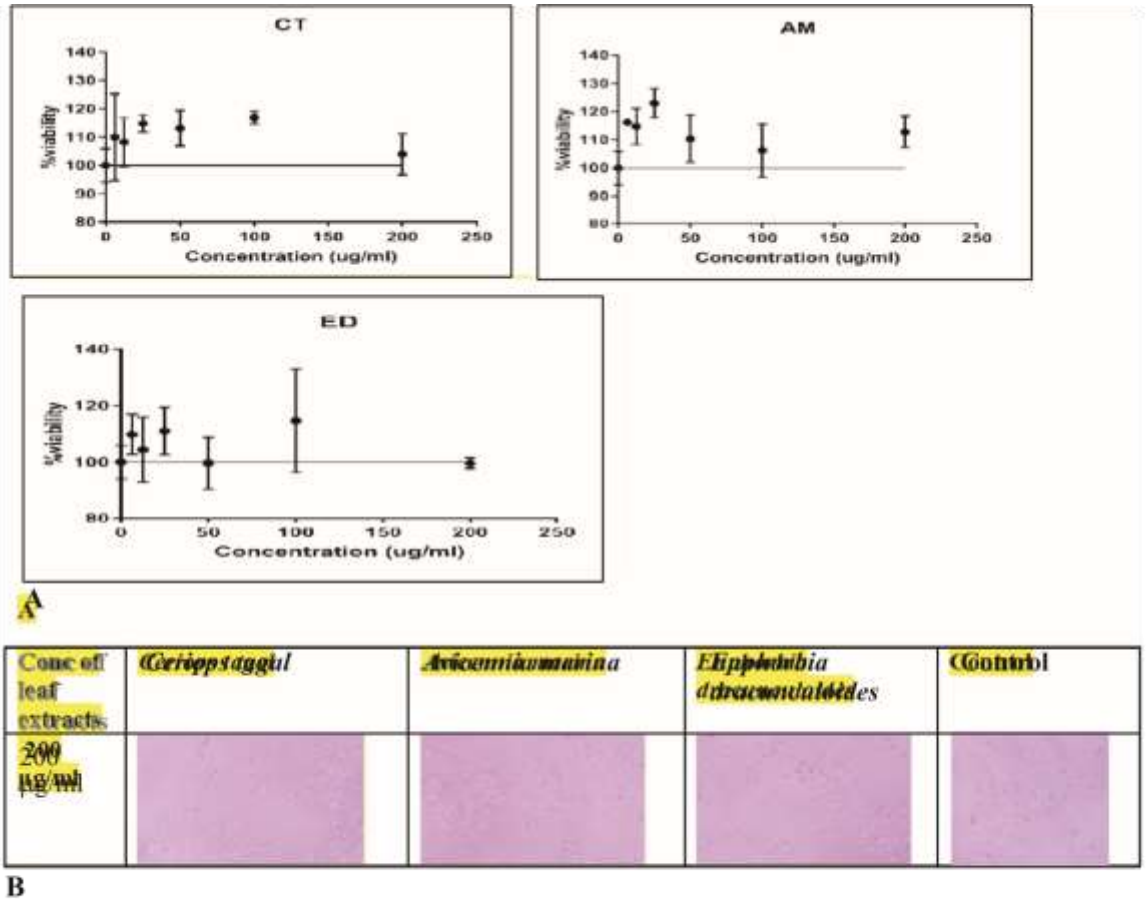

Fig. 3. A. Percent viability of SH-SY5Y cell line after treatment with the leaf extracts of *C. tagal, A. marina* and *E. dracunculoides* (100% viable cells in all concentrations in all three plants) has been shown. **B.** Effect of the leaf extracts of *C. tagal, A. marina* and *E. dracunculoides* at a conc. of 20 µg/ml on SH-SY5Y cell lines have been shown. No cytotoxicity was observed in any plant extracts.

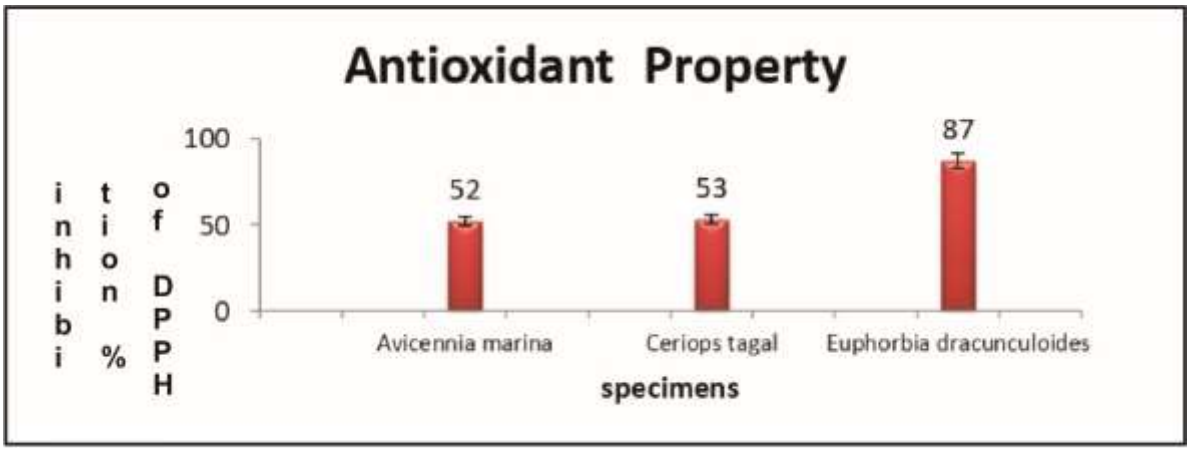

Fig.4. Free radical scavenging activity of *C. tagal, A. marina* and *E. dracunculoides* has been shown.

positive results FD, HSA and PPA. *Ceriops tagal* leaf extract was found to inhibit FD and PPA, *Euphorbia dracunculoide* and *Avicennia marina* inhibited HSA and the inhibition was comparable to standard drugs like Miglitol or Acarbose. The three plant extracts were rich in secondary metabolites like flavonoids etc. and showed no cytotoxicity against neuroblastoma cell line. *Euphorbia dracunculoides* showed high free radical scavenging activity followed by the other two plant extracts. Detailed examination of the secondary metabolites and identification of the potential compounds will help to the discovery of novel α-amylase blockers from these plants.

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FULL LENGTH ARTICLE

Pollination biology and pollen morphology of *Alangium salvifolium* (L.f.) Wangerin (Alangiaceae)

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The family Alangiaceae represents a single genus *Alangium* in the tropical and subtropical part of the old World. The genus comprises about 24 species and occurs in the form of trees and shrubs. Only two species occur in India. *Alangium salvifolium* flowers during February to April. Opened flowers are evident throughout the day and night with higher frequency and offer pollen and nectar as the reward to their insects visitors. Altogether 18 insects species (*Apis florae, Trigona* sp., *Amegilla* sp., *Xylocopa latipes, Xylocopa pubescens*) were found foraging at the flowers. The bees collected pollen as well as nectar. The wasps while foraging for nectar their dorsal side touches the anthers and cause pollination. *Xylocopa latipes* and *Xylocopa pubescens* are the regular and effective pollinators throughout the flowering period. Pollen grains are oblate spheroidal and brevicolporate. Exine has sculptured ornamentations.

Key Words: Pollination, nectar, brevicolporate.

INTRODUCTION

Alangiaceae, in the basal Asterid clade, consists of only the genus *Alangium*, with approximately 24 species mostly distributed in tropical and subtropical regions of eastern and southeastern Asia, extending to most of the western and southwestern Pacific Islands (Bloembergen, 1939; Cronquist, 1981; Fedina *et al.*, 2002). *Alangium* is characterized by the combination of alternately arranged simple leaves, 5-9-merous polypetalous epigynous flowers with stamens equal or half in number to the petals, a 1- or 2-car-pellate ovary and a drupe-like fruit. The genus has usually been treated in or close to Cornaceae. It was included as a member of Cornaceae by Harms (1897), but since 1910 (Wangerin, 1910) the genus has been commonly recognized as a monogeneric family in the Cornales (Takhtajan, 1980, 1997; Cronquist, 1981; Thorne, 1992). Recent molecular phylogenetic studies confirmed that the genus is a member of Cornales and sister to the dogwood genus Cornus (Chase *et al*., 1993; Xiang *et al*., 1998, 2002; Fan and Xiang, 2003). The flowers being available day and night foraged by diurnally active insects. The insects include species of bees, wasps, flies and butterflies (Layek *et al*., 2015). The bees collect both pollen and nectar, while the remaining groups forage for nectar only. The flower-form by exposing the stamens and stigma is promiscuous to foraging by any kind of visitor species.

The large-sized bees are perfect and appropriate for the flowers of *A. salvifolium*. The flowers standout visually and greatly attract the bees from several

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metres, Further the flowers are very conspicuous by their larger display and by the shedding of leaves. The bees make inter-tree movements and exhibit 'traplining' and 'territorial' foraging behavior. The traplining bees by foraging continuously or alternately greatly promote cross-pollination. The bees that exhibit territoriality select a population of flowers from one to few, conspecific trees of an area and guard the floral food of those flowers from intruder insect species by repelling them (Van der Pijl 1954; Janzen 1964; Barrows 1980; Frankie *et al*., 1983; Aluri and Subba Reddi, 1989). As the anthesis rate is almost uniform, the amount of nectar flow temporally, is uniform throughout. Consequently, the bees appear on the flowers at the same frequency and exploit floral forage from sunrise to sunset. Further, the nectar volume and sugar concentration per flower is in accordance with other *Xylocopa*-pollinated flowers such as *Anisomeles indica, A. malabarica* and *Gliricidia sepium* (Aluri and Subba Reddi, 1989, 1993; Pal, A. 2022, 2023). The continuous foraging of bees on the tree species effects pollination in numerous flowers daily. The bees forage alternately or shift from one to another species for sometime depending on the richness of floral reward. The other plant species that the bees preferentially foraging in the area during the flowering period of *A. salvifolium* include *Gliricidia* sp., *Peltophorum pterocarpum, Cassia* sp., *Moringa oleifera* and *Cochlospermum religiosum*. The plant species exhibit different dates of peak flower production. As the carpenter bees are swift fliers and have great capacity to exploit floral reward in a very short time on a single foraging bout, their simultaneous or alternate foraging on the above mentioned plant species do not in any way appear to influence the success of self-or-cross-pollination in those plants.

The fast flying sphingid, *M. gyrans* (hawkmoth) forages equally efficiently as *Xylocopa*. The hawkmoth forages in the dim light before sunrise and again after sunset. Since the tree antheses throughout the night too, the amount of fresh flowers accumulated by early morning are so great that it stimulates the intensity of foraging by *M. gyrans*. The hawkmoth forages the flowers very rapidly and moves at speed from one flower to another. It exploits floral forage of almost all of the flowers available by that time and effects pollination. All other foragers appear by sunrise and close their foraging activities by sunset as soon as they stop foraging, several individuals of hawkmoth appear foraging until it is dark. Although the hawkmoth has limited foraging period, it is as equally efficient a pollinator as *Xylocopa*. Its services in this context assume great significance in the pollination of *A. salvifolium*. From this account, it suggests that the *Xylocopa* sp. and *M. gyrans* are the principal pollinators of this tree. Since, comparable data from other species of *Alangium* are lacking, the present observations on *A. salvifolium* cannot be compared to other species. However, it is not unreasonable to suggest that the pollination system described here for *A. salvifolium* may be occurring in allied species as they do not differ much in their floral morphology and floral features (Stone and Kochummen, 1975; Ananthakrishnan *et al*., 1981).

MATERIALS AND METHOD

Pollen output per anther of *Alangium salvifolium* was assessed by counting all the pollen grains in a sample obtained by gently crushing and tapping the anther on a clean microscope slide spreading the pollen mass uniformly. The longevity of pollen and stigma was based on the fruit set success from hand-pollinations at regular intervals. To assess pollen amounts transferred on the stigma in a single visit by a particular kind of insects, bagged flowers just before anthesis were opened one by one for the insects to visit. When such exposed flowers received the first visit, their stigmas were examined for pollen. Similarly the pollen deposited on the stigmas was assessed at regular intervals.

RESULTS

The plants begin to bloom soon after the cold season. The blooming season extends from February to April every year. Flowers are white or yellowish white, hermaphrodite in axillary fascicles bearing 2-3 flowers. The root bark is anthelmintic and purgative. It is useful for fevers and skin diseases and is generally administered in the form of powder.

Pollination biology

The process of pollination begins when the pollen grains from the respective flowers lands on the stigma and form a pollen tube with the style length, which connects both the stigma and ovary. After the completion of the pollen tube, the pollen grain starts transmitting sperm cells from the grain to the ovary. Later the process of fertilization in plants will take place when the sperm cells reach the ovary and egg cells. The seed is then released from the parent plant and making it able to grow into a plant and continue the reproductive cycle with the use of the pollination method.

Floral biology and reproductive ecology

Phenology of anthesis:

Opened flowers are evident throughout the day and night with a higher frequency. A bud takes 20-30 min. to become fully open. Concomitant with the gradual opening of flower, the process of nectar secretion is also started. Petals covered the stamens and stigma, but are yellowish at the time of anthesis. The petals were excited by this time and are consequently reflexed downwards as a sudden mechanism. Then, they expose the anthers and stigmas which happens to be longer than the former to the visitors.

Flower morphology:

Flowers are considerably large and hermaphrodite. Calyx tube is adnate to the overy; limb is truncate or 4-10 toothed. Petals 4-10 linear, light green, polypetalous, valvate, thickend and recurved in flower. Flower length ranges from 1.6-2.2 and is 0.5mm wide, stamens ranging from 20-30. The anthers are dithecous and introrse. Stigma is large capitates, projecting beyond the anthers. Ovules are solitary and pendulous.

Stigma receptivity:

Stigma remains receptive for 36 h beginning with anthesis. On hand pollination 0,6,12,24 and 36 h old stigmas get 80,70,60,40 and 30% fruit set respectively and still older stigmas were not receptive.

Nectar dynamics:

Nectar is situated around the ovary base. Nectar volumes measured at 2 h intervals indicating that the rate of production is not consistent throughout flower life.

Flower visitor activity:

During the study period, 18 insect species were found foraging at the flowers (Table 1) of this 12 are Hymenoptera (3 Apidae; 4 Anthophoridae, 2 Xylocopidae, 3 Eumenidae), 1 Diptera and 5 Lepidoptera (1 Sphingidae, 2 Danaidae, 1 Pieridae and 1 Hesperidae).

Diurnal activity:

All the flower visitors are diurnal in their activity.

Insect behavior at the flowers:

The bees collect pollen as well as nectar. *Amegilla* and *Apis sp.* collect pollen on their ventral side of the body while touching the essential organs. *Xylocopa, Delta* and *Rhynchium* species visit the flowers and nectar and effecting the nototribic pollination. The small bodied bees such as *Trigona sp.* and *Ceratina sp.* concentrated on pollen collection. They alighted on the upper side of each anther and collected pollen by virtue of which their abdominal surfaces got smeared.

Description of pollen grain:

Alangium salvifolium (L.f) Wangerin.

Pollen grains oblate spheroidal, PXE = 45-51 x 48-56 µm, amb sub-triangular, angles rounded, sides conves, brevicolporate- pororate to porate, colpi small, apices acute to rounded, endoaperture circular or slightly lolongate, 2-2.5 µm in diameter, exine 1.5-2 µm thick, sexine thicker than nexine; surface reticulate, muri low, lumina polygonal, 1-1.5 µm across.

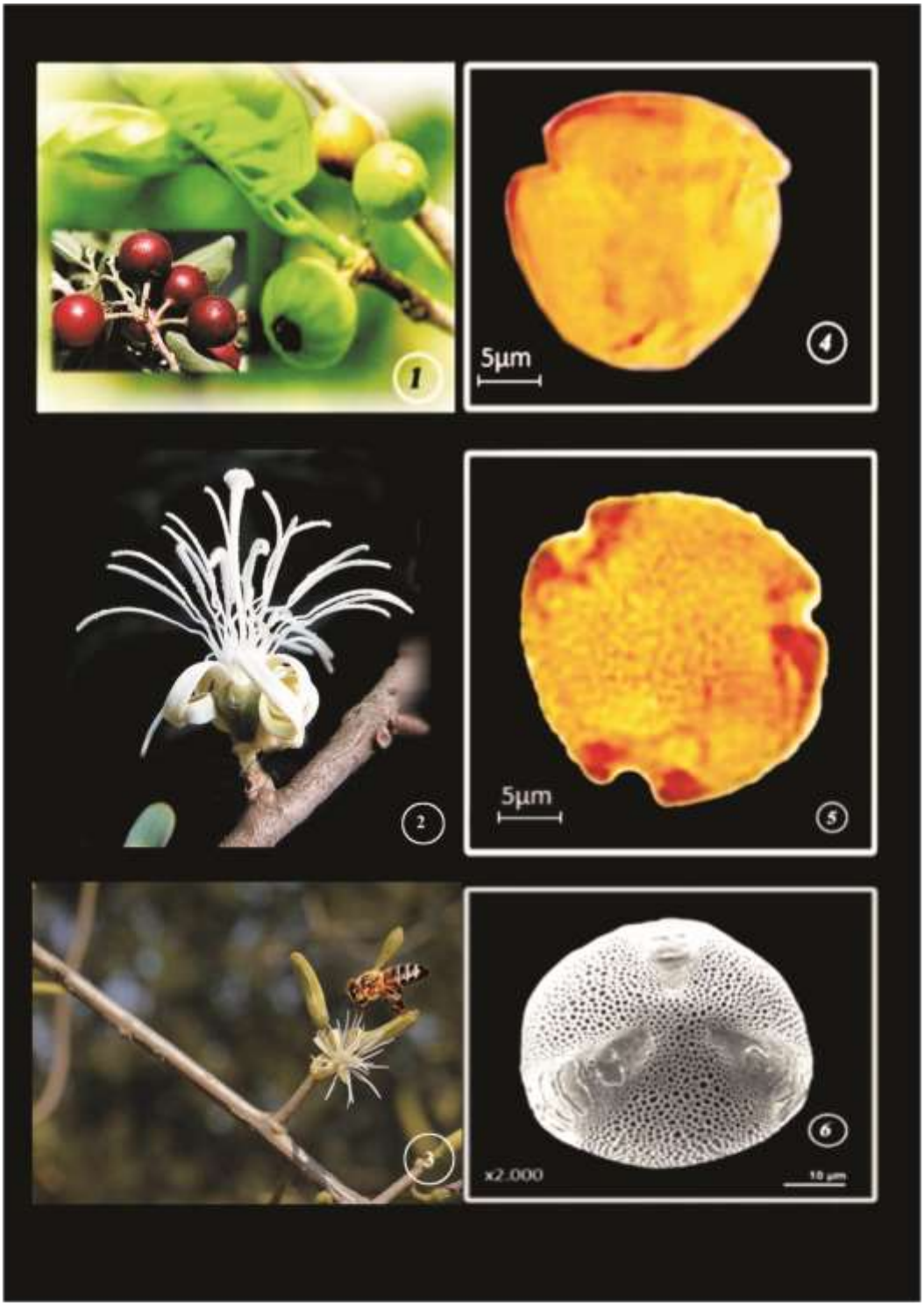

Plate 1. Fig 1: Fruit of *Alangium salvifolium*; **Fig 2:** Blooming flower of *Alangium salvifolium*; **Fig 3:** *Apis dorsata* visiting flowers; **Fig 4-5:** Pollen grains of *Alangium salvifolium* x 1180, **4:** Polar view in optical section x 1180, **5:** Polar view showing sub-triangular amb with trizonocolporate aperture and reticulate surface with polygonal lumina; **Fig 6:** Scanning electron microscope photomicrograph of *Alangium salvifolium* pollen grain in polar view. The scale bar represents 10 µm.

Table 1. Particulars of flower visitors on *A. salvifolium*

Visitor species	Forage type		Body region of pollen deposition
	Pollen	Nectar	
Hymenoptera			
Apidae			
Apis cerana indica	+	+	Head ventral side
A. florae	+	+	--"--
Trigona sp.	+	+	--"--
Anthophoridae			
Amegilla sp.	+	+	--"--
Ceratina sp.	+	+	--"--
Thyreus histrio	+	+	--"--
Pithitis bingami	+	+	--"--
Xylocopidae			
Xylocopa latipes	+	+	Head dorsal ventral side
X. pubescens	+	+	--"--
Eumenidae			
Delta sp.	–	+	Dorsal side head
Ropalidia spatulata	–	+	--"--
Rhynchium metallicum	–	+	
Diptera			
Muscidae			
Musca sp.	–	+	Ventral side
Lepidoptera			
Sphingidae			
Macroglossum gyrans	–	+	Proboscis legs
Danaidae			
Danaus chrysippus	–	+	--"--
Euploea core	–	+	--"--
Pieridae			
Catopsilia pyranthe	–	+	--"--
Hesperiidae			
Pelopidas mathias	–	+	--"--

DISCUSSION

Opened flowers are evident at any time of the day. The flowers are hermaphrodite and homogamous. Both selfing through geitonogamy and out-crossing appear to play a role in the reproduction of *Alangium salvifolium* as revealed by hand-pollination experiments, but to a varying degrees. The essential parts of the flowers are placed in the centre of the blossom. The stigma is located a little above the anthers. The insects approach them with equal convenience from almost any side, and work on or from top of them.

The bees *Amegilla* sp., *Apis* sp., *Caratina* sp., *Trigona* sp. and *Thyreus* foraged on pollen and collected the pollen through ventral side of the body, and then effected srernotribic pollination. The large bodies *Xylocopa* sp., the wasps *R. metallicum* and *Delta* sp., foraged on nectar, and then their back of head thorex contacted the essential flower parts and received pollen grains. The butterflies visited the essential flowers for nectar only. The contact of proboscis with the essential organs are unlikely because the space between essential organs and the basal part of the flower is wide, there is no way of transferring pollen on to the stigma with proboscis.

Among the 18 flower visitors, the bees such as *A. florae, Amegilla* sp., *Ceratina* sp., *Trigona* sp., the wasps *Delta* sp. and *R. metallicum* are the major pollinators (Baker *et al*., 1971) because their visits are consistent and more frequent, more mobile at the flowers; picked up and transferred sufficient number of pollen. The remainder of species recorded at the flowers are to be treated as minor pollinators. Of the minor pollinators the butterflies *C. pyranthe* and *P. mathias* also made substantial visits but the contact of proboscis with the essential organs is supposedly unlikely.

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FULL LENGTH ARTICLE

New records of unicellular cyanobacteria from India

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Unicellular Cyanobacteria from aquatic as well as aerial and subaerial habitats of West Bengal were explored in the current survey. A total of 7 taxa namely *Chroococcidiopsis thermalis, Snowella fennica, Snowella lacustris, Chlorogloea tuberculosa, Chlorogloea novacekii, Hydrococcus cesatii* and *Aphanocapsa hyalina* were found new to India.

Key words: Cyanoabcteria, Unicellular, New record, India

INTRODUCTION

The Chroococcales is one of the most important order of Cyanobacteria distributed in aquatic as well as terrestrial regimes. Due to simpler morphology and less identifying characters, this group is taxonomically problematic (Komarek, 1999). However several works have been conducted throughout world regarding exploration of the unicellular or colonial form of Cyanobacteria. Silva and Pienaar (1999) surveyed entire South Africa and recorded 40 taxa consisting of 18 genera from 6 families of this order. Planktic Cyanobacteria of SauPaulo, Brazil was investigated and 26 taxa including natural as well as cultural samples of Chroococcales were recorded by Sant'Anna (2004). Extensive studies from north western Australia revealed 54 species having dominant taxa like, *Aphanocapsa, Cyanodictyon, Myxobaktron, Merismopedia* and *Microcystis* (Mc Gregor, 2007). A total of 27 taxa from Horsund area of Spitsbergen were recorded by Matula (2007). The dominant genera were *Aphanocapsa, Aphanothece, Gloeocapsa, Gloeothece, Chroococcus, Gomphosphaeria, Snowella, Cyanobium, Cyanothece* and *Coelomoron*. A number of 61 coccoid cyanobacteria consisting of seven new species (*Asterocapsa aerophytica, Chroococcus subviolaceus, Gloeocapsopsis chroococcoides, Nephrococcus shilinensis , Chondrocystis dermochroa, Gloeocapsa novacekii* and *G. stegophila*) from North America were recorded after survey of Atlantic rain forest of Saupaulo state (Brazil) by Gama (2014).In India, distributional records of coccoid cyanobacteria were relatively poor. A total of 29 species of coccoidal cyanobacteria were found from the Ganges of Kanpur (Rishi *et al*., 2016). Anand and Hopper (1987) reported 11 taxa of unicellular blue green algae from paddy fields of India, whereas, 12 taxa showing dominance of *Microcystis,*

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Aphanothece, Aphanocapsa and *Gloeothece* from different rice fields throughout India were recorded by Tiwari (1972). In West Bengal coccoid Cyanobacteria from different regions of Kolkata were recorded by SinghaRoy *et al*. (2015), Banerjee and Pal (2017).Gupta and Kumar (2005) worked out the bloom of *Microcystis aeruginosa* in Malda district. Das and Keshri (2017) recorded 11 Chroococcalean genera including *Aphanothece, Eucapsis, Merismopedia* and *Microcystis* from Himalayan region. In the present study several sites were investigated from different parts of West Bengal and new records of Chroococcalean taxa from India were described. Besides aquatic biotopes, sub-aerophytic and aerophtic habitats were also surveyed.

MATERIAL AND METHOD

Samples were collected from 7 districts of West Bengal namely Hoogly, Bankura, South 24 Parganas, Murshidabad, Purulia, Malda and Uttar Dinajpur. Samplings were done from rice fields, soil crusts, tree barks, stagnant waters of grass lands with the help of sterilized scalpels and forceps during 2017-2019. Samples were preserved in 4% formalin; voucher specimens were properly labeled and deposited in Calcutta University Herbarium. Aerial or subaerial samples were recorded with acronym CUH/AL/AE/CYANO and fresh water samples were recorded as CUH/AL/FW/CYANO and maintained in Calcutta University Algae Herbarium. Samples were observed under Carl-Zeiss Axiostar microscope. Photomicrographs were taken using Canon T2-T2 1, 6x SLR 426115. The morphological identification of samples was done using available literatures viz. Desikachary (1959) and Komarek (1999). The present status of the names were also confirmed from Algaebase (Guiry and Guiry, 2014), a listing of the world's algae.

RESULTS AND DISCUSSION

Total 25 species from 15 genera were collected in our survey among which 7 taxa were found new to India and described in this section. Our group also worked earlier on investigation of cyanobacterial diversity from West Bengal (Barman *et al*., 2015; Banerjee *et al*., 2020) but these taxa were not detected so far.

After investigating earlier research works from different states, the new records were confirmed from India. Kant and Tiwari (2004) surveyed different places of Uttarpradesh and Bihar. A total of 51 species under 18 genera of unicellular and colonial cyanobacteria were reported which included, *Aphanocapsa, Aphanothece, Asterocapsa, Chlorogloea, Chroococcus, Coelosphaerium, Cyanobacterium, Gloeocapsa, Gloeothece, Hyella, Merismopedia, Microcystis, Myxosarcina, Pleurocapsa, Stanieria, Synechococcus, Synechocystis* and *Xenococcus*. Species level identification was not done in their study. Eastern part of Uttar Pradesh was surveyed and diverse morphological variations of cyanobacteria were found by Yadav *et al*. (2021). Among unicellular taxa, they reported *Chroococcus micrococcus* and *C. gigantus* whereas rest of the taxa belonged to filamentous non-heterocystous and heterocystous genera. Sai River of Lucknow was surveyed for cyanobacterial diversity by Srivastava *et al.* (2014). They reported 23 fresh water cyanobacterial species belonged to 14 taxa among which unicellular members were *Chroococcus minor, C. turgidus, Gloeothece rupestris, Aphanothece saxicola, Gloeocapsa gelatinosa, Microcystis aeruginosa* etc. Bharati *et al*. (2020) investigated diversity of cyanobacteria from four districts namely Patna, Nalanda, Vaishali and Samastipur from central Bihar. Fourteen fresh water habitats were surveyed and the recorded taxa belonged to 11 genera, among them, *Microcystis smithii, M. botrys, M. aeruginosa, M. flos-aquae, Sphaerocystis schroeteri, Merismopedia glauca, M. elegans, Synechococcus elongatus* and *Coelosphaerium dubium* were reported from unicellular cyanobacteria. In south India, estuarine region of southestern coast of Tamilnadu was surveyed by Ramanathan *et al.* (2014). They reported several unicellular cyanobacteria which included *Chroococcus minutus, C. turgidus, Microcystis litoralis, Aphanothece microscopica, A. littoralis, Aphanocapsa marina, Microcystis aeruginosus, Synechocystis salina, S. pevalekii, Synechococcus aeruginosus, S. elongatus, Myxosarcina burmensis* and *M. spectabilis* along with other filamentous cyanobacteria. Koodalmanikyam

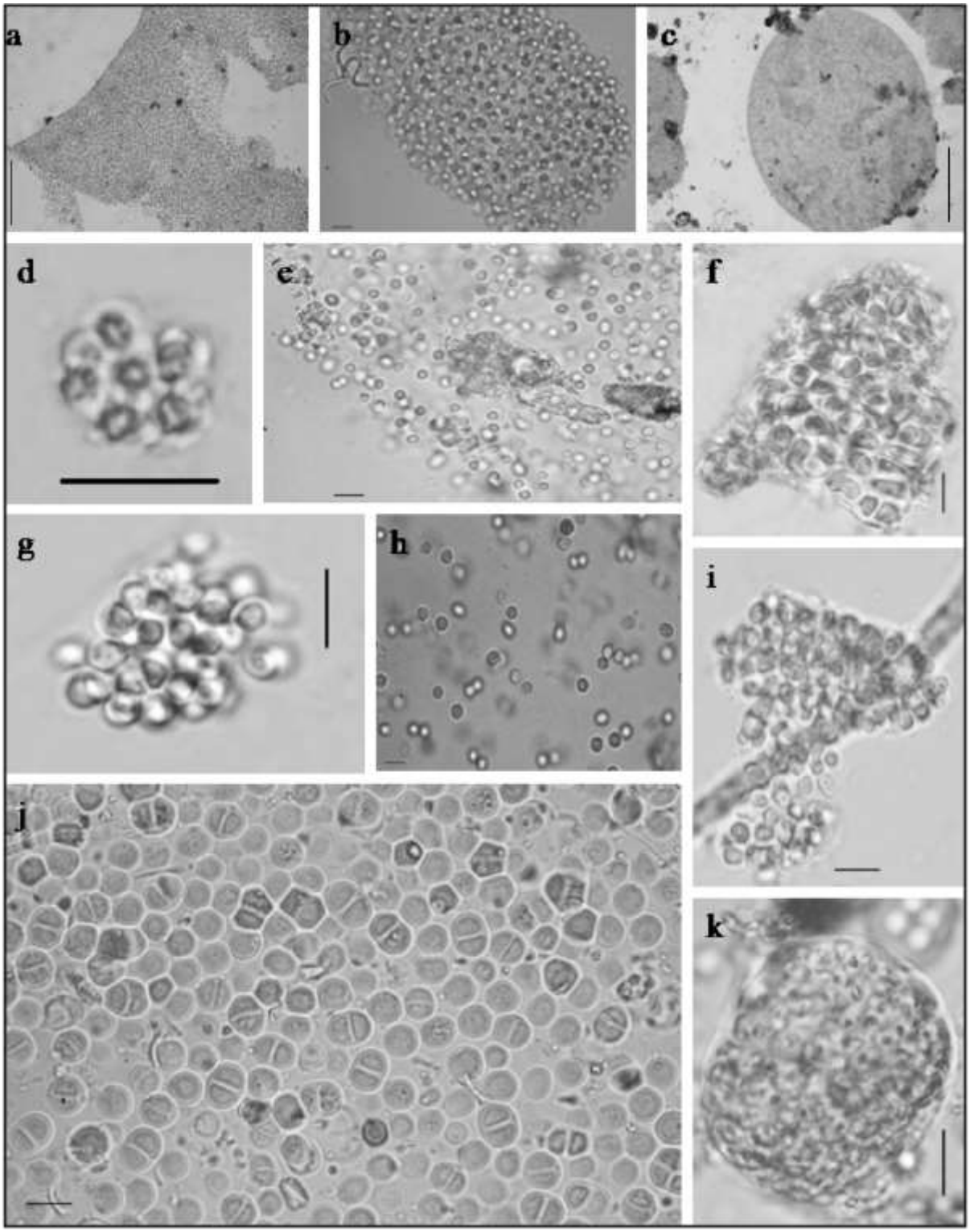

Figure 1: a-c *Aphanocapsa hyalina*; **d-** *Snowella fennica*; **e-** *Aphanocapsa hyalina*; **f-** *Hydrococcus cesatii*; **g-** *Snowella lacustris*; **h-** *Aphanocapsa hyalina*; i- *Chlorogloea tuberculosa*; j- *Chroococcidiopsis thermalis*; k- *Chlorogloea novacekii*. Scale bar-a-20 µm, b-5 µm, c-20 µm, d-i-5 µm, j-10 µm, k-5 µm.

and Kodungallur temple ponds in Thrissur district, Kerala was surveyed and 54 species of blue green algae from 17 genera were recorded mostly belonging to filamentous heterocystous and non-heterocystous genera. Among them *Microcystis* was the only unicellular blue-green algae reported by them (Jissa and Tessy, 2014). However, in our investigation, 7 taxa namely *Chroococcidiopsis thermalis, Snowella fennica, Snowella lacustris, Chlorogloea tuberculosa, Chlorogloea novacekii, Hydrococcus cesatii* and *Aphanocapsa hyalina* were found new to India and not reported earlier as per best of our knowledge.

Systematic accounts of recorded taxa are as follows-

PHYLUM -CYANOBACTERIA
CLASS- CYANOPHYCEAE
ORDER- CHROOCOCCALES
FAMILY- MERISMOPEDIACEAE

Aphanocapsa hyalina (Lyngbye) Hansgirg [Fig.1a-c,e,h].

Komarek and Anagnostidis 1999, p.158, fig.192.

Basionym: *Palmella hyalina* Lyngbye; Homotypic Synonym(s): *Palmella hyalina* Lyngbye .

Colonies more or less spherical, gelatinous, blue green or dirty olive green with densely arranged cells, free living. Mucilage thick, gelatinous, colourless. Cells spherical 1.5-2.5 µm in diameter.

Voucher No.-CUH/AL/FW/CYANO 159,164.

Location 24°52'56.3736" N, 88°7'44.3928"E.

Snowella lacustris (Chodat) Komárek and Hindák [Fig.1g].

Komarek and Anagnostidis 1999; p.207, fig.270.

Basionym: *Gomphosphaeria lacustris* Chodat.

Homotypic Synonym(s): *Gomphosphaeria lacustris* Chodat; *Coelosphaerium lacustre* (Chodat) Ostenfeld. Colonies microscopic, ellipsoidal, 15-20 µm in diameter with cells densely arranged in old colonies. Mucilaginous envelope diffluent, stalks thin, clearly visible at the centre of the colony, cells obovoid, 1-4x1-3.5 µm, blue green without gas vesicles.

Voucher No.-CUH/AL/FW/CYANO 127.

Location 23°20'22.6068"N, 86°21'24.9444"E.

Snowella fennica Komárek and Komárková-Legnerová [Fig.1d].

Komarek and Anagnostidis 1999, p.205, fig.268.

Colonies free floating, solitary, sphaerical, multicellular with cells distant from one another and radially displaced, joined to the end of thin, mucilaginous pseudo-dichotomously branched stalks, radiating from the center of the colony. Old cells slightly elongate drop shaped,1-4.2x0.8-2 µm.

Voucher No.-CUH/AL/FW/CYANO 127.

Location 23°20'22.6068"N, 86°21'24.9444"E.

FAMILY- XENOCOCCACEAE

Chroococcidiopsis thermalis Geitler [Fig.1j].

Komarek and Anagnostidis 1999, p.421, fig.549,550.

Cells spherical, in groups,in colonies usually of varied size. Mucilaginous envelopes thin but distinct, firm, colourless. Cells 5.5-10 µm in diameter; after irregular division of cells baeocyte production proceeds occasionally,1-3.5 µm in diameter, sphaerical; cell content homogenous, blue green.

Voucher No.-CUH/AL/FW/CYANO 19.

Location 23°13'58.008"N, 87°3'33.4044"E.

FAMILY- HYDROCOCCACEAE

Hydrococcus cesatii Rabenhorst [Fig.1f].

Komarek and Anagnostidis 1999, p.354, fig.468.
Colonies flat, usually blastoparenchymatous with

usually elongate cells (particularly in marginal parts); marginal creeping rows of cell usually grow radially, terminal cells are arcuate. Cells in upper layers usually isodiametric, 1.2-2.5 µm in diameter. Cells violet in colour. Mucilaginous sheath thin firm colourless.

Voucher No.-CUH/AL/FW/CYANO 152.

Location 25°13'51.4164"N, 88°45'26.6544"E.

FAMILY- ENTOPHYSALIDACEAE

Chlorogloea tuberculosa (Hansgirg) Wille [Fig.1i].

Komarek and Anagnostidis 1999, p.328, fig.435.

Colonies epiphytic, composed of numerous irregular, more or less short rows of cells, oriented radially from the colonial center or vertically, enveloped by fine, thin, homogenous, colourless mucilage. Cells spherical, slightly irregular–rounded, 1-2.5x1-1.5 µm, bluish green in colour.

Voucher No.-CUH/AL/FW/CYANO 152.

Location 22°22'30.4464"N, 88°26'8.2608"E.

Chlorogloea novacekii Komárek and Montejano [Fig.1k].

Komarek and Anagnostidis 1999, p.331, fig.438.

Colonies mucilaginous, microscopic, small, more or less spherical, yellowish with cells densely and irregularly arranged, distant from one another, surrounded by individual firm, but structureless and at the margin slightly diffluent. Slightly refractive envelopes; mucilage colourless in old parts yellowish brown. Cells sub-spherical 1-2.5 µm in diameter; diameter of gelatinous envelopes around solitary cells 5-12 µm.

Voucher No.-CUH/AL/FW/CYANO 46.

Location 25°13'51.4164"N, 88°45'26.6544"E.

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SHORT COMMUNICATION

The protective action of alcoholic extract of Sabina (*Juniperus sabina*) on Chlorpyrifos induced immune-pathogenic changes in *Gallus gallus domesticus* embryo

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Chlorpyrifos, as an organophosphate, is one of the most widely used insecticides in agriculture to control many insect pests (Giesy *et al*., 1999). As protection against termites, Chlorpyrifos is also applied beneath buildings (Barron and Woodburn, 1995). Numerous studies have examined the chronic toxicity of Chlorpyrifos (CPF) which reported adverse effects on fertility, hatchability and embryo deformities, including a reduction in body weight, twisted necks and eggshell thickness, egg production, egg weight and hatching weight (Gile and Meyers, 1986). Chlorpyrifos is an Ach (Acetylcholine) inhibitor with widespread nervous activity disruption (George *et al*., 2014) although this is not related to our study. Most of the studies of CPF toxicity done on rats and data regarding toxicity in broiler birds are not well known. The biochemical aspect in a single study reported the relationship between CPF and cholinesterase activity in chicks (Mohammad *et al*., 2008). CPF induced a significant difference in health biomarkers of chicken than control birds, and produced a considerable decrease in Ach concentration and internal organ damage. It directly or indirectly affects the poultrysector. *Juniperus sabina* (Cupressaceae) is a low shrub with ascending branches with oblique stem, older bark red-brown, exfoliating, branches very dense; branchlets thin, hardly 1 mm thick, more rounded than angular. Leaves are very sharp, unpleasant odour when crushed with needle and scale-like leaves, 4 mm long, sharply acuminate, blue-green above with a distinctly raised midrib, scale leaves opposite, ovate (Franco, 1964). The young shoots are abortifacient, diuretic, emetic, emmenagogue and irritant. The plant is rarely used internally but is helpful as an ointment, dressing for blisters, etc., to promote discharge. The powdered leaves are used in the treatment of warts. Leaves are used as insect repellent. Essential oil from the leaves and shoots are strong diuretic properties. This oil is also used as an insecticide. *Juniperus sabina* is a juniper native to the mountains of Central and Southern Europe. The shrub is very variable in shape, up to 1-4 m tall, 50-67 members' present Juniperus species (Hampe and Petit, 2010). Essential oil of the plant was reported to possess antimicrobial activity (Akimov *et al*., 1977). *J. sabina* resulted in the isolation of α-cedrol, coumasabin, isocupressic acid, skimming, undulatoside A, hypolaetin 7-0-β-D-xylopyranoside and quercetin 3-0-α-L-rhamnoside (Zhao *et al*., 2008). Essential oils of *J. sabina* were proved to possess inhibitory activity against oxidative stress and protein

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glycation (Asgary *et al*., 2013). This plant contains abundant bioactive compounds such as lignans, flavonoids, terpene and essential oil (Zhao *et al*., 2008). CPF was linked to the onset of respiratory asthma like symptoms in villagers in rural China (Zhang *et al*., 2002). IFN-Y is the signature cytokine (Mattern *et al*., 2005). Pro-inflammatory cytokines enhanced due to chronic exposure to low levels of the organophosphate insecticide (Gordon *et al.*, 1997). To the best of our knowledge, no data on cytokine changes associated with CPF have been reported for *Gallus gallus domesticus* embryo.

The Embryonated eggs

Embryonated *Gallus gallus domesticus* eggs were procured from Government State poultry Farm, Kolkata, India. The surface of the eggs was cleaned with sterile distilled water. All the eggs were candled and incubated at 38ºC ± 0.5ºC with 75 % humidity. The eggs were arranged in six experimental sets, with three eggs in each group.

Group I – on the 3rd day, Chlorpyrifos (25 µg) was administered into the egg through a puncture on the air sac with a long needle.

Group II – on the 3rd day, Sabina was administered, followed by Chlorpyrifos after 1 hour.

Group III – on the 5th day, Chlorpyrifos (25 µg) was administered into the egg through a puncture on the air sac.

Group IV – on the 5th-day administration of Sabina followed by administration of Chlorpyrifos after 1 hour.

Group V – on the 7th day, Chlorpyrifos (25 µg) was administered into the egg through a puncture on the air sac.

Group VI – on the 7th day, Sabina's administration was followed by Chlorpyrifos after 1 hour.

One control group was also studied where only ethanol (100 µL) was injected instead of the extract.

All the eggs were kept at 38ºC up to the 14th day and were harvested on the same day. Pictorial evidence for any deformity was recorded. In case of any death of the embryo, it was recorded.

Chemicals: The Chlorpyrifos (C9H11Cl3NO3PS)) was purchased from Sigma, CAS -2921-88-2.

The extract: Juniperus sabina ethanolic extract (dried plant, 10g/dL) was procured from HAPCO – a government approved pharmaceutical industry where one Botanist identified the plant before extraction.

Doses and intoxication

The egg shell was pierced with a very fine needle near the air space and then through this puncture, the required amount of pesticide solution was administered. The puncture sites were sealed with sterile stickers before incubation. The experiment was started with one dose (25 µg) of pesticide solution. 10 mL of a stock solution of Chlorpyrifos was prepared by dissolving 100 mg of Chlorpyrifos in ethanol. It was stored in 10 aliquots 1mL each in a glass container at ambient room temperature in a dark place. 1 mL of stock solution was diluted to 10 mL with ethanol, and 25 µL was administered in each egg which contained 25 µg of the chemical.

Incubation

Both treated and control eggs were incubated in an incubator at 38ºC ± 0.5ºC with 75 % relative humidity.

Gene expression studies of Cytokines

Comparative gene expression studies were done in real time PCR (Bio-Rad CFX96, Singapore) with SYBR Green tagged primers, dNTPs, Taq polymerase, $MgCl_2$, buffer etc. after nucleic acid extraction. The changes of expressions were calculated as fold increase or decrease from the value of the normal control.

In the control group normal development of the embryo occurred and for interpretation we compared observations of the control group with other experimental sets.

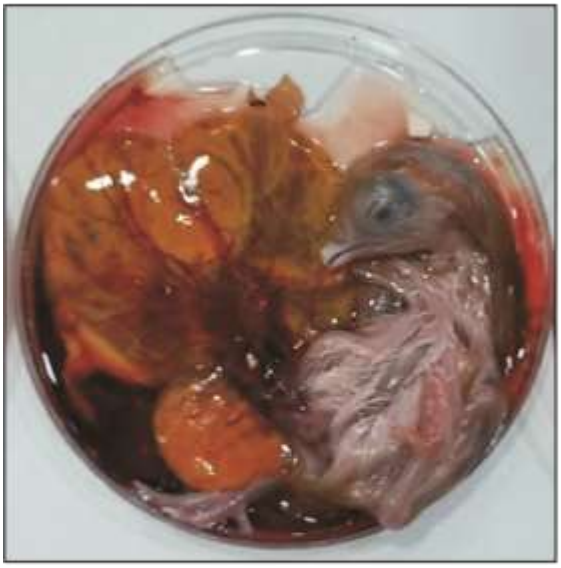

Fig. 1. CONTROL SET (14th day embryo).

On the 3rd Day set: After the application of 25 µg of Chlorpyrifos in the *Gallus gallus* fertilized egg on the 3rd day, there is no further progress in the development and formation of the embryo. Ethanolic extract of crude Sabina (*Juniperus Sabina*, 10 % concentration), 100 µl, is injected 1hr before application of Chlorpyrifos. There was a marked improvement in the embryo development with distinct eyecup development and some other embryonic structures (Fig.2).

On the 5th Day set: On the 5th day, after application of 25 µg of Chlorpyrifos, the embryo's development mildly progressed, however, there was comparatively good eye cup formation and development of blood vessels after activation of crude Sabina (*Juniperus Sabina*, 10% concentration, 100 µl was injected one hr. before application of Chlorpyrifos (Fig.3).

On the 7th Day set: On the 7th day, after the application of Chlorpyrifos, initially the development of the embryo was good, and there was no significant alteration after the application of a crude extract of *Juniperus Sabina* one hour before administration of Chlorpyrifos. However, the development of the embryo was altered afterwards and delayed with the appearance of some deformities (Fig.4).

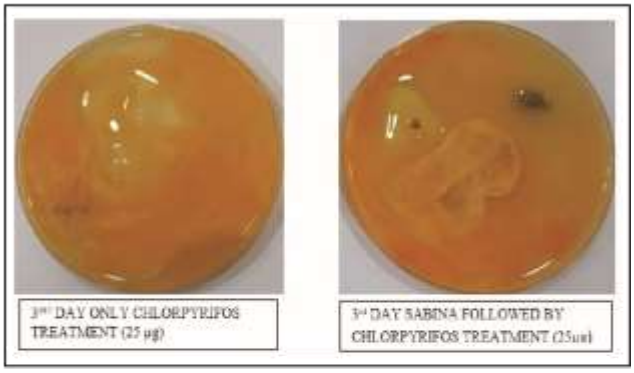

Fig. 2. Embryo in 3rd day experimental sets.

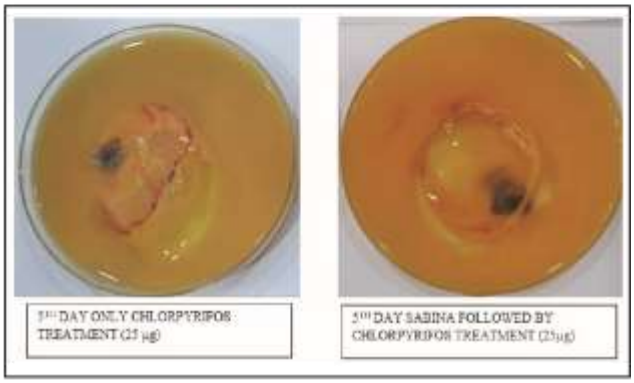

Fig. 3. Embryo in 5th day experimental sets.

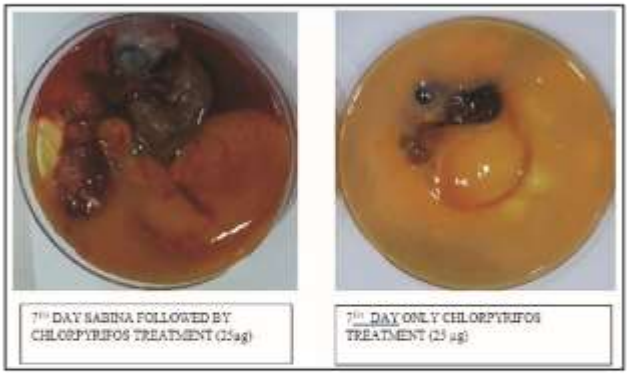

Fig. 4. Embryo in 7th day experimental sets.

The results of this experiment revealed very large amounts of interferon alpha expression (Fig. 5) which protects against viral infection. In group VI (the 7th day set, Sabina's administration was followed by Chlorpyrifos after 1hr) there was 3.25 fold increase of

IFN-α (Interferon alpha) than the control. IFN-β which generally reduces inflammation also markedly increased. In group I (on the third day CPF was administered) there was 48.79 fold and in group IV 160.09 fold and in case of group VI 1000.08 fold increase of IFN-β (Interferon beta)than the control (Fig. 6). IL-8 which activates neutrophils in inflammatory region. In group VI there was 14.51 fold increases in comparison to control (Fig. 9). IL-1β which helps the lymphocytes to fights the infection was also increased. In group IV and group VI there were 17.30 and 17.59 fold increase in comparison to control. Probably sabina has an anti-inflammatory properties because in group VI we have observed an increase value of IFN α, IFN β, IL-8 and IL-1β compared to control. Details of all gene expression changes are given in Figs. 5-11.

In this experiment, it was clear that if Chlorpyrifos is applied in fertilized *Gallus gallus* eggs within five days, it prevents embryo development. But if it is used after the 5th day, there is no detrimental effect, and the embryo development was more or less normal. There were also no structural deformities in the chick embryo. Thus Chlorpyrifos cannot produce any deformation in the *Gallus gallus* embryo at this stage. Still, it prevents the embryo's development and can even stop any growth if applied in early embryogenic life. These findings indicate that Chlorpyrifos should not be used in houses if pregnant women are present. However, further study is necessary in higher animals to prove it. Another important finding of this study is

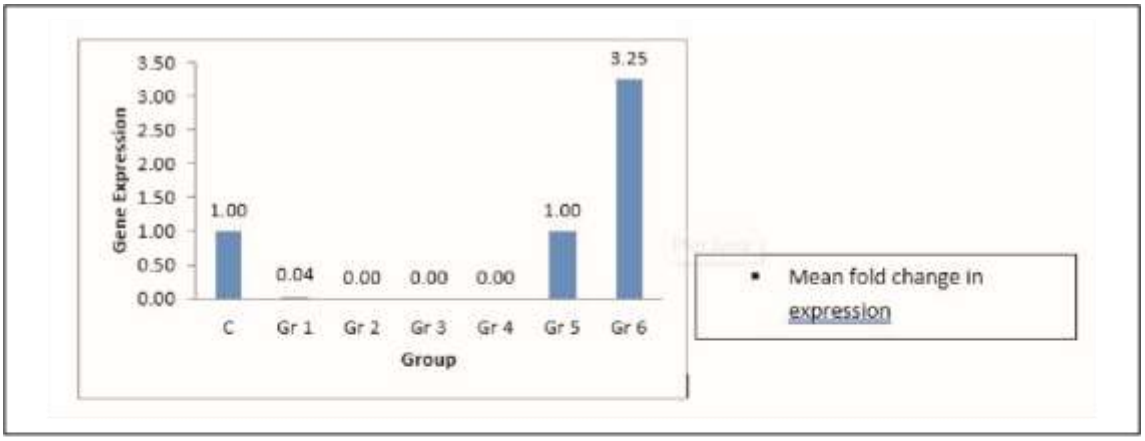

Fig. 5. Mean fold change of IFN-α gene expression in different experimental sets.

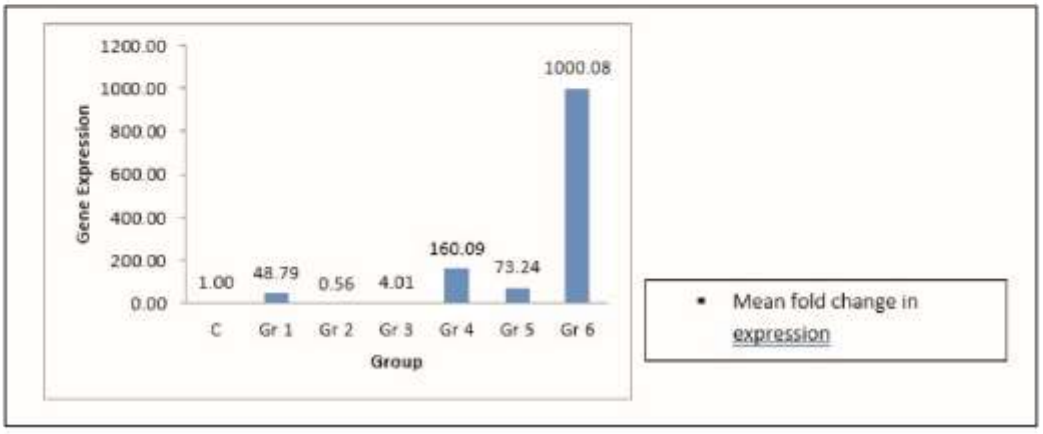

Fig. 6. Mean fold change of IFN-β gene expression in different experimental sets.

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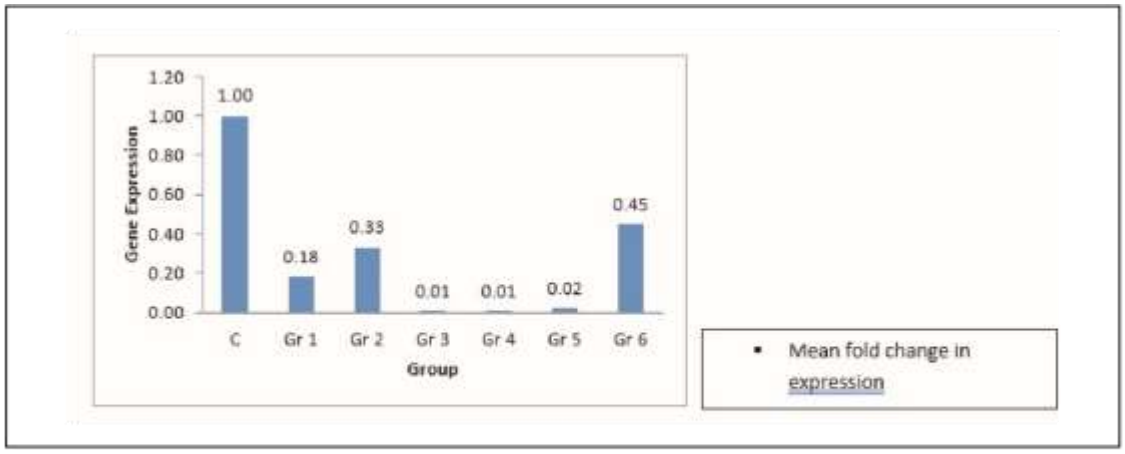

Fig. 7. Mean fold change of IFN-γ gene expression in different experimental sets.

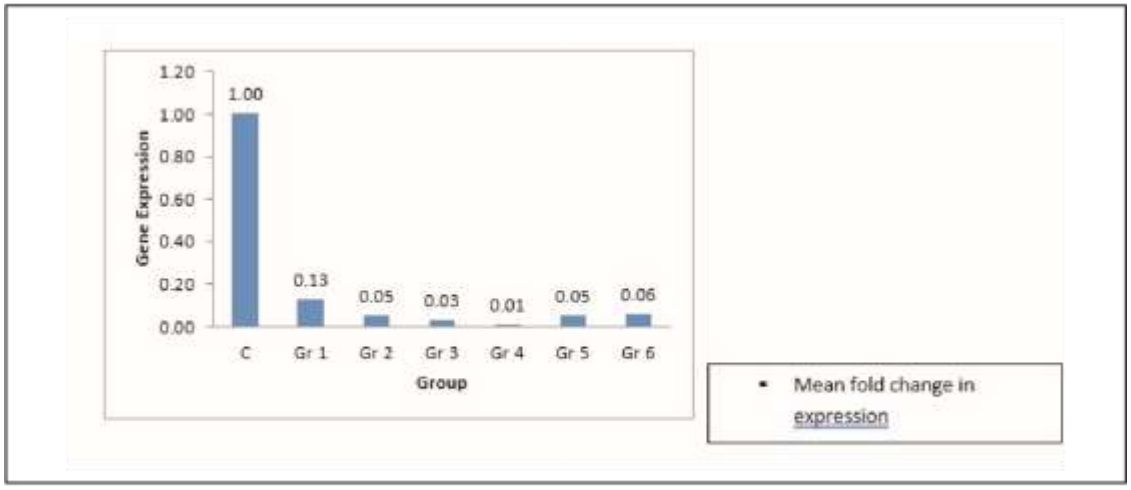

Fig. 8. Mean fold change of IL-6 gene expression in different experimental sets.

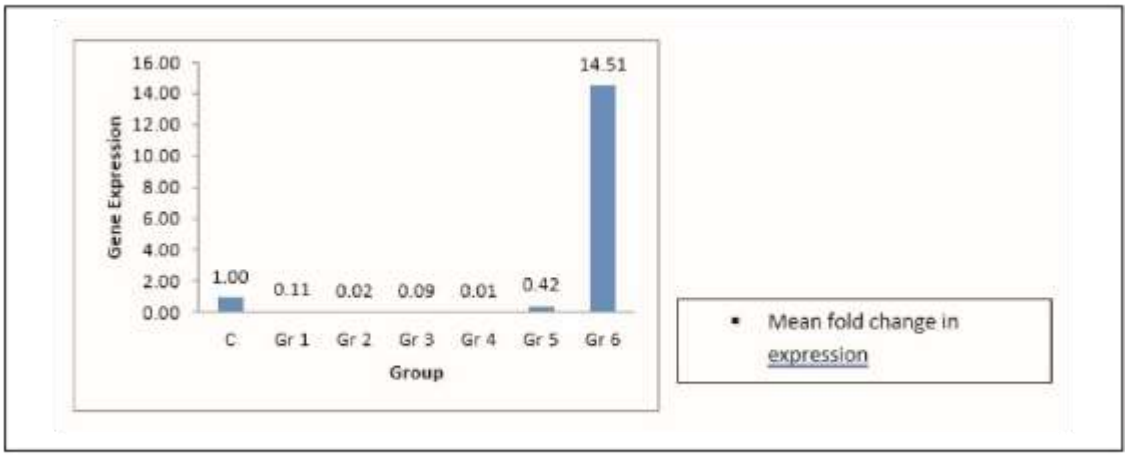

Fig. 9. Mean fold change of IL-8 gene expression in different experimental sets.

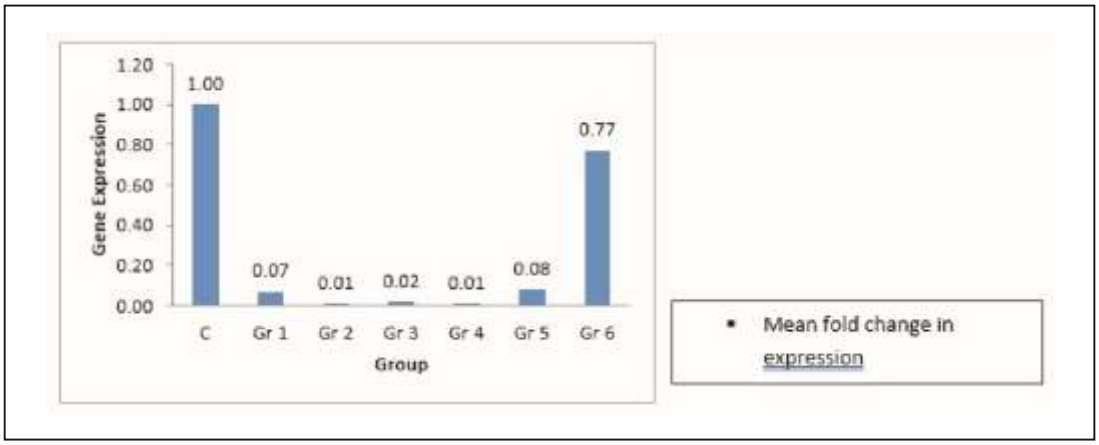

Fig. 10. Mean fold change of IL-10 gene expression in different experimental sets.

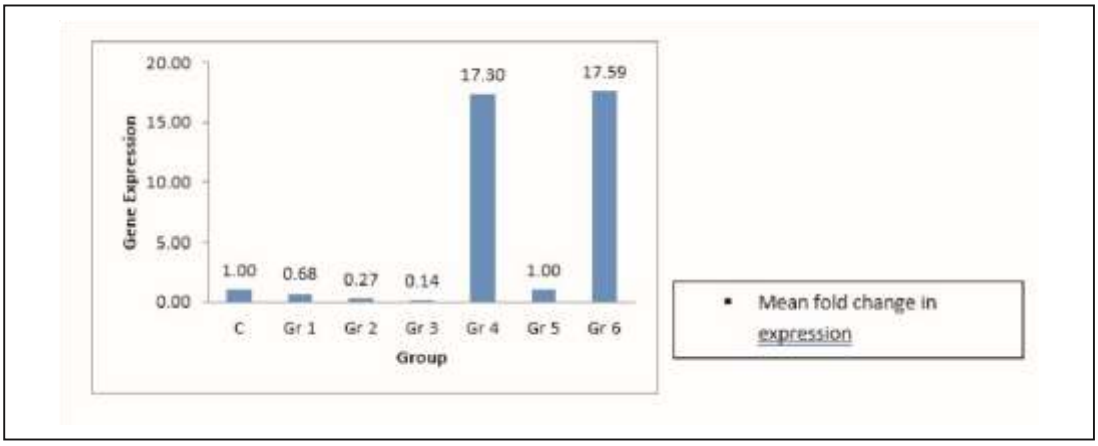

Fig. 11. Mean fold change of IL-1β gene expression in different experimental sets.

that although crude *Juniperus sabina* extract is known to prevent developmental anomalies in higher mammals, including humans, it is not effective in the avian system except for a very mild improvement if applied in the twilight zone of the avian embryo development. INF-α, INF-β and IL-1β were significantly increased in 7th day in experimental preventive set in comparison to 3rd day and 5th day experimental sets which clearly indicate their beneficial role by immune-modulation in Chlorpyrifos induced pathogenic changes.

Crude extract of *Juniperus sabina*, if applied early in the embryogenic life, can somehow prevent the complete stoppage of development induced by chlorpyrifos. Still, after some developmental progression with the formation of eye cups, found no further progress. When applied the extract on the 5th day, it was ineffective and could not produce any demonstrable improvement in the embryo's development. On the 7th day, the extract and Chlorpyrifos showed a marked detrimental effect on the embryo's development. INF-α, INF-β and IL-1β were significantly increased in 7th day in experimental preventive set in comparison to 3rd day and 5th day experimental sets. This may indicate

increased immune-modulating activities of the embryo with maturity in an attempt to balance cytokine activities in tissue damage.

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1ST BOTANICAL CONGRESS, 2023 – A REPORT

Botanical Society of Bengal in collaboration with the Department of Botany, University of Calcutta organized the 1st Botanical Congress from 23rd to 25th March, 2023 at Ballygunge Science College Campus, Calcutta University, Kolkata. More than 300 participants took part in the Congress. Academicians from different Universities and Colleges and Scientists from different National Institutes of our Country participated and delivered lectures on various important topics of botany. Dr. Subhra Chakraborty, Director, NIPGR, New Delhi. Dr. A. A. Mao, Director, BSI, Kolkata, Dr. D.K. Upreti, Emeritus Scientist, NBRI, Lucknow, Prof. Uma Shankar, NEHU, Shillong, Dr. Rakesh Maurya, ex Chief Scientist, CDRI, Lucknow, Dr. Shasi Kumar, ICGEB, New Delhi, Dr. Niraj Rai, Senior Scientist, BSIP, Lucknow, Prof. A.P. Das. RGU, Arunachal Pradesh, Prof. Sudhendu Mandal, Advisor (Academic and Administrative), Central University of Odisha, Koraput were some of the delegates who came from other states.

Honourable Vice Chancellor, University of Calcutta, Prof. Asis Kumar Chattopadhyay inaugurated the Congress. The Plenary lecture was given by Prof. Uma Shankar on "Incredible Botanical Knowledge at Crossroads: Opportunities on the Horizon". On the first day in the Cryptogamic World section Keynote lecture was delivered by Dr. D.K. Upreti, on "Indian Lichen diversity and its Bioprospection" and Dr. A. A. Mao spoke on "The Flowering Plants" in the Flowering Plants: The Blossoms section. In the Medicinal Plants Section Dr. Rakesh Maurya spoke on "Application of Traditional Knowledge in Search of Potential leads for drug discovery", while Dr. Niraj Rai, enlightened the participants on "In search of molecular past: Reconstructing floral and faunal population histories using ancient DNA" in the Evolutionary and Environmental botany section. Dr. Subhra Chakraborty gave the Keynote lecture on "Decoding organeller control of multi-host resistance" and the Keynote lecture on "Fresh water algal biodiversity of India" in the Technical session was delivered by Prof. Jai Prakash Keshri of the University of Burdwan. Simultaneously oral presentations by young scientists in all the sections continued. This was followed by the Poster Session by the faculty and research scholars. The day ended with the Cultural program and the Congress Dinner. The second day started with the inauguration of the Science Fair by Prof. Debasis Das, Registrar, University of Calcutta. Dr. Shashi Kumar gave an illuminating talk on "Plant synthetic biology for terpenoid drug biosynthesis" under the Engineering resilient plant for better future section. This was followed by Popular lectures for students, particularly the school students. Forty students from 8 schools of West Bengal participated in the Congress. Eminent scientists from different branches of botany spoke on various aspects of plant science. Prof. S.P. Adhikari (Ex. Professor Visva Bharati University and Ex. Vice-Chancellor, F.M. University, Balasore, Odisha) spoke on "Studying botany with special reference to biology and utilization of algae: prospects and challenges in India". Dr. Kanad Das (Botanical Survey of India) highlighted on "Diversity, exploration and taxonomy of wild mushrooms of India". Prof. Binay Chaubey (University of Calcutta) gave the students an idea of "SARS CoV-2: the virus, the vaccine and future". Prof. Sudhendu Mandal (Ex. Professor Visva- Bharati University and Advisor, Academic and Administration, Central University of Odisha, Koraput) spoke on "Aerobiology and human health" while Dr. Biswajit Ghosh (Ramakrishna Mission Vivekananda Centenary College) gave the students the concept of "Plant tissue culture: a basic tool of plant biotechnology". In the Microbial World section Prof. Sanjoy Guha Roy of West Bengal State University highlighted on "Tackling plant endemics in the genomics era: late blight of potato-

a case study". In the post lunch session there was Pavilion visit of the Science Fair for students, scientific deliberations (oral presentation) and the Poster session. The third day began with the Interactive Session with Foreign Alumni and oral presentations running in parallel sessions. Running simultaneously was the session on Entrepreneurship and Interactive session with farmers and NGO. Fifty farmers joined this session from the different villages of West Bengal. The keynote Lecture was delivered by Prof. Krishnendu Acharya of the University of Calcutta, who lucidly explained how farmers can start mushroom culture on small scale basis, prepare their own biofertilizers and start entrepreneurship in organic culture for sustainable rural development. The post lunch session was the Valedictory session which ended with distribution of prizes to the young scientists and certificates to all the participants.

The main aim of the 1st Botanical Congress 2023 was to broaden the horizon of knowledge of plant sciences among young minds, touching upon diverse domains of the subject. Interactive sessions with professors, renowned scientists and professionals not only benefitted young researchers but also created an interest in the subject among school children, the future of our nation. This congress ensures to establish a platform for the unhindered flow of ideas, innovations and concepts that would benefit mankind. Along with the gain of knowledge, societal responsibility of plant scientists and entrepreneurship development among the youth have been attempted.

Subir Bera
Ashalata D'Rozario
Conveners

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