Journal of Plant Development Sciences
Meerut, India

RESEARCH ARTICLE

NATURAL PRESERVATION OF POLLEN IN HONEY AND BEE BREAD: DIVERSITY, VIABILITY, AND *IN VITRO* GERMINATION IN THE INDIAN SUNDARBANS MANGROVE ECOSYSTEM

Nirmallya Ghosh, MD Ashfaque Molla, Priyasi Ray, Radhakanta Nandi, Sarupa Barman, Anisha Satpati, and Sudha Gupta*

Department of Botany, University of Kalyani, Kalyani-741235, West Bengal, India
Email: sudhaguptain@gmail.com

Received-18.06.2026, Revised-11.07.2026, Accepted-29.07.2026

Abstract: Pollen preservation is important for understanding plant reproductive biology, pollination ecology and biodiversity, but conventional storage methods often require controlled environmental conditions and specialised infrastructure. The present study investigated the diversity, viability and *in vitro* germination potential of pollen naturally recovered from bee bread (BB) and honey (HN) in the Sundarbans mangrove ecosystem of India. Bee bread and honey samples collected from five bee hives representing four regions of the Sundarbans were subjected to palynological analysis. Pollen viability was assessed using Aniline Blue, Acetocarmine and 2,3,5-triphenyl tetrazolium chloride (TTC), while germination was evaluated under *in vitro* conditions at different sucrose concentrations and incubation periods. A total of 20 pollen taxa representing 12 plant families were identified, comprising mangrove, mangrove-associated and terrestrial taxa and reflecting the diverse floral resources exploited by honey bees. Viability varied among pollen taxa, staining methods and storage matrices, with bee-bread pollen generally showing higher viability than pollen recovered from honey. Significant differences in viability between BB and HN were observed for all three staining methods, whereas the responses among staining methods varied according to the pollen matrix. *In vitro* germination was highest in 20% sucrose and reached a maximum at 72 h of incubation. Germination frequency was consistently higher in BB than in HN pollen, ranging from 70.46–87.64% in BB and 62.32–73.42% in HN. Linear regression indicated strong positive associations between incubation time and germination frequency in both BB (R^2 = 0.8941) and HN (R^2 = 0.9107). Thus, pollen recovered from bee bread showed greater retention of viability and germination potential than pollen recovered from honey, although responses were species-specific. The findings identify bee bread as a promising natural biological archive for investigating pollen biology, bee–plant interactions, pollination ecology and biodiversity, while providing a basis for future research on natural pollen preservation.

Keywords: Bee bread, Honey; Pollen preservation, pollen viability, Sundarbans, Mangrove ecosystem

INTRODUCTION

Honey bees (*Apis* spp.) are among the most important pollinators, contributing significantly to ecosystem stability, biodiversity conservation, and agricultural productivity. During foraging, worker bees collect nectar and pollen from flowering plants. Nectar undergoes enzymatic modification and dehydration within the hive to produce honey, whereas pollen is mixed with nectar, honey, and glandular secretions and packed into comb cells, where microbial fermentation, particularly involving lactic acid bacteria, leads to the formation of bee bread—the principal protein-rich food reserve of the colony (Bogdanov et al., 2008; Da Silva et al., 2016; Kieliszek et al., 2018). Within the hive, bee bread is generally stored adjacent to brood cells, facilitating rapid access by nurse bees, while honey serves as the colony's primary carbohydrate reserve and is commonly stored in the upper and peripheral regions of the comb (Seeley, 1995).

Honey is a complex natural product valued for its nutritional, medicinal, ecological, and commercial importance (Edo *et al*., 2023). It is composed mainly of carbohydrates together with water, proteins, amino acids, enzymes, vitamins, minerals, organic acids, phenolic compounds, flavonoids, and varying quantities of pollen grains (Bogdanov *et al*., 2008). The pollen spectrum of honey reflects the floral resources exploited by foraging bees and serves as a reliable indicator of its botanical and geographical origin (Ponnuchamy *et al*., 2014). The identification and analysis of pollen grains in honey, known as melissopalynology, is widely used for honey authentication, determination of floral sources, and reconstruction of regional vegetation (Louveaux *et al*., 1978; Bryant & Jones, 2001). Melissopalynological studies also provide valuable information on bee foraging behaviour, seasonal floral availability, habitat characteristics, and ecosystem health, making them indispensable in apicultural, ecological, and biodiversity research

*Corresponding Author

(Ahmad *et al*., 2024). Similarly, bee bread contains pollen collected from diverse flowering plants and has attracted increasing attention because of its nutritional value, ecological significance, and commercial potential (Baky *et al*., 2023). The fermentation process alters the biochemical characteristics of pollen and may contribute to its preservation within the bee bread matrix, although its influence on pollen physiological viability remains insufficiently understood (Kieliszek *et al*., 2018). Together, honey and bee bread represent naturally occurring storage environments in which pollen grains are retained under distinct physicochemical conditions, including low water activity, high sugar concentration, and fermentation-associated biochemical changes. Whether these natural storage conditions maintain pollen viability and germination potential, however, remains poorly understood.

The preservation of viable pollen is of considerable importance in plant sciences because stored pollen serves as a valuable resource for plant breeding, hybridization, germplasm conservation, *ex situ* genetic resource management, crop improvement, fertility assessment, and studies of plant reproductive biology (Dinato *et al*., 2020). Conventional methods for pollen preservation include desiccation, refrigeration, freezing, and cryopreservation, each requiring careful optimization of moisture content and storage conditions to maintain pollen viability (Dinato *et al*., 2021). However, these approaches often require specialised equipment, controlled environments, and continuous maintenance. Consequently, the search for naturally occurring and sustainable systems capable of retaining pollen structure and physiological competence has attracted increasing scientific interest.

Pollen viability is fundamental to successful sexual reproduction in flowering plants. Viable pollen grains retain the ability to germinate on compatible stigmas, produce pollen tubes, and achieve fertilization (Dafni, 2000). Their physiological competence depends on the integrity of cellular structures and the availability of stored carbohydrates, proteins, lipids, and other reserve materials that support pollen tube growth. Therefore, the assessment of pollen viability and germination has broad applications in reproductive biology, germplasm conservation, pollen storage, fertility evaluation, and studies of pollen–stigma interactions (Althiab-Almasaud, 2024). Cytochemical staining methods, including Acetocarmine, Aniline Blue, and 2,3,5-triphenyltetrazolium chloride (TTC), provide rapid estimates of pollen viability, whereas *in vitro* germination assays offer a functional measure of pollen performance by directly evaluating the ability of pollen grains to produce pollen tubes under controlled conditions (Ballantyne *et al*., 1974; Tushabe & Rosbakh, 2021).

The Sundarbans, a UNESCO World Heritage Site, constitute the world's largest mangrove ecosystem, extending across the Ganga–Brahmaputra delta of India and Bangladesh (Ghosh *et al*., 2015). The Indian Sundarbans are characterized by exceptional floral diversity and abundant melliferous plant species that support extensive foraging by wild honey bees and the production of multifloral honey (Chakraborti *et al*., 2019). The region comprises a rich assemblage of mangrove and associated plant species, including *Avicennia marina, Bruguiera gymnorhiza, Rhizophora mucronata, Ceriops decandra, Sonneratia apetala*, and *Excoecaria agallocha*, which provide abundant nectar and pollen resources for bees (Naskar & Mandal, 1999). Consequently, honey and bee bread produced in this ecosystem contain pollen derived from a diverse range of mangrove and associated flora, making them valuable materials for melissopalynological and ecological investigations.

Although several studies have examined the botanical origin, melissopalynological characteristics, and physicochemical properties of Sundarbans honey (Refat *et al*., 2026), much less is known about the physiological status of pollen naturally retained within honey and bee bread. In particular, whether pollen grains recovered from these bee products remain viable and retain the capacity for *in vitro* germination remains poorly understood. Comparative information on pollen diversity, viability, and germination potential in honey and bee bread from the Sundarbans mangrove ecosystem is especially limited. Addressing this knowledge gap may improve our understanding of pollen behaviour under natural storage conditions and provide new insights into the biological quality and potential applications of bee-derived products.

Therefore, the present study investigated the botanical diversity, pollen viability, and *in vitro* germination potential of pollen isolated from honey and bee bread collected from different locations in the Sundarbans. By integrating melissopalynological analysis with cytochemical viability assays and *in vitro* germination tests, this study provides new insights into the diversity of floral resources exploited by honey bees and evaluates the physiological integrity of pollen retained within bee products. The findings improve our understanding of pollen persistence under natural storage conditions in honey and bee bread and highlight the potential significance of these bee-derived products for plant reproductive biology, biodiversity assessment, melissopalynological research, and conservation studies. To the best of our knowledge, this is among the first studies to simultaneously investigate the botanical composition, viability, and *in vitro* germination potential of pollen recovered from both honey and bee bread collected from the Sundarbans mangrove ecosystem.

MATERIALS AND METHODS

Material

Bee bread (BB) and honey (HN) samples were collected during the summer season (May 2026) directly from wild honey bee colonies at four locations in the Indian Sundarbans (Fig. 1, 2). The sampling locations and corresponding sample codes were: Kumirmari (BB1/KUM, HN1/KUM, BB2/KUM, HN2/KUM), Parghumti (BB3/PGM, HN3/PGM), Shamsernagar (BB4/SMN, HN4/SMN), and Hemnagar (BB5/HMN, HN5/HMN). Samples were collected using sterile tools to minimize external contamination, immediately transferred into sterile containers, and stored at −20 °C until further analysis.

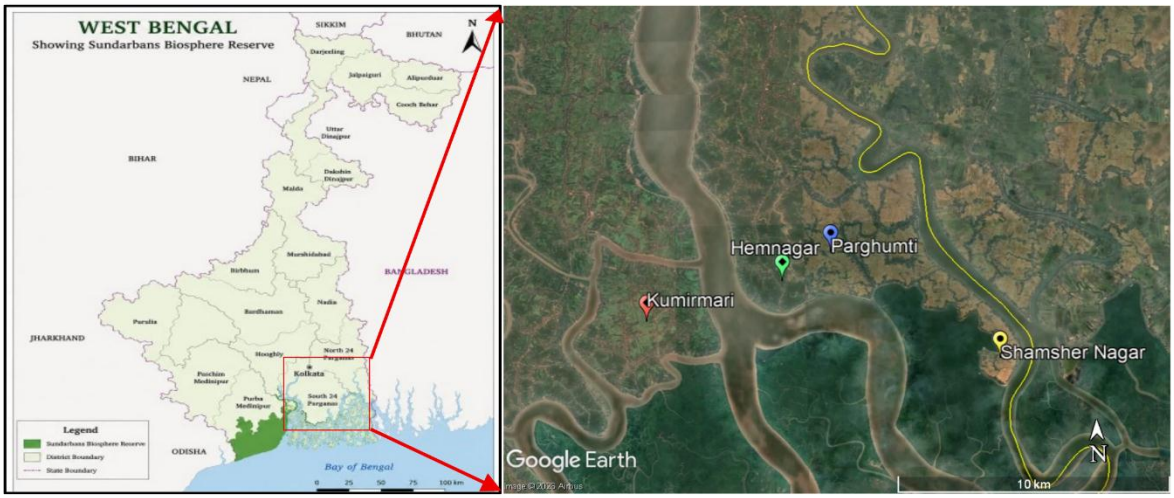


Fig. 1: Map of the sampling locations of bee bread and honey in the Indian Sundarbans.

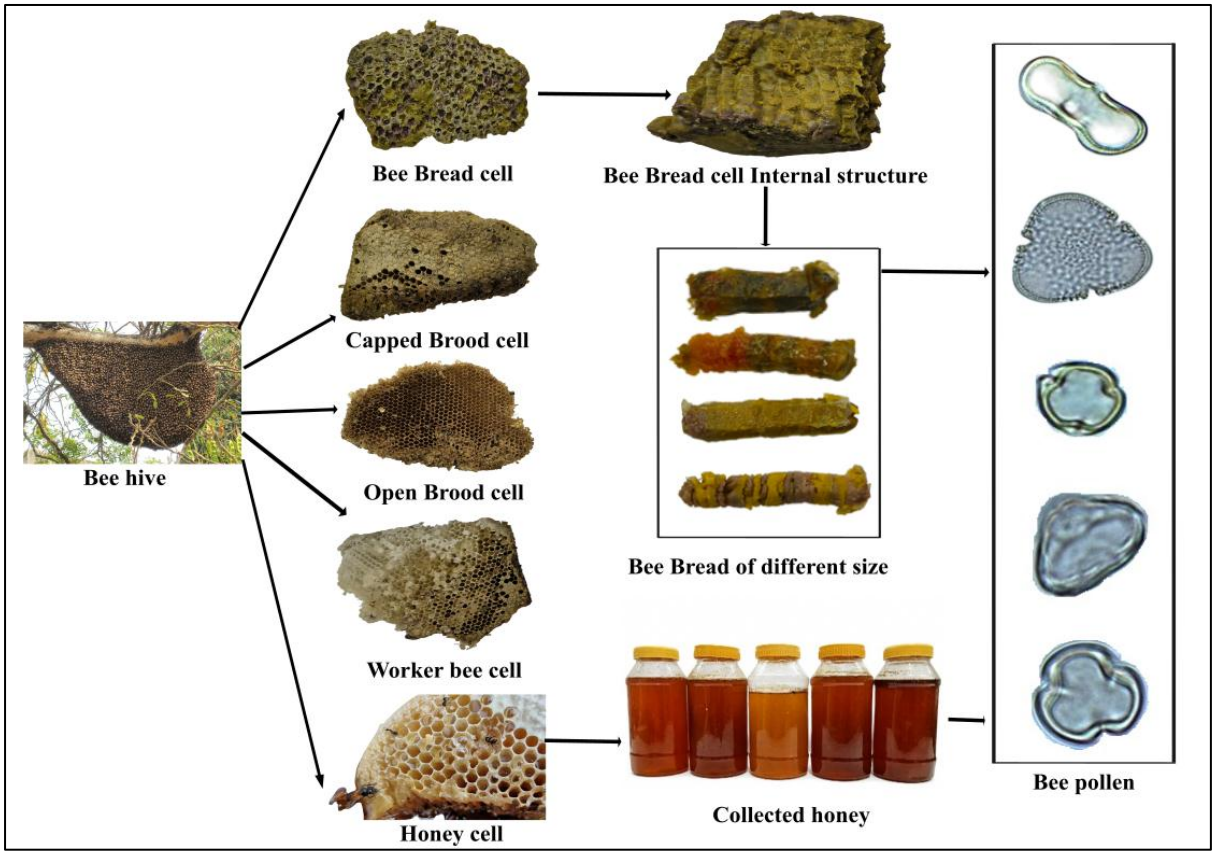


Fig. 2. Photographic workflow showing the collection and characterization of bee-hive materials, different cell types, bee-bread structures, honey, and representative pollen grains recovered from these materials.

METHODS

Sample Preparation

For each bee-bread sample, 20 bee-bread pellets were randomly collected from a single hive at each sampling location, pooled, and thoroughly homogenized. From the homogenized bee bread, 1 g was taken for pollen isolation. For honey, 10 g of sample was collected directly from the same hive and processed separately. Each sample was suspended in distilled water and thoroughly homogenized. The resulting suspensions were centrifuged at 2500 rpm for 10 min using a Remi RM-02 centrifuge to concentrate the pollen fraction. Following each centrifugation step, the supernatant was carefully decanted, and the sediment was resuspended in distilled water. The washing and centrifugation steps were repeated until a relatively clean pollen pellet was obtained. The recovered pollen fraction was subsequently used for palynological analysis, pollen viability assessment, and *in vitro* germination studies.

Pollen Viability Assessment

For viability assessment, the isolated pollen fraction obtained from each sample was divided into three sterile Eppendorf tubes, representing three technical replicates. Pollen viability was assessed using three cytochemical staining methods: 1% Aniline Blue, 2% Acetocarmine, and 5% 2,3,5-triphenyltetrazolium chloride (TTC) (El-Sofany *et al*., 2020). The pollen suspensions were incubated with the respective staining solutions for 5–6 h at room temperature. Following incubation, approximately 2000 pollen grains from each bee-bread sample and 1200 pollen grains from each honey sample were examined for each staining treatment under a Leitz Laborlux light microscope equipped with an E3 Scientific digital camera. Pollen grains showing intense blue, red, and red-to-yellow staining following treatment with Aniline Blue, acetocarmine, and TTC, respectively, were considered viable, whereas unstained or weakly stained pollen grains were considered non-viable. The percentage of pollen viability was calculated using the following equation (Bots & Mariani, 2005):

Pollen Viability (%) =

$$\left(\frac{\text{Number of viable pollen grains}}{\text{Total number of pollen grains observed}}\right) \times 100$$

In Vitro Pollen Germination Assay

The germination potential of pollen grains recovered from bee bread and honey was evaluated in vitro using a modified Brewbaker and Kwack (BK) germination medium (Brewbaker & Kwack, 1963). The germination medium was prepared in distilled water and consisted of 20% (w/v) sucrose, 100 mg L^{-1} boric acid, 100 mg L^{-1} calcium nitrate, 100 mg L^{-1} magnesium sulphate, and 100 mg L^{-1} potassium nitrate. Prior to the key germination experiment, the effect of sucrose concentration on pollen germination was evaluated using media containing 0, 10, 15, 20, and 25% (w/v) sucrose. The medium containing 20% sucrose showed the highest germination rate across the tested bee-bread and honey samples and was therefore selected as the standard germination medium for the subsequent experiments. The isolated pollen fraction from each sample was equally distributed into sterile Eppendorf tubes, and 1 mL of the germination medium was added to each tube. The pollen suspensions were incubated under laboratory conditions for 24, 48, 72, and 96 h. Three technical replicates were maintained for each sample at each incubation period. Following incubation, a drop of the pollen suspension was placed on a clean glass slide and examined under a Leitz Laborlux light microscope equipped with an E3 Scientific digital camera. Approximately 2,000 pollen grains from bee-bread samples and 1,200 pollen grains from honey samples were examined at each incubation period. A pollen grain was considered germinated when a pollen tube emerged through the germination aperture. The percentage of pollen germination was calculated using the following equation (Kakani et al., 2002):

Pollen Germination (%) =

$$\left(\frac{\text{Number of germinated pollen grains}}{\text{Total number of pollen grains observed}}\right) \times 100$$

Statistical Analysis

All experimental data are presented as mean ± standard deviation (SD). Pollen viability percentages obtained using Acetocarmine, Aniline Blue, and 2,3,5-triphenyltetrazolium chloride (TTC) staining were compared among the three staining methods. Similarly, *in vitro* pollen germination percentages were compared among the four incubation periods (24, 48, 72, and 96 h). One-way analysis of variance (ANOVA), followed by Tukey's Honestly Significant Difference (HSD) post hoc test, was used to determine significant differences among the pollen viability estimates obtained using the three staining methods and among pollen germination percentages recorded at different incubation periods. Linear regression analysis was performed to examine the relationship between incubation time and pollen germination percentage. Statistical analyses were performed using RStudio (version 2024.12.0+467), and differences were considered statistically significant at $p < 0.05$. Graphical representations, including heatmaps, box plots, and regression plots, were generated using RStudio.

RESULTS

Pollen Diversity and Distribution in Bee Bread and Honey

Five bee hives comprising bee bread (BB) and honey (HN) samples collected from four regions of the Indian Sundarbans were subjected to palynological analysis. A total of 20 pollen taxa belonging to 12 plant families were identified from the BB and HN samples. Of these, 12 taxa were common to both bee bread and honey, whereas four taxa were recorded

exclusively in bee bread and four taxa exclusively in honey (Table 1). Thus, 16 pollen taxa were identified in bee bread and 16 in honey, indicating substantial overlap in pollen composition between the two sample types along with differences in their representation among localities.

The identified pollen assemblage comprised both mangrove and associated terrestrial taxa. Rhizophoraceae was the most taxon-rich family, represented by *Bruguiera gymnorhiza*, *Ceriops decandra*, *Kandelia candel* and *Rhizophora apiculata*, followed by Malvaceae, represented by *Bombax ceiba*, *Heritiera fomes* and *Thespesia* sp. Among the identified taxa, *Ceriops decandra* was the most frequently represented, occurring in seven of the ten individual BB and HN samples. It was recorded in both BB and HN samples from Parghumti and Shamshernagar, in BB from Kumirmari and in both BB and HN from Shamshernagar, while it was absent from both samples at Hemnagar. *Avicennia* sp. was the second most frequently represented taxon, occurring in six of the ten samples and being recorded in both BB and HN from Kumirmari, Parghumti and Hemnagar, but absent from both samples at Shamshernagar. Twelve pollen taxa—*Acanthus ilicifolius*, *Avicennia* sp., *Bombax ceiba*, *Bruguiera gymnorhiza*, *Ceriops decandra*, *Coriandrum sativum*, *Desmodium* sp., *Excoecaria agallocha*, *Heritiera fomes*, *Lumnitzera littorea*, *Sonneratia apetala* and *Xylocarpus granatum*—were common to both bee bread and honey. Their occurrence in both sample types indicates substantial overlap in the floral resources represented in bee bread and honey. Four pollen taxa, namely *Aegiceras corniculatum*, *Laguncularia racemosa*, *Rhizophora apiculata* and *Thespesia* sp., were detected exclusively in bee bread. In contrast, *Kandelia candel*, *Nypa fruticans*, *Phoenix paludosa* and *Psidium guajava* were detected exclusively in honey. The occurrence of pollen from both mangrove and associated terrestrial plants demonstrates the diversity of floral resources represented in the samples. The differences in pollen composition among localities and between bee bread and honey suggest variation in the floral resources utilized by honey bees across the study area. The occurrence of several pollen taxa in both sample types further indicates overlap in the floral resources contributing to bee bread and honey, whereas sample-specific taxa may reflect differences in resource availability, collection site or the timing of foraging.

Table 1: Pollen taxa recovered from bee bread and honey samples collected from different localities of the Indian Sundarbans

IDENTIFIED POLLEN GRAINS	FAMILY	KUMIRMARI				PARGHUMTI		SHAMSHERNAGAR		HEMNAGAR	
		BB1/ KUM	HN1/ KUM	BB2/ KUM	HN2/ KUM	BB3/ PGM	HN3/ PGM	BB4/ SMN	HN4/ SMN	BB5/ HMN	HN5/ HMN
Acanthus ilicifolius L.	Acanthaceae	-	-	+	-	-	+	+	+	-	-
Aegiceras corniculatum (L.) Blanco	Primulaceae	-	-	-	-	-	-	+	-	+	-
Avicennia sp.	Acanthaceae	+	+	+	-	+	+	-	-	+	+
Bombax ceiba (L.)	Malvaceae	-	-	-	-	-	-	+	+	+	-
Bruguiera gymnorhiza (L.) Lam.	Rhizophoraceae	-	-	-	-	+	+	-	-	-	-
Ceriops decandra (Griff.) W.Theob.	Rhizophoraceae	+	-	+	+	+	+	+	+	-	-
Coriandrum sativum L.	Apiaceae	-	-	-	+	-	-	-	-	+	+
Desmodium sp.	Fabaceae	-	-	-	-	+	-	+	+	-	-
Excoecaria agallocha L.	Euphorbiaceae	-	-	-	+	-	-	-	-	+	+
Heritiera fomes Buch.	Malvaceae	-	+	+	+	-	-	+	-	-	-
Kandelia candel (L.) Druce	Rhizophoraceae	-	-	-	-	-	+	-	-	-	+
Laguncularia racemosa (L.) C.F.Gaertn.	Combretaceae	-	-	-	-	-	-	+	-	+	-
Lumnitzera littorea (Jack) Voigt	Combretaceae	-	-	-	+	-	-	-	-	+	-
Nypa fruticans Wurmb	Arecaceae	-	-	-	-	-	-	-	+	-	+
Phoenix paludosa Roxb.	Arecaceae	-	+	-	-	-	-	-	+	-	-
Psidium guajava L.	Myrtaceae	-	+	-	-	-	+	-	-	-	-
Rhizophora apiculata Blume	Rhizophoraceae	+	-	+	-	-	-	-	-	-	-
Sonneratia apetala Buch-Ham.	Lythraceae	+	+	-	+	+	-	-	-	-	-
Thespesia sp. L.	Malvaceae	+	-	+	-	-	-	-	-	-	-
Xylocarpus granatum J. Koenig	Meliaceae	+	-	-	-	+	-	-	+	+	+

Pollen Viability and Germination in Bee Bread and Honey

Pollen viability of the identified pollen taxa recovered from bee bread (BB) and honey (HN) samples collected from different localities of the Indian Sundarbans was assessed using three staining methods—Aniline Blue, Acetocarmine and 2,3,5-triphenyl tetrazolium chloride (TTC)—along with *in vitro* germination analysis. The viability and germination values presented in Table 2 represent the mean values obtained across the respective BB and HN samples from the study localities. Thus, the values provide an overall comparison of pollen viability and germination between the two mediums rather than locality-specific estimates.

Aniline Blue staining showed mean viability values ranging from 81.53% in *Acanthus ilicifolius* to 92.24% in *Heritiera fomes* for bee bread pollen. In honey pollen, the corresponding mean viability ranged from 82.33% in *Nypa fruticans* to 89.24% in *Excoecaria agallocha* and *Xylocarpus granatum*. Among the pollen taxa represented in both matrices, *Bruguiera gymnorhiza* showed identical mean viability (85.46%) in BB and HN, whereas *Acanthus ilicifolius* and *Xylocarpus granatum* showed higher mean viability in honey (86.34% and 89.24%, respectively) than in bee bread (81.53% and 83.33%, respectively).

Acetocarmine staining yielded mean viability values ranging from 80.67% in *Acanthus ilicifolius* to 93.32% in *Desmodium* sp. for bee bread pollen. In honey pollen, mean viability ranged from 82.34% in *Bruguiera gymnorhiza* to 90.75% in *Avicennia* sp. *Acanthus ilicifolius* and *Xylocarpus granatum* again showed higher mean viability in honey (89.47% and 85.47%, respectively) than in bee bread (80.67% and 81.19%, respectively).

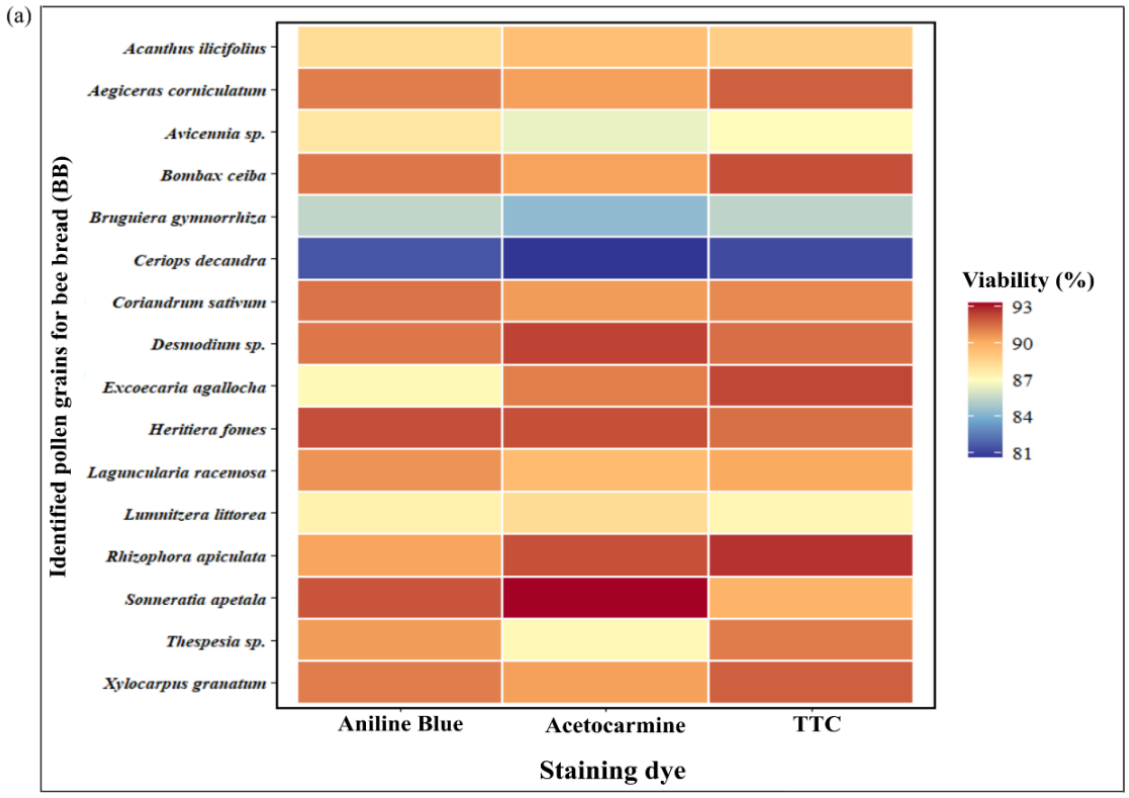
TTC staining recorded mean viability values ranging from 81.22% in *Acanthus ilicifolius* to 92.78% in *Laguncularia racemosa* in bee bread, whereas honey pollen showed mean values ranging from 81.01% in *Avicennia* sp. to 86.88% in *Kandelia candel*. *Acanthus ilicifolius* and *Xylocarpus granatum* again showed higher mean viability in honey (84.91% and 84.88%, respectively) than in bee bread (81.22% and 82.22%, respectively). Thus, among the taxa represented in both matrices, these two taxa consistently exhibited higher mean viability in honey across all three staining methods, whereas the other comparable taxa generally showed higher mean viability in bee bread.

Heatmaps (Fig. 3a, b) provide a comparative visualization of the mean pollen viability values obtained using Aniline Blue, Acetocarmine and TTC staining methods. Fig. 3a represents pollen recovered from bee bread, whereas Fig. 3b represents pollen recovered from honey. The colour intensity reflects the magnitude of pollen viability, with lighter (blue) gradients indicating relatively lower viability and darker (brown) gradients indicating relatively higher viability. Distinct gradient variations are evident not only between bee bread and honey pollen but also among the three staining methods within each matrix, indicating taxon- and stain-dependent differences in the estimated viability. In bee bread pollen (Fig. 3a), comparatively lighter gradients are observed for *Bruguiera gymnorhiza* and *Ceriops decandra*, particularly in comparison with taxa showing darker gradients, although the intensity varies among the three staining methods. In honey pollen (Fig. 3b), most taxa show relatively lighter gradients compared with the bee bread heatmap; however, some taxa exhibit distinct stain-specific patterns. For example, *Sonneratia apetala* shows noticeable differences in colour intensity among Aniline Blue, Acetocarmine and TTC, reflecting variation in its estimated viability according to the staining method. As such, the heatmaps demonstrate matrix-, taxon- and staining-method-dependent variation in pollen viability and provide a visual representation of the patterns presented quantitatively in Table 2.

The box plots in Fig. 4 illustrate the variation in pollen viability determined using Aniline Blue, Acetocarmine and TTC staining methods. Pairwise comparisons among the three staining methods showed no statistically significant differences in pollen viability for bee bread pollen (Fig. 4a). In contrast, significant differences were observed among the staining methods for honey pollen: viability obtained using Aniline Blue differed significantly from that obtained using Acetocarmine ($p < 0.05$) and TTC ($p < 0.01$), whereas the difference between Acetocarmine and TTC was not statistically significant. Pairwise comparisons between bee bread and honey pollen for each staining method revealed significantly higher viability in bee bread pollen (Fig. 4b). The difference between BB and HN was significant at $p < 0.05$ for both Aniline Blue and Acetocarmine, whereas a highly significant difference was observed for TTC staining ($p < 0.001$). These comparisons indicate that the choice of staining method did not significantly affect the estimated viability of bee bread pollen, whereas staining method influenced the viability estimates obtained for honey pollen, with bee bread pollen consistently exhibiting higher viability than honey pollen across all three staining methods.

Table 2: Mean pollen viability (%) ± standard deviation of 20 identified pollen taxa assessed using Aniline Blue, Acetocarmine, and TTC staining methods, together with the maximum germination frequency observed after 72 h of incubation.

Identified Pollen Taxa	Mean Pollen viability in different staining dyes						Germination (%)	
	Aniline Blue		Acetocarmine		TTC			
	Bee bread	Honey	Bee bread	Honey	Bee bread	Honey	Bee bread	Honey
Acanthus ilicifolius L.	81.53 ± 4.33	86.34 ± 2.13	80.67 ± 4.90	89.47 ± 1.78	81.22 ± 5.15	84.91 ± 4.66	70.46 ± 5.03	64.74 ± 3.22
Aegiceras corniculatum (L.) Blanco	91.24 ± 3.52	-	90.47 ± 4.16	-	91.88 ± 4.07	-	82.28 ± 4.45	-
Avicennia sp.	87.92 ± 3.35	87.52 ± 2.06	86.47 ± 3.83	90.75 ± 2.22	87.12 ± 3.72	81.01 ± 4.63	84.68 ± 4.42	71.68 ± 3.34
Bombax ceiba (L.)	91.37 ± 3.64	87.37 ± 2.33	90.39 ± 4.34	84.39 ± 1.15	92.23 ± 4.23	83.23 ± 4.56	78.56 ± 4.70	63.56 ± 2.98
Bruguiera gymnorhiza (L.) Lam.	85.46 ± 3.58	85.46 ± 2.21	84.34 ± 4.30	82.34 ± 1.42	85.38 ± 4.17	82.38 ± 4.47	76.34 ± 4.51	67.34 ± 2.83
Ceriops decandra (Griff.) W.Theob.	88.34 ± 3.33	84.53 ± 2.19	89.47 ± 3.88	85.67 ± 0.88	88.91 ± 3.78	86.22 ± 4.59	76.74 ± 4.32	67.46 ± 2.31
Coriandrum sativum L.	91.46 ± 3.46	87.46 ±2.22	90.56 ± 4.03	86.56 ± 1.37	90.98 ± 3.94	83.98 ± 4.61	83.42 ± 4.45	73.42 ± 3.29
Desmodium sp.	92.11 ± 3.29	88.37± 2.04	93.32 ± 5.08	84.47 ± 2.17	89.91 ± 4.74	84.58 ± 4.59	84.32 ± 5.22	63.58 ± 3.36
Excoecaria agallocha L.	90.56 ± 3.22	89.24 ± 2.45	87.26 ± 2.46	86.23 ± 1.18	91.26 ± 1.28	86.38 ± 4.68	82.18 ± 2.10	64.24 ± 3.00
Heritiera fomes Buch.	92.24 ± 3.27	87.24 ± 1.98	92.2 ± 3.77	85.20 ± 2.10	91.52 ± 3.55	86.52 ± 4.62	87.64 ± 4.69	71.64 ± 3.44
Kandelia candel (L.) Druce	-	88.24 ± 2.47	-	86.47 ± 1.02	-	86.88 ± 4.68	-	71.56 ± 2.61
Laguncularia racemosa (L.) C.F.Gaertn.	90.36 ± 3.28	-	92.18 ± 1.77	-	92.78 ± 1.80	-	78.54 ± 2.56	-
Lumnitzera littorea (Jack) Voigt	90.76 ± 3.42	83.56 ± 2.23	89.67 ± 2.42	84.38 ± 1.22	90.23 ± 2.54	84.35 ± 4.51	82.16 ± 2.48	64.68 ± 3.08
Nypa fruticans Wurmb	-	82.33 ± 2.13	-	86.19 ± 1.11	-	83.22 ± 4.51	-	68.68 ± 1.98
Phoenix paludosa Roxb.	-	86.36 ± 1.32	-	86.18 ± 1.34	-	86.78 ± 4.66	-	68.54 ±1.58
Psidium guajava L.	-	85.56 ± 0.57	-	84.26 ± 1.36	-	84.26 ± 4.58	-	65.18 ± 2.38
Rhizophora apiculata Blume	91.24 ± 3.33	-	90.47 ± 4.28	-	91.88 ± 4.27	-	80.56 ± 3.95	-
Sonneratia apetala Buch.Ham.	91.37 ± 3.64	88.11 ± 2.25	92.47 ± 3.81	83.32 ± 1.27	91.58 ± 3.63	85.91 ± 4.61	83.58 ± 4.40	62.32 ± 3.19
Thespesia sp. L.	87.56 ± 3.96	-	88.38 ± 4.71	-	87.35 ± 4.41	-	82.68 ± 5.03	-
Xylocarpus granatum J.Koenig	83.33 ± 3.62	89.24 ± 2.30	81.19 ± 4.71	85.47 ± 1.35	82.22 ± 4.72	84.88 ± 4.64	72.68 ± 4.48	68.28 ± 2.75



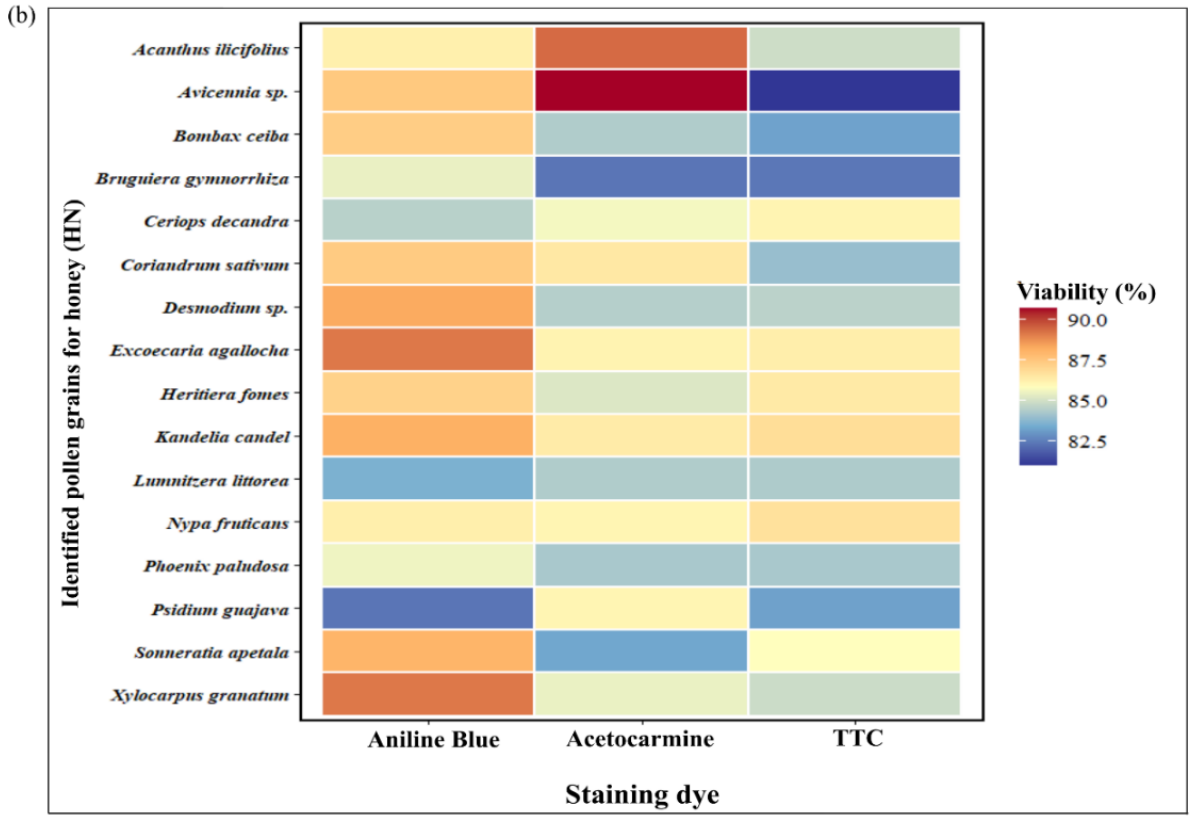
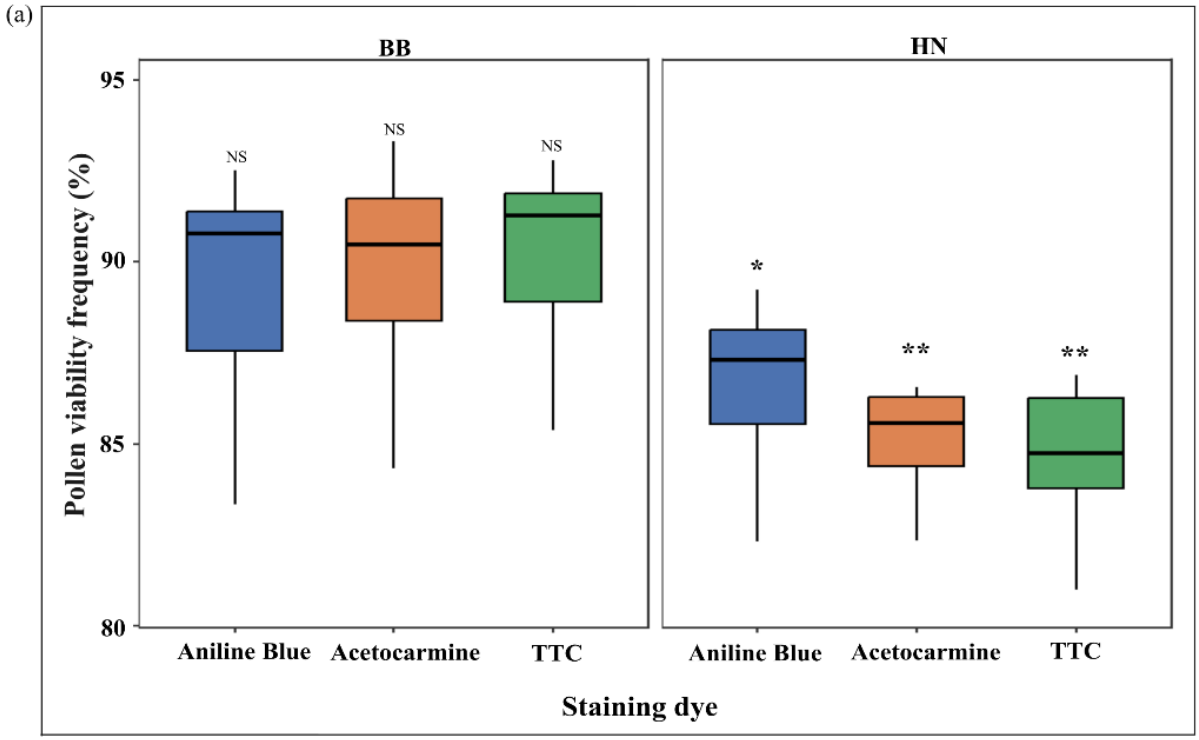


Fig. 3: Heatmap illustrating the pollen viability (%) of 16 identified pollen taxa recovered from **(a)** bee bread (BB) and **(b)** paired honey (HN), as assessed using three cytochemical staining methods: Aniline Blue, Acetocarmine, and TTC (2,3,5-triphenyltetrazolium chloride). The colour gradient from blue to dark brown represents lower to higher pollen viability (%), respectively.



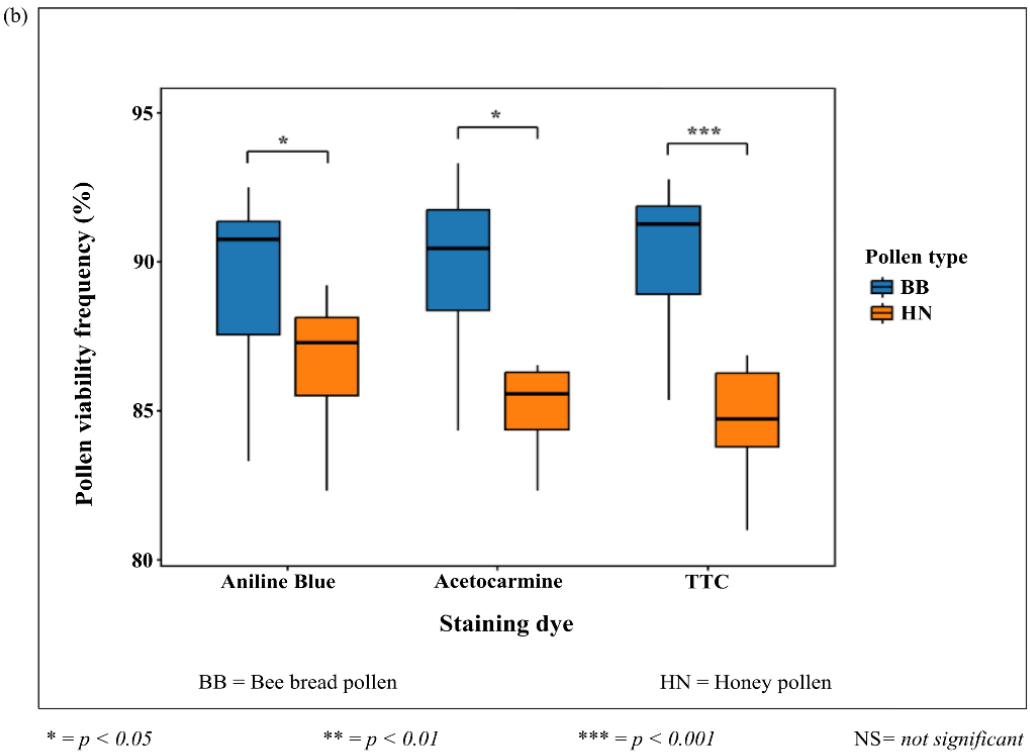


Fig. 4. Comparative assessment of pollen viability among staining methods and between bee bread and honey pollen. **(a)** Boxplots showing pollen viability (%) estimated using Aniline Blue, Acetocarmine, and TTC staining in bee bread (BB) and honey (HN) pollen. No significant differences were observed among the staining methods for BB pollen, whereas significant pairwise differences were observed between Aniline Blue and Acetocarmine and between Aniline Blue and TTC for HN pollen. **(b)** Boxplots comparing pollen viability between BB and HN pollen for each staining method. Significant differences were observed between BB and HN for Aniline Blue and Acetocarmine at $p < 0.05$ and for TTC at $p < 0.001$. Asterisks indicate statistical significance: $p < 0.05$, *$p <$ 0.01, and **$p < 0.001$.

The *in vitro* germination of pollen recovered from bee bread (BB) and honey (HN) samples (Fig. 5) was evaluated at 24, 48, 72 and 96 h of incubation using different sucrose concentrations (Fig. 6a, b). Germination frequency increased with increasing incubation time and reached its maximum at 72 h in both BB and HN pollen, followed by a slight decline at 96 h (Fig. 6b). The sucrose-concentration experiment showed that 20% sucrose produced the highest germination response under the experimental conditions, and this concentration was therefore selected as the working germination medium for subsequent experiments (Fig. 6a). Analysis of variance (ANOVA), followed by Tukey's Honest Significant Difference (HSD) test at $p \leq 0.05$, demonstrated significant differences in germination frequency among the incubation periods (Fig. 6b). Linear regression analysis demonstrated a strong positive association between incubation time and pollen germination frequency, with R^2 values of 0.8941 for bee bread pollen and 0.9107 for honey pollen (Fig. 6c, d). The corresponding regression equations were y = 1.0906x for BB pollen and y = 0.893x for HN pollen. Thus, the regression models explained 89.41% and 91.07% of the variation in germination frequency, respectively.

The mean germination frequency was consistently higher in pollen recovered from bee bread than in pollen recovered from honey. For BB pollen, germination frequency ranged from 70.46% in *Acanthus ilicifolius* to 87.64% in *Heritiera fomes*, whereas HN pollen showed values ranging from 62.32% in *Sonneratia apetala* to 73.42% in *Coriandrum sativum*. The relative germination frequencies of the identified pollen taxa recovered from BB and HN are presented in Fig. 7. For all pollen taxa represented in both matrices, the mean germination frequency was higher in BB than in HN. The difference was particularly pronounced for *Heritiera fomes*, *Desmodium* sp., *Excoecaria agallocha*, *Coriandrum sativum* and *Avicennia* sp., whereas the difference was comparatively smaller for *Xylocarpus granatum* and *Acanthus ilicifolius*. As such, the higher germination performance of pollen recovered from BB, together with its generally higher viability, indicates that the bee-bread matrix may provide more favourable conditions for maintaining pollen viability and germination potential than honey.

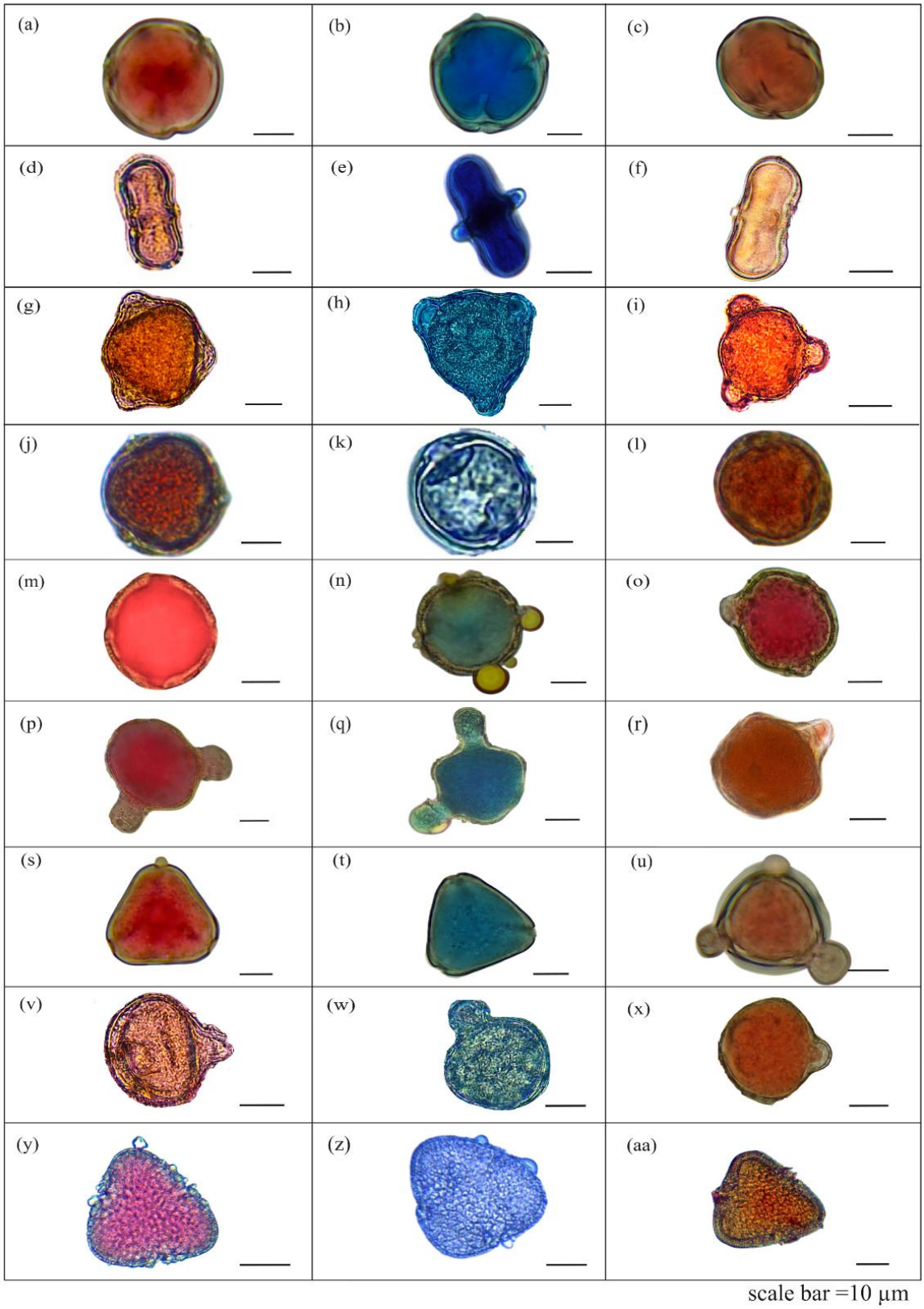


Fig. 5: Photomicrographs of pollen grains from 20 plant species stained with three cytochemical dyes for assessment of pollen viability, along with pollen germination and pollen tube formation. Each pollen type is represented under three staining protocols: **Acetocarmine** (left column), **Aniline Blue** (centre column), and **TTC [2,3,5-triphenyltetrazolium chloride]** (right column). **(a–c)** *Heritiera fomes*; **(d–f)** *Coriandrum sativum*; **(g–i)** *Sonneratia apetala*; **(j–l)** *Rhizophora apiculata*; **(m–o)** *Aegiceras corniculatum*; **(p–r)** *Ceriops decandra*; **(s–u)** *Bruguiera gymnorhiza*; **(v–x)** *Xylocarpus granatum*; **(y–aa)** *Bombax ceiba*.

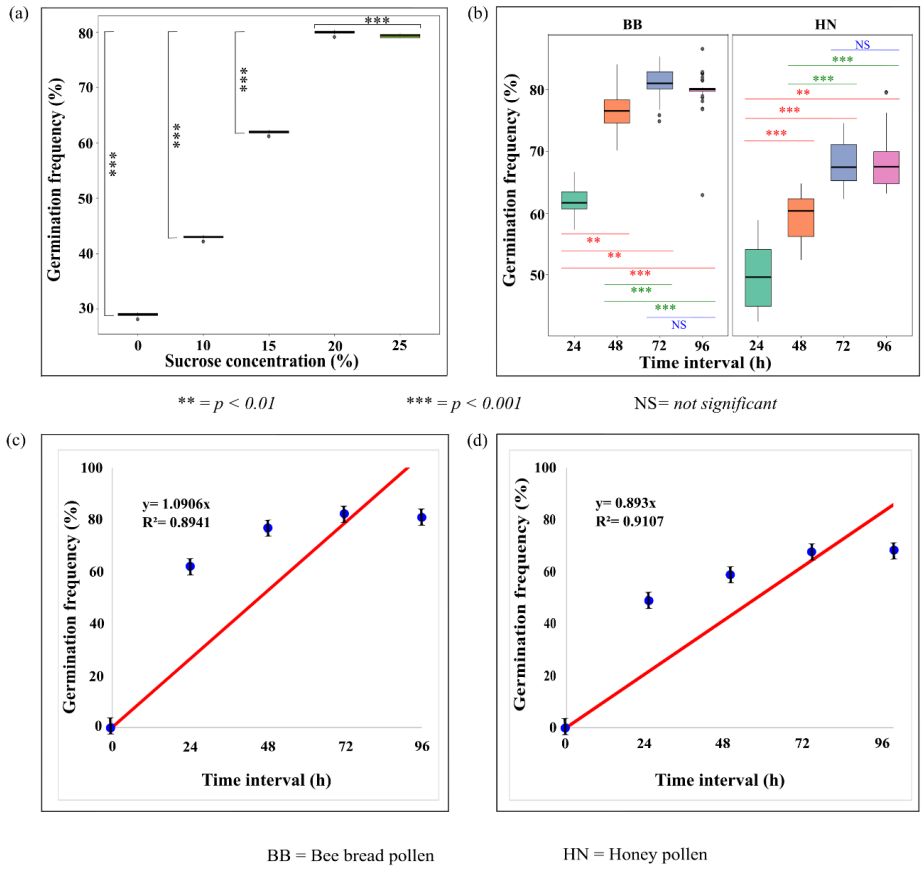


Fig 6: Effect of sucrose concentration and incubation time on pollen germination and comparative germination frequency of bee bread and honey pollen. **(a)** Germination frequency of pollen grains at different sucrose concentrations (0–25%). **(b)** Boxplots showing the temporal progression of germination frequency (%) at 24, 48, 72, and 96 h of incubation, with statistical significance brackets indicating pairwise comparisons among incubation periods. **(c–d)** Linear regression between incubation time and germination frequency for bee bread (BB) and honey (HN) pollen, respectively.

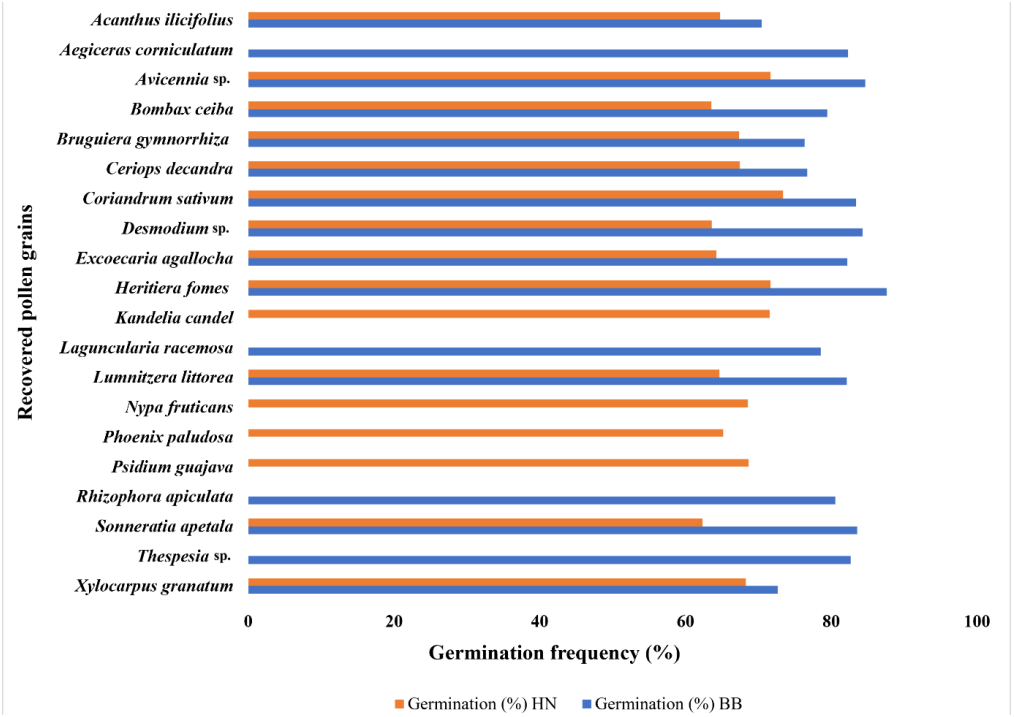


Fig. 7: Comparative germination frequency of identified pollen taxa recovered from bee bread and honey samples.

DISCUSSION

Conventional pollen preservation methods include chemical fixation, refrigeration, freezing, desiccation and cryopreservation, with the choice of method depending on whether the primary objective is preservation of morphological integrity, physiological viability or genetic material (Aguilera & Karel, 1997; Grizzle *et al*., 2008; Pegg, 2009; Huang & Yeung 2015; Parihar, 2023). Cryopreservation at −196 °C is widely regarded as an effective approach for long-term pollen storage when pollen moisture content is appropriately controlled. Excessive moisture may promote ice-crystal formation, whereas excessive dehydration can damage cellular membranes and reduce viability (Dinato *et al*., 2021). In addition, conventional preservation approaches may require specialised infrastructure, controlled environmental conditions and continuous maintenance. These limitations highlight the importance of exploring naturally occurring, low-cost systems that may contribute to the maintenance of pollen quality. In this context, bee-derived matrices such as bee bread and honey provide an interesting natural system for investigating the persistence of pollen viability and germination potential under hive-associated conditions.

The present analysis recovered 20 pollen taxa belonging to 12 families from bee bread and honey collected from different localities of the Indian Sundarbans. The assemblages included mangrove taxa such as *Acanthus ilicifolius, Aegiceras corniculatum, Avicennia* sp., *Bruguiera gymnorhiza, Ceriops decandra, Excoecaria agallocha, Heritiera fomes, Kandelia candel, Rhizophora apiculata, Sonneratia apetala* and *Xylocarpus granatum*, together with cultivated and associated taxa such as *Coriandrum sativum, Desmodium* sp., *Bombax ceiba* and *Psidium guajava*, reflecting the broad foraging landscape available to honey bees. The occurrence of taxa differed among localities and between bee bread and honey, indicating that the two hive products provide overlapping but not identical records of floral visitation. The presence of pollen taxa restricted to one matrix may reflect differences in the biological origin and accumulation of pollen in bee bread and honey. Bee bread represents pollen actively collected by worker bees and stored in comb cells, whereas pollen in honey may enter the product during nectar collection and subsequent hive processing. Consequently, the pollen spectra of the two matrices need not be identical. Differences in pollen production, nectar availability, flowering phenology and bee foraging behaviour may further contribute to the contrasting pollen assemblages.

The diversity of pollen types recorded in the present study is relevant to the interpretation of pollen preservation because the taxa differed considerably in their subsequent viability and germination responses. Mean viability values obtained using Aniline Blue, Acetocarmine and TTC were generally high, but the magnitude varied among pollen taxa and between the two matrices. For example, *Heritiera fomes, Desmodium* sp., *Coriandrum sativum* and several other taxa showed relatively high viability in bee bread, whereas *Acanthus ilicifolius* consistently showed comparatively lower viability in bee bread. Conversely, *Acanthus ilicifolius* and *Xylocarpus granatum* showed higher mean viability in honey than in bee bread across the three staining methods. These taxon-specific responses indicate that the effect of the storage matrix is not uniform across all pollen types and may depend on intrinsic pollen characteristics.

The three staining procedures used in the present study should be regarded as indirect indicators of pollen viability rather than direct measures of reproductive competence. Positive staining reflects particular aspects of cellular or physiological integrity, but staining alone does not establish the ability of pollen to germinate and successfully participate in fertilization (Stokes & Geitmann, 2025). Aniline Blue is particularly useful for detecting callose associated with pollen germination and pollen tube development (Currier, 1957; Heslop-Harrison, 1987), whereas Acetocarmine provides an indication of cytological integrity through staining of nuclear and cytoplasmic components (Stanley & Linskens, 1974; Shivanna & Rangaswamy, 1992). TTC staining provides a physiological indicator based on the reduction of tetrazolium salts by metabolically active cells and associated dehydrogenase activity (Lakon, 1949; Shivanna & Heslop-Harrison, 1981). Thus, the three methods provide complementary rather than interchangeable information about the condition of stored pollen.

Importantly, the present results do not support the conclusion that one staining method was universally superior to the others. In bee bread, no statistically significant difference was detected among the three staining methods, whereas significant differences were observed among staining methods in honey pollen, particularly between Aniline Blue and Acetocarmine and between Aniline Blue and TTC. The comparisons between the two matrices further showed significantly higher viability in bee bread than in honey for all three staining methods, with the difference being particularly strong for TTC. Therefore, the statistical results suggest that the storage matrix had a stronger and more consistent influence on pollen viability than the choice of staining method, although the magnitude of the staining response varied among taxa and matrices. This interpretation is more consistent with the heatmap and box-plot results than the earlier conclusion that Aniline Blue was universally the best staining method.

The taxon-specific patterns observed in the heatmaps further support this interpretation. The colour gradients varied not only between bee bread and honey but also among Aniline Blue, Acetocarmine and TTC within each matrix. In bee bread, *Bruguiera gymnorhiza* and *Ceriops decandra* showed comparatively lower viability profiles than several other taxa, whereas in honey *Sonneratia apetala* displayed noticeable variation among the three staining methods. Conversely, *Acanthus ilicifolius* and *Xylocarpus granatum* showed relatively higher viability in honey than in bee bread across the three staining approaches. These patterns indicate that pollen response to storage conditions is taxon-dependent and that no single staining method completely captures all aspects of pollen physiological status.

The consistently higher viability observed for many taxa in bee bread raises the possibility that the bee-bread matrix provides conditions favourable for maintaining pollen integrity. Bee bread is formed when worker bees collect pollen and incorporate nectar and glandular secretions before compacting it into comb cells. Subsequent microbial activity, particularly involving lactic acid bacteria, contributes to fermentation and changes in pH and other physicochemical properties (Herbert & Shimanuki, 1978). Such conditions may restrict the proliferation of undesirable microorganisms and contribute to the preservation of the stored pollen. However, the present study did not directly measure microbial communities, fermentation intensity, pH, water activity, antioxidant activity or biochemical degradation. Therefore, fermentation should be regarded as a plausible mechanism rather than a demonstrated explanation for the higher viability observed in bee bread.

Honey represents a substantially different storage environment. Pollen occurring in honey is not stored as a dedicated pollen reserve but may become incorporated during nectar collection and hive processing. The high sugar concentration and low water availability of honey can restrict microbial growth, while its acidic environment and other chemical properties may affect the physiological condition of entrapped pollen (Bogdanov *et al*., 2008). The present results suggest that pollen retained sufficient structural or cytochemical integrity to produce relatively high staining responses in honey, but its germination potential was generally lower than that of pollen recovered from bee bread. This distinction is important because it demonstrates that positive viability staining does not necessarily translate into equivalent germination competence.

The *in vitro* germination experiment provided a functional assessment that complemented the staining-based viability estimates. Germination increased with incubation time and reached its maximum at 72 h, followed by a slight decline at 96 h. The 20% sucrose medium produced the highest germination response under the conditions tested and was therefore selected for subsequent experiments. Linear regression showed strong positive associations between incubation time and germination frequency, with R^2 values of 0.8941 for bee bread pollen and 0.9107 for honey pollen. These values indicate that approximately 89.41% and 91.07%, respectively, of the variation in germination frequency was explained by the fitted regression models. The regression results therefore support a strong association between incubation duration and germination response over the experimental period, although the slight decline at 96 h indicates that the relationship should not be interpreted as indefinite or continuously increasing germination.

A clear difference between the two natural matrices was evident in the germination results. Mean germination frequency was higher for bee-bread pollen than for honey pollen across the taxa represented in both matrices. In bee bread, germination ranged from 70.46% in *Acanthus ilicifolius* to 87.64% in *Heritiera fomes*, whereas honey pollen ranged from 62.32% in *Sonneratia apetala* to 73.42% in *Coriandrum sativum*. The differences were particularly pronounced for *Heritiera fomes, Desmodium* sp., *Excoecaria agallocha* and *Bombax ceiba*, whereas the difference between the two matrices was comparatively smaller for *Xylocarpus granatum*. These taxon-specific differences again indicate that pollen response to natural storage conditions is not uniform.

The difference between staining-based viability and *in vitro* germination is particularly informative. In several taxa, viability estimates were substantially higher than germination frequencies. This is expected because staining methods detect selected aspects of cellular integrity or metabolic activity, whereas germination provides a more functional measure of the ability of pollen to initiate pollen-tube growth under the experimental conditions. Consequently, a pollen grain may retain sufficient structural or metabolic characteristics to stain positively while having reduced capacity to germinate. The present findings therefore support the use of both staining-based viability assessment and *in vitro* germination when evaluating the biological condition of naturally stored pollen.

The total pattern observed in this study suggests that bee bread has considerable potential as a natural matrix for maintaining pollen viability and germination competence. However, the results should not be interpreted as definitive evidence that bee bread acts as a long-term pollen preservation system. The study was based on naturally occurring pollen recovered from bee bread and honey and did not include freshly collected pollen as an initial control or a controlled time-course experiment in which the same pollen taxa were stored under defined bee-bread and honey conditions. Therefore, the observed

differences demonstrate an association between matrix and pollen condition, rather than directly establishing a causal preservation effect. Controlled experiments involving freshly collected pollen, defined storage periods, physicochemical characterization, microbial profiling and repeated viability and germination measurements would be necessary to determine the mechanisms and temporal stability of pollen preservation in bee bread.

Finally, the species-specific differences observed in both viability and germination indicate that bee bread should not be considered a uniformly effective natural preservative for all pollen taxa. Some pollen types, such as *Heritiera fomes* and *Coriandrum sativum*, maintained relatively high viability and germination responses, whereas others showed lower or contrasting responses between the two matrices. *Acanthus ilicifolius* and *Xylocarpus granatum*, in particular, exhibited comparatively unusual patterns in which honey-associated pollen showed higher viability than bee-bread pollen despite the overall trend favouring bee bread. These findings emphasize that the interaction between pollen characteristics and storage matrix should be considered on a taxa-specific basis. Thus, the Sundarbans bee-bread system provides a promising natural model for studying pollen preservation, while also highlighting the need for species-specific evaluation of pollen viability and germination.

CONCLUSION

The present study from the Sundarbans mangrove ecosystem provides baseline evidence of the diversity, viability and germination potential of pollen naturally preserved within bee bread and honey. Pollen recovered from bee bread generally exhibited higher viability and *in vitro* germination potential than pollen recovered from honey, suggesting that bee bread provides a more favourable natural environment for maintaining pollen physiological integrity and germination capacity. The study also revealed considerable pollen diversity associated with mangrove and surrounding vegetation, with taxon-specific differences in viability and germination between the two matrices. These findings highlight the potential of bee bread as a natural biological archive for investigating pollen biology, bee–plant interactions, pollination ecology, floral-resource availability and biodiversity, while also providing a promising basis for future research on natural pollen preservation and *ex situ* pollen conservation. Further controlled studies using freshly collected pollen and defined storage periods are needed to determine the long-term stability of pollen in bee bread and to elucidate the factors contributing to its preservation.

Credit Statement

Nirmallya Ghosh (NG): Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft. MD Ashfaque Molla (AM): Data curation, Methodology; Formal analysis; Investigation. Priyasi Ray (PR): Data Curation, Methodology; Formal analysis; Investigation. Radhakanta Nandi (RN): Data Curation; Methodology; Formal analysis; Investigation. Validation; Visualization; Writing – original draft. Sarupa Barman (SB): Data curation; Validation; Visualization; Writing – original draft. Anisha Satpati (AS): Data curation; Validation; Visualization; Writing – original draft. Sudha Gupta (SG): Conceptualization; Resources; Supervision; Validation; Visualization; Writing – review & editing.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the Department of Botany, University of Kalyani, for providing the necessary facilities and support to carry out this work. The authors also sincerely thank the Sundarbans honey collectors, Mr Tapan Mondal, Mr Bapi Mondal, Mr Abhijit Sarkar, Mr Manab Gayen, and Mr Tarapada Malik, for their valuable assistance during the field visits and for helping with the collection of honey and bee bread samples from the Sundarbans. Financial support received by the authors is also gratefully acknowledged. NG acknowledges support from the UGC–JRF fellowship, RN from the UGC–JRF fellowship, SB from the UGC–NFSC fellowship, and AS from the CSIR–JRF fellowship.

Funding

SG gratefully acknowledges the financial support received from the University of Kalyani, Kalyani, India, in the form of a Faculty Research Grant (MS No. Rev/957 of 2025–26). NG and RN acknowledge the financial support provided by the Joint CSIR–UGC in the form of Senior Research Fellowships, with NTA Reference Nos. 211610038625 and 211610192813, respectively. SB acknowledges the financial support provided by the UGC–NFSC in the form of a Senior Research Fellowship, with NTA Reference No. 221610161067. AS acknowledges the financial support provided by the Joint CSIR–UGC in the form of a Senior Research Fellowship, with Award No. CSIRAWARD/JRF-NET2023/14141.

REFERENCES

Aguilera, J. M. and Karel, M. (1997). Preservation of biological materials under desiccation. *Critical Reviews in Food Science & Nutrition*, **37**(3), 287-309. https://doi.org/10.1080/10408399709527776

Google Scholar

Ahmad, M., Fahad, S., Pieroni, A., Zafar, M., Sultana, S. and Majeed, S. (2024). *Melissopalynology: Bee Flora, Pollen and Honey. Elsevier*.
Google Scholar

Althiab-Almasaud, R., Teyssier, E., Chervin, C., Johnson, M. A. and Mollet, J. C. (2024). Pollen viability, longevity, and function in angiosperms: key drivers and prospects for improvement. *Plant Reproduction,* **37**(3), 273-293.
Google Scholar

Amit, S. K., Uddin, M. M., Rahman, R., Islam, S. R. and Khan, M. S. (2017). A review on mechanisms and commercial aspects of food preservation and processing. *Agriculture & food security,* **6**(1), 51.
Google Scholar

Baky, M. H., Abouelela, M. B., Wang, K. and Farag, M. A. (2023). Bee pollen and bread as a super-food: A comparative review of their metabolome composition and quality assessment in the context of best recovery conditions. *Molecules,* **28**(2), 715.
https://doi.org/10.3390/molecules28020715
Google Scholar

Ballantyne, D. J. (1974). Pollen Viability Testing Storage and Related Physiology.
Google Scholar

Bogdanov, S., Jurendic, T., Sieber, R. and Gallmann, P. (2008). Honey for Nutrition and Health: A Review. *Journal of the American College of Nutrition,* **27**(6), 677–689.
https://doi.org/10.1080/07315724.2008.10719745
Google Scholar

Bots, M. and Mariani, C. (2005). Pollen viability in the field. *COGEM, Radbound University Nijmegen, The Netherlands,* **52**.
Google Scholar

Brewbaker, J. L. and Kwack, B. H. (1963). The essential role of calcium ion in pollen germination and pollen tube growth. *American journal of botany,* **50**(9), 859-865.
Google Scholar

Bryant Jr, V. M. and Jones, G. D. (2001). The r-values of honey: Pollen coefficients. *Palynology,* **25**(1), 11-28.
Google Scholar

Chakraborti, U., Mitra, B. and Bhadra, K. (2019). Diversity and ecological role of insect flower visitors in the pollination of mangroves from the Indian Sundarbans. *Current science,* **117**(6), 1060-1070.
Google Scholar

Currier, H. B. (1957). Callose substance in plant cells. *American Journal of Botany*, 478-488.
Google Scholar

Dafni, A. and Firmage, D. (2000). Pollen viability and longevity: practical, ecological and evolutionary implications. *Plant Systematics and Evolution,* **222**(1), 113-132.
Google Scholar

Da Silva, P. M., Gauche, C., Gonzaga, L. V., Costa, A. C. O. and Fett, R. (2016). Honey: Chemical composition, stability and authenticity. *Food chemistry,* **196**, 309-323.
Google Scholar

Dinato, N. B., Imaculada Santos, I. R., Zanotto Vigna, B. B., de Paula, A. F. and Fávero, A. P. (2020). Pollen cryopreservation for plant breeding and genetic resources conservation. *CryoLetters,* **41**(3), 115-127.
Google Scholar

Edo, G. I., Onoharigho, F. O., Akpoghelie, P. O., Akpoghelie, E. O., Agbo, J. J., Agoh, E. and Lawal, R. A. (2023). Natural honey (raw honey): insights on quality, composition, economic and health effects: a comprehensive review. *Food Science and Engineering,* **4**(2), 265-293.
Google Scholar

Ghosh, A., Schmidt, S., Fickert, T. and Nüsser, M. (2015). The Indian Sundarban mangrove forests: history, utilization, conservation strategies and local perception. *Diversity,* **7**(2), 149-169.
Google Scholar

Herbert Jr, E. W. and Shimanuki, H. (1978). Chemical composition and nutritive value of bee-collected and bee-stored pollen. *Apidologie,* **9**(1), 33-40.
Google Scholar

Heslop-Harrison, J. (1987). Pollen germination and pollen-tube growth. *International review of cytology,* **107**, 1-78.
Google Scholar

El-Sofany, A., Naggar, Y. A., Naiem, E., Giesy, J. P., and Seif, A. (2020). Authentication of the botanical and geographic origin of Egyptian honey using pollen analysis methods. *Journal of Apicultural Research,* **59**(5), 946-955.
Google Scholar

Gopal, B., & Chauhan, M. (2006). Biodiversity and its conservation in the Sundarban mangrove ecosystem. *Aquatic sciences*, **68**(3), 338-354.
Google Scholar

Grizzle, W. E., Fredenburgh, J. L. and Myers, R. B. (2008). Fixation of tissues. *Theory and practice of histological techniques,* **6**, 53-74.
Google Scholar

Herbert Jr, E. W., & Shimanuki, H. (1978). Chemical composition and nutritive value of bee-collected and bee-stored pollen. *Apidologie*, **9**(1), 33-40.
Google Scholar

Huang, B. Q. and Yeung, E. C. (2015). Chemical and physical fixation of cells and tissues: an overview. *Plant micro techniques and protocols,* 23-43.
Google Scholar

Kakani, V. G., Prasad, P. V., Craufurd, P. Q. and Wheeler, T. R. (2002). Response of in vitro pollen germination and pollen tube growth of groundnut

(Arachis hypogaea L.) genotypes to temperature. *Plant, Cell & Environment,* **25**(12), 1651-1661.
Google Scholar

Kieliszek, M., Piwowarek, K., Kot, A. M., Błażejak, S., Chlebowska-Śmigiel, A. and Wolska, I. (2018). Pollen and bee bread as new health-oriented products: A review. *Trends in Food Science & Technology,* **71**, 170-180.
Google Scholar

Lakon, G. (1949). The topographical tetrazolium method for determining the germinating capacity of seeds. *Plant physiology,* **24**(3), 389.
Google Scholar

Layek, U., Mondal, R., & Karmakar, P. (2020). Honey sample collection methods influence pollen composition in determining true nectar-foraging bee plants. *Acta Botanica Brasilica*, **34**(3), 478-486.
Google Scholar

Leponiemi, M., Freitak, D., Moreno-Torres, M., Pferschy-Wenzig, E.-M., Becker-Scarpitta, A., Tiusanen, M., Vesterinen, E. J., & Wirta, H. (2023). Honeybees' foraging choices for nectar and pollen revealed by DNA metabarcoding. *Scientific Reports, 13*, Article 14753. https://doi.org/10.1038/s41598-023-42102-4
Google Scholar

Louveaux, J., Maurizio, A. and Vorwohl, G. (1978). Methods of melissopalynology. *Bee world,* **59**(4), 139-157.
Google Scholar

Naskar, K. and Mandal, R. (1999). Ecology and biodiversity of Indian mangroves (Vol. 1). *Daya Books.*
Google Scholar

Parihar, A., Kumar, A., Panda, U., Khan, R., Parihar, D. S. and Khan, R. (2023). Cryopreservation: a comprehensive overview, challenges, and future perspectives. *Advanced Biology,* **7**(6), 2200285.
Google Scholar

Pegg, D. E. (2009). Principles of Cryopreservation. Preservation of Human Oocytes. *CRC Press,* 34-46.
Google Scholar

Ponnuchamy, R., Bonhomme, V., Prasad, S., Das, L., Patel, P., Gaucherel, C. and Anupama, K. (2014). Honey pollen: using melissopalynology to understand foraging preferences of bees in tropical South India. *Plos one,* **9**(7), e101618.
Google Scholar

Refat, M. K. H., Salekeen, R., Jahan, S., Motalab, M., Mim, S. H., Liza, A. A. and Islam, M. N. (2026). Physicochemical Characteristics and Bioactive Properties of Honey from the Sundarbans Mangrove Forest, Bangladesh. *Next Research,* 102203.
Google Scholar

Sakhraoui, A., Arrari, F., Sakhraoui, A., Aylanc, V., Rodriguez-Flores, M. S., Seijo, M. C. and Falcão, S. I. (2025). Characterization of Multifloral Bee Pollen Collected from Geographically and Botanically Distinct Regions in Tunisia. *Foods,* **14**(23), 3986.
Google Scholar

Shivanna, K. R. and Heslop-Harrison, J. (1981). Membrane state and pollen viability. *Annals of Botany,* **47**(6), 759-770.
Google Scholar

Shivanna, K. R. and Rangaswamy, N. S. (1992). Pollen germination and pollen tube growth in vitro. In: Pollen Biology: A Laboratory Manual. *Springer, Berlin, Heidelberg,* 23-31.
Google Scholar

Seeley, T. D. (1995). The foraging abilities of a colony. 46-68.
Google Scholar

Smati, N. (2021). Bee bread preservation methods: physical, chemical and microbial stability throughout storage (*Master's thesis, Instituto Politecnico de Braganca* (Portugal)).
Google Scholar

Stanley, R. G. and Linskens, H. F. (1984). Pollinosis. In Pollen: Biology Biochemistry Management. *Springer, Berlin, Heidelberg,* 164-181.
Google Scholar

Stokes, M., & Geitmann, A. (2025). Screening methods for thermotolerance in pollen. *Annals of Botany,* **135**(1-2), 71-88.
Google Scholar

Tushabe, D. and Rosbakh, S. (2021). A compendium of in vitro germination media for pollen research. *Frontiers in Plant Science*, **12**, 709945.
Google Scholar

Journal of Plant Development Sciences
Meerut, India

RESEARCH ARTICLE

MORPHOLOGICAL, PHENOLOGICAL AND MEIOTIC ANALYSIS OF *BUDDLEJA CRISPA* BENTH. FROM JAMMU PROVINCE

Rabia Farooq Bajran[1], Hrishi Manchanda[1] and Geeta Sharma[1]*

[1]Department of Botany, University of Jammu, Jammu, India
Email: geetaji@yahoo.com

Received-22.06.2026, Revised-13.07.2026, Accepted-27.07.2026

Abstract: *Buddleja crispa* Benth. is an important species growing on high altitudinal sites of Jammu province. Presently, two populations from Pouni and Batote of Jammu province were analysed for morphological, phenological and meiotic aspects. The plants of this species remain in vegetative stage from late September to mid-February, form buds from mid-February to early March, show full blooming from early March to mid-May and fruiting from May to June. Morphological comparison using one way ANOVA reveals Batote population is vigorous in comparison of Pouni population which seems to be an outcome of prevailing climatic conditions. Meiotic studies revealed all PMCs having diploid count of 38. These chromosomes paired as 19 bivalents at diakinesis and metaphase I and separated equally (19:19) at anaphase I. The second meiotic division was also regular. The percentage pollen stainability and viability as determined using 1% acetocarmine, triphenyl tetrazolium chloride (TTC) and fluorescein diacetate (FDA) respectively valued as 88.65%, 53.82% and 62.6% which points towards cytological stability of the species.

Keywords: *Buddleja crispa*, Meiosis, Phenology, Jammu province

INTRODUCTION

The genus *Buddleja* Houst. ex L. of family Scrophulariaceae is represented by 11 species from India (Santapau and Henry, 1973). *Buddleja crispa* is an important shrubby species of this genus, which grows on high elevations of Himalaya (Polunin and Stainton, 1984). Because of releasing compounds like benzaldehyde and lilac aldehyde, this species is highly attractant to butterflies (Gong *et al*., 2015). Its leaf extracts, containing anti-oxidant and anti-inflammatory components are known to lower blood pressure (Ahmad *et al*., 2008; Gilani *et al*., 2009; Bukhari *et al*., 2016).

While studying diversity aspects of *Buddleja* species growing in Jammu province, we found two populations of *B. crispa* at Pouni and Batote. Lack of information on morphological, phenological and cytological aspects of this species prompted us to study these features at population level.

MATERIALS AND METHODS

Study sites

Field surveys conducted in different areas of Jammu Province during 2023-2025 led to identification of two populations from Pouni and Batote of Reasi and Ramban districts respectively (Table 1).

Table 1. Details of study sites of two *B. crispa* populations

District	Location	Latitude (N)	Longitude (E)	Altitude (masl)
Reasi	Pouni	33.080˚	74.836˚	1200
Ramban	Batote	33.112˚	75.350˚	1390

Morphological and phenological analysis

The plants of tagged populations were screened for qualitative traits like leaf shape, apex, base, margin, pubescence, inflorescence colour and type. The quantitative traits studied included plant height, leaves per 30 cm branch, leaf size, petiole length, inflorescence length, flower length, sepal and petal length as well as dimensions of anther, stigma and style. Besides, onset and duration of different phenological events as vegetative stage, bud stage, flowering and fruiting were recorded by regularly visiting the study sites. For the data generated on quantitative traits, the values of mean and standard error were determined and significant variation in traits across populations was determined by employing one way ANOVA using MS Excel.

Meiotic analysis

For meiotic studies, young floral buds were collected at 9 am and fixed in Carnoy's fluid (absolute ethanol; glacial acetic acid as 3:1) for 24 hrs (Sharma and Gohil, 2013) and preserved in 70% ethanol (4°C). The anthers were squashed in 0.7% propino-carmine.

*Corresponding Author

For determining pollen stainability, pollen grains from freshly dehisced anthers were squashed in 1% acetocarmine. Similarly, pollen viability was determined using triphenyl tetrazolium chloride (TTC) and fluorescein diacetate (FDA) wherein filled and coloured pollen were considered viable, whereas empty and shriveled were taken as non-viable.

RESULTS AND DISCUSSION

Morphology and Phenology

The studied *B. crispa* plants (Fig 1a) have simple, opposite, sub-sessile, ovate, pubescent leaves with obtuse apices, cordate bases and undulated margins. The inflorescence of this species is an indeterminate spike (Fig 1b). The purple flowers (Fig 1c) of this species have 4 fused sepals and petals as accessory organs. The pistil of *B. crispa* is represented by clavate stigma, minute style and ovoid ovary (Fig 1d) showing axile placentation (Fig 1e) and containing translucent ovules (Fig 1f). Male reproductive organs are represented by four epipetalous stamens with anthers being basifixed and dithecous (Fig 1g). The plants of *B. crispa* remain in vegetative phase for nearly 5 months (late September to mid-February) and differentiate floral buds from mid-February to March. The blooming occurs from early March to mid-May and fruit formation from May to June (Table 2).

Figure 1 (a) Plant in full bloom; (b) inflorescence; (c) V.S. of flower; (d) clavate stigma, style and ovary; (e) T.S. of ovary; (f) ovules; (g) dehisced and undehisced anthers and (h) different developmental stages of flower from bud to fruit initiation.

Table 2. Duration of different phenological phases: vegetative ( ), bud ( ), blooming ( ) and fruiting phase ( ) of *B. crispa*

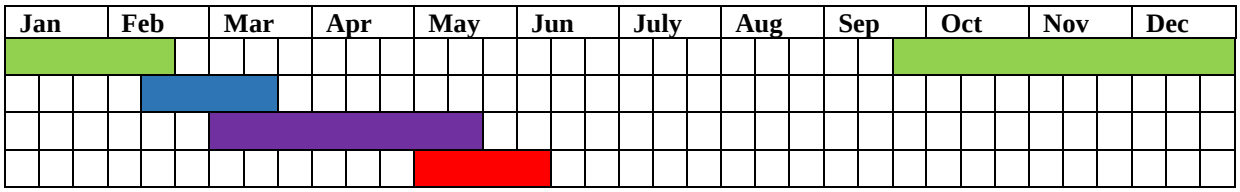


Table 3. Matrices of quantitative traits of *B. crispa*

Traits	Pouni	Batote	F- value
Tree height (m)	2.83±0.13	2.07±0.19	9.44
Leaves/unit branch	50.3±1.18	62.5±2.51	17.33*
Inflorescence length (cm)	2.24±0.17	2.81±0.21	3.67
Flowers per inflorescence	38.5±1.07	42.6±2.85	1.625
Petiole length (cm)	0.46±0.03	0.57±0.07	1.58
Leaf length (cm)	1.94±0.1	4.97±0.18	186.73*
Leaf width (cm)	2±0.04	2.04±0.12	36.87*
Internode length (cm)	4.72±0.29	3.28±0.26	11.72*
Flower length (mm)	11.8±0.16	11.3±0.26	2.31
Petal length (mm)	5.78±0.12	8.45±0.19	121.49
Petal width (mm)	2.86±0.07	2.56±0.13	3.391
Sepal length (mm)	4.75±0.12	3.4±0.13	46.53*
Anther length (mm)	2.1±0.03	1.95±0.04	7.10*
Anther width (mm)	1.24±0.06	0.99±0.05	7.47*

Stigma length (mm)	1.05±0.06	0.99±0.04	0.49
Ovary length (mm)	1.91±0.15	1.96±0.22	0.03

*asterisk marked indicates the traits showing significant differences ($p<0.05$)

Analysis of quantitative traits of Pouni and Batote populations of *B. crispa* showed marked differences in various foliar and floral traits. Significant variation among these two populations exists in leaves per unit branch, leaf size, internode length, sepal length and anther size (Table 3). Of the two populations, the plants of Batote bearing greater number of large sized leaves per unit branch and larger inflorescences supporting more flowers seem more vigorous compared to these of Pouni population. The noted difference in phenotypic traits of studied populations may be the outcome of varying climatic conditions of the two locations as also recorded in *Mentha longifolia* (Devi and Sharma, 2022).

Meiotic Studies

Meiotic studies in *B. crispa* revealed their pollen mother cells uniformly having 2n=38 chromosomes, the count earlier recorded for this species by Moore (1961), Bedi *et al.* (1981), Khatoon and Ali (1993) and Chen *et al*. (2007) who referred it as diploid. This is unlike *B. candida*, *B. fallowiana*, *B. davidii* showing tetraploidy (Moore, 1947, 1960, Chatha and Bir, 1987), *B. delavayi*, *B. nivea*, *B. albiflora*, *B. forestii* depicting hexaploidy (Moore, 1947, 1960) and *B. colvilei* plants displaying higher ploidy levels represented by 2n=152, 300 and 456 chromosomes (Moore, 1947, Janaki Ammal, 1954, Gadella, 1980).

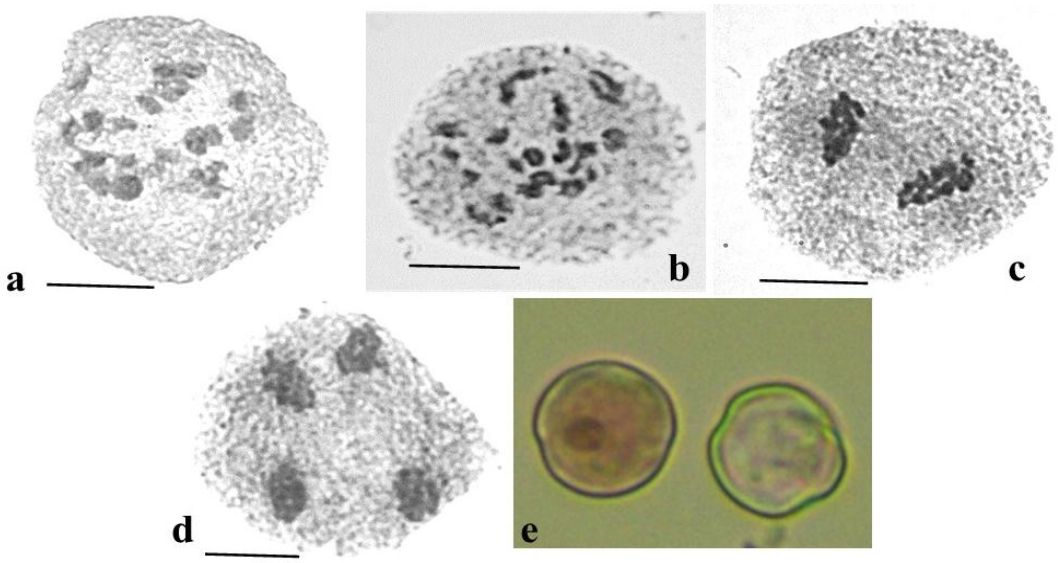


Figure 2: PMCs of *B. crispa* at showing 19 II at (a) diakinesis; (b) metaphase; (c) anaphase I displaying 19:19; (d) anaphase II showing 19:19:19:19 segregation; (e) viable and non-viable pollen grain; Scale=10 µm.

So far behaviour of chromosomes of this species is concerned, 38 chromosomes were present as 19 bivalents at diakinesis and metaphase I (Fig 2a-b). At anaphase I (Fig 2c), equal disjunction patterns were met. Likewise, regular 19:19:19:19 separation of chromatids was seen at anaphase II (Fig 2d). The pollen stainability as determined using 1% acetocarmine and pollen viability as estimated using triphenyl tetrazolium chloride (TTC) and fluorescein diacetate (FDA) came out to be 88.65%, 53.82% and 62.6% respectively (Fig 2 e). As such, *B. crispa* on account of showing normal chromosome behaviour during meiosis and depicting high pollen stainability and good viability seems to be cytologically stable.

ACKNOWLEDGEMENTS

Rabia Farooq Bajran is thankful to RUSA 2.0 for providing one time scholarship vide order no. RA/26/5230-58 dated 07-01-2026. Hrishi Manchanda is thankful to University of Jammu for providing financial assistance under University Research Scholarship (URS). Senior author acknowledges the financial assistance for the research project vide no. RA/25/4714-20 dated 09-12-2025. Thanks, are also due to Special Assistance Programme-Departmental Research Support II (SAP-DRS II), RUSA and Promotion of University Research for providing financial assistance to Department.

REFERENCES

Ahmad, I., Chen, S., Peng, Y., Chen, S. and Xu, L. (2008). Lipoxygenase inhibiting and antioxidant iridoids from *Buddleja crispa*. *J. Enzyme Inhib. Med Chem*, **1**:140–3.

Google Scholar

Bedi, Y.S., Bir, S.S. and Gill, B.S. (1981). Chromosome number reports LXXIII. *Taxon*, 30: 843.

Google Scholar

Bukhari, I.A., Gilani, A.H., Me, S.A. and Saeed, A. (2016). Analgesic, anti-inflammatory and anti-platelet activities of *Buddleja crispa*. *BMC complementary and alternative medicine*, **16**: 102.

Google Scholar

Chatha, G.S. and Bir, S.S. (1987). Population analysis of some woody species from Palni Hills, south India. *J. Cytol. Genet.*, **22**: 83–94.

Google Scholar

Chen, G., Sun, W.B. and Sun, H. (2007). Ploidy variation in *Buddleja* L (Buddlejaceae) in the Sino-Himalayan region and its biogeographical implications. *Bot. J. Linn. Soc.*, **154**: 305-312.

Google Scholar

Devi, A. and Sharma, G. (2022). Morphological, phenological and cytological comparison of *Mentha longifolia* and *M. spicata* from sub-tropical and temperate regions of Jammu province (J&K). *Vegetos*, **35**(1), 179-187.

Google Scholar

Gadella, T.W.J. and Norman, E.M. (1986). Chromosome number reports XCI. *Taxon*, **35**: 404-405.

Google Scholar

Gilani, A.H., Bukhari, I.A., Khan, R.A., Shah, A.J., Ahmad, I. and Malik, A. (2009). Presence of blood pressure lowering and spasmolytic constituents in *Buddleja crispa*. *Phytother. Res.*, **4**:492–7.

Google Scholar

Gong, W., Chen, G., Vereecken, N.J., Dunn, B.L., Ma, Y.P. and Sun, W.B. (2015). Floral scent composition predicts bee pollination system in five butterfly bush (*Buddleja*, Scrophulariaceae) species. *Plant Biology*, **17**(3): 1-9.

Google Scholar

Janaki Ammal, E.M. (1954). The cytogeography of the genus *Buddleja* in Asia. *Science and Culture*, **19**: 578-581.

Google Scholar

Khatoon, S. and Ali, S.I. (1993). Chromosome atlas of the angiosperms of Pakistan. Karachi: Department of Botany, University of Karachi Press.

Google Scholar

Moore, R. (1947). Cytotaxonomic studies in the Loganiaceae I Chromosome numbers and phylogeny in the Loganiaceae I. *American Journal of Botany*, **34**: 527-538.

Google Scholar

Moore, R. (1960). Cytotaxonomic notes on *Buddleja*. *Amer. J. Bot.*, **47**: 511-517.

Google Scholar

Moore, R. (1961). Polyploidy, phylogeny and photoperiodism in Old World *Buddleja*. *Evolution* **15**: 272-280.

Google Scholar

Polunin, O. and Stainton, A. (1984). Flowers of the Himalaya Oxford, Oxford University Press**;** 264-266.

Google Scholar

Santapau, A. and Henry, A.N. (1973). A dictionary of the flowering plants in India. Publication and Information Directorate, CSIR, New Delhi.

Google Scholar

Sharma, G. and Gohil, R.N. (2013). Double hypoploid of *Allium tuberosum* (2n=4x=30): its origin and cytology. *Genet. Resour. Crop Evol.*, **60**: 2283-2292.

Google Scholar

Journal of Plant Development Sciences
Meerut, India

RESEARCH ARTICLE

EVALUATION OF PHYTOCHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIES OF LEAF AND ROOT EXTRACTS OF *JUSTICIA SANTAPAUI* BENNET

Dhanya S.R.[1]* and P.M. Radhamany[2]

[1]*Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram, Kerala, India.*
[2]*Emeritus Professor, Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram, Kerala, India*
Email: dhanyaresearch85@gmail.com

Received-26.06.2026, Revised-10.07.2026, Accepted-28.07.2026

Abstract: The present study evaluated the phytochemical composition and pharmacological potential of leaf and root extracts of *Justicia santapaui* Bennet, an endemic medicinal plant of the Western Ghats, Kerala. Plant materials were sequentially extracted using petroleum ether, chloroform, ethyl acetate, methanol, and distilled water. Qualitative and quantitative phytochemical analyses were performed using standard protocols. Antioxidant activity was evaluated through DPPH and FRAP assays; anti-inflammatory potential via protein denaturation, LOX, and COX inhibition assays; antidiabetic activity using α-amylase inhibition assay; and cytotoxicity against SH-SY5Y human neuroblastoma cells using SRB assay. Methanol exhibited the highest extraction yield for both leaves (15.83%) and roots (48%). Leaves demonstrated greater phytochemical diversity (10 classes) compared to roots (6 classes), with alkaloids as the most abundant constituent (65.03 mg/g) in the methanolic leaf extract. The leaf extract exhibited superior antioxidant activity (DPPH IC_{50} = 381.84 µg/mL; FRAP IC_{50} = 482.84 mg/mL), potent anti-inflammatory activity (LOX IC_{50} = 85.34 µg/mL; COX IC_{50} = 92.67 µg/mL), significant α-amylase inhibition (IC_{50} = 137.20 µg/mL), and moderate cytotoxicity against SH-SY5Y cells (LC_{50} = 127.69 µg/mL). The superior bioactivity of leaves correlated with their richer phytochemical composition, including higher alkaloid, phenolic, and flavonoid contents, along with the exclusive presence of cardiac glycosides and steroids. This study provides the first scientific validation of *J. santapaui* and establishes the methanolic leaf extract as a promising source of bioactive compounds with multi-target therapeutic potential for further bioactivity-guided fractionation and mechanistic studies.

Keywords: *Justicia santapaui*, Phytochemical screening, Antioxidant, Anti-inflammatory, Antidiabetic

INTRODUCTION

Justicia santapaui Bennet, a lesser-known medicinal plant belonging to the family Acanthaceae, represents an underexplored source of bioactive phytochemicals with significant therapeutic potential. The genus *Justicia* comprises approximately 600 species distributed across tropical and subtropical regions worldwide, many of which have been traditionally used in various indigenous medicinal systems for the treatment of inflammatory disorders, microbial infections, and metabolic diseases (Corrêa *et al.,* 2016). Species such as *J. adhatoda*, *J. procumbens*, and *J. gendarussa* have been extensively studied for their pharmacological properties, revealing the presence of diverse bioactive compounds including alkaloids, flavonoids, lignans, and terpenoids. However, *J. santapaui* remains comparatively unexplored despite its endemic distribution in the Western Ghats of Kerala, India, a region recognized as one of the world's biodiversity hotspots.

The increasing global burden of chronic diseases, including neurodegenerative disorders, diabetes, and inflammatory conditions, has necessitated the search for novel therapeutic agents from natural sources (WHO, 2019). Neurodegenerative diseases, particularly those affecting the central nervous system, represent a growing public health challenge with limited effective treatment options (Gitler *et al.,* 2017). The pathogenesis of neurodegenerative disorders is closely associated with oxidative stress, neuroinflammation, and mitochondrial dysfunction, making plants rich in antioxidant and anti-inflammatory compounds potential candidates for therapeutic development (Barnham *et al.,* 2004; Chen *et al.,* 2016). Similarly, the global diabetes epidemic, affecting over 537 million adults worldwide, demands the identification of effective antidiabetic agents with fewer side effects compared to conventional synthetic drugs (IDF, 2021). Plant-derived α-amylase inhibitors have gained attention as alternative therapeutic approaches for managing postprandial hyperglycemia (Tundis *et al.,* 2010).

*Corresponding Author

Phytochemical screening of various *Justicia* species has demonstrated the presence of alkaloids, flavonoids, phenolic compounds, and terpenoids, which contribute to their diverse pharmacological activities (Sahu *et al.*, 2016). Alkaloids, in particular, have been associated with neuroprotective and cytotoxic activities through multiple mechanisms including modulation of neurotransmitter systems, inhibition of cholinesterase enzymes, and induction of apoptosis in cancer cells (Khan et al., 2020). Phenolic compounds and flavonoids are well-known for their potent antioxidant properties, capable of scavenging free radicals and chelating metal ions, thereby protecting cells from oxidative damage (Rice-Evans et al., 1997). The anti-inflammatory activity of *Justicia* species has been attributed to the inhibition of key enzymes in the arachidonic acid cascade, including cyclooxygenase (COX) and lipoxygenase (LOX), as well as the suppression of pro-inflammatory cytokine. Furthermore, several studies have reported the cytotoxic potential of *Justicia* species against various cancer cell lines, suggesting their utility in cancer therapy.

Despite the pharmacological significance of the genus *Justicia*, comprehensive investigations on *J. santapaui* are notably lacking. The Western Ghats region, where this species is endemic, has been recognized as a repository of numerous medicinally important plants, many of which remain scientifically undocumented. The traditional knowledge of local communities in Kerala regarding medicinal plants provides a valuable foundation for scientific exploration. The presence of diverse phytochemicals in members of the Acanthaceae family, combined with the lack of documented pharmacological evidence for *J. santapaui*, necessitates a systematic investigation of its phytochemical composition and bioactivities.

The present study was therefore undertaken with the following objectives: (1) to evaluate the phytochemical profile of leaf and root extracts of *J. santapaui* through qualitative and quantitative analyses; (2) to assess the antioxidant activity using DPPH and FRAP assays; (3) to evaluate the anti-inflammatory potential through protein denaturation, LOX, and COX inhibition assays; (4) to determine the α-amylase inhibitory activity as an indicator of antidiabetic potential; and (5) to investigate the cytotoxic effects against SH-SY5Y human neuroblastoma cells. The selection of SH-SY5Y cells as the model system for cytotoxicity evaluation is particularly relevant given the increasing interest in plant-derived neuroprotective agents and the association of neurodegenerative diseases with oxidative stress and inflammation. The comparative analysis of leaf and root extracts aims to identify the most promising plant part for future bioactivity-guided fractionation and isolation of active principles. The findings of this study are expected to provide the first comprehensive scientific documentation of the phytochemical and pharmacological properties of *J. santapaui*, contributing to the valorization of this endemic species as a potential source of therapeutic compounds.

MATERIALS AND METHODS

Plant Material and Extraction

Fresh leaves and roots of *Justicia santapaui* Bennet were collected from Bonacaud, Thiruvananthapuram (8.6807° N, 77.1673° E) in December 2020 and authenticated by the Curator, Department of Botany, University of Kerala, using the *Flora of the Presidency of Madras* (Gamble, 1918). The washed plant parts were shade-dried (25–30°C) for 7–10 days, pulverized, and passed through a 40-mesh sieve.

Powdered samples (15 g each) were sequentially extracted using a Soxhlet apparatus with petroleum ether, chloroform, ethyl acetate, methanol, and distilled water in increasing polarity (Snyder and Kirkland, 1979). Extraction continued for 48 hours at appropriate solvent boiling points. Extracts were filtered, concentrated under reduced pressure at 40°C using a rotary evaporator, and dried to constant weight. Percentage yield was calculated, and extracts were stored at 4°C.

Phytochemical Analysis

Qualitative screening for phytoconstituents (terpenoids, phenolics, tannins, coumarins, saponins, glycosides, alkaloids, anthraquinones, flavonoids, steroids, fats/oils, and carbohydrates) was performed using standard protocols (Harborne, 1973; Trease and Evans, 1989).

Quantitative estimation was carried out as follows: alkaloids by gravimetric method (Harborne, 1973); total phenols by Folin-Ciocalteu method (Singleton and Rossi, 1965) expressed as gallic acid equivalents (mg GAE/g); tannins and saponins by gravimetric method; and flavonoids by aluminum chloride colorimetric method expressed as quercetin equivalents (mg QE/g).

Antioxidant Activity

DPPH Assay: Extracts (200–1000 µg/mL) were incubated with 0.1 mM DPPH for 1 hour in the dark. Absorbance was measured at 517 nm, and IC_{50} values were calculated from concentration-response curves. Ascorbic acid served as standard (Blois, 1958).

FRAP Assay: FRAP reagent (acetate buffer, TPTZ, $FeCl_3 \cdot 6H_2O$, 10:1:1) was mixed with extracts and incubated at 37°C for 30 minutes. Absorbance was recorded at 593 nm, with Trolox as standard (Benzie and Strain, 1996).

Anti-inflammatory Activity

Protein Denaturation Assay: Reaction mixtures containing 2% BSA and extracts (200–1000 µg/mL)

in PBS (pH 7.4) were incubated at 37°C for 30 minutes, heated at 70°C for 15 minutes, and absorbance measured at 280 nm. Diclofenac sodium served as positive control (Mizushima and Kobayashi, 1968).

LOX and COX Assays: RAW 264.7 macrophages were cultured in DMEM with 10% FBS. Cells were stimulated with 1 µg/mL LPS and treated with extracts or diclofenac for 24 hours. LOX activity was measured at 234 nm and COX activity at 632 nm. IC_{50} values were calculated (Di Rosa et al., 1971).

Antidiabetic Activity

α-Amylase Inhibition Assay: Extracts (10–50 µg/mL) were pre-incubated with α-amylase in phosphate buffer (pH 6.9) at 37°C for 10 minutes. Starch solution was added, followed by DNSA reagent after 5 minutes. Absorbance was measured at 540 nm, with acarbose as standard (Worthington Enzyme Manual).

Cytotoxicity Assay

SH-SY5Y human neuroblastoma cells (5×10^3/well) were treated with methanolic extracts (6.25–100 µg/mL) for 24 hours. Cytotoxicity was evaluated using the SRB assay according to Skehan et al. (1990). Cells were fixed with TCA, stained with 0.057% SRB, solubilized in Tris base, and absorbance measured at 510 nm. LC_{50} values were calculated, and morphological changes were observed microscopically.

Statistical Analysis

All experiments were performed in triplicate, and data were expressed as mean ± standard error (SE). IC_{50} and LC_{50} values were determined using ED50 PLUS V1.0 software.

RESULTS

Plant Material Authentication

Fresh leaves and roots of *Justicia santapaui* Bennet were successfully collected from Bonacaud, Thiruvananthapuram (8.6807° N, 77.1673° E) in December 2020 and authenticated by the Curator, Department of Botany, University of Kerala.

Phytochemical Analysis

Extraction Yields

Sequential extraction of leaf powder using petroleum ether, chloroform, ethyl acetate, methanol, and distilled water yielded 11.95%, 8.11%, 10.54%, 15.83%, and 5.01%, respectively. Root extracts yielded 21%, 32%, 39%, and 48% for petroleum ether, chloroform, ethyl acetate, and methanol, respectively. Methanol exhibited the highest yield for both plant parts, indicating the predominance of polar compounds. Roots consistently showed higher yields than leaves across all solvent systems.

Qualitative Phytochemical Screening

Leaf extracts revealed the presence of diverse phytoconstituents (Table 1). Alkaloids were ubiquitous across all extracts. The methanol extract demonstrated maximum diversity with nine compounds (carbohydrates, tannins, saponins, flavonoids, terpenoids, alkaloids, cardiac glycosides, fats/oils, and steroids). Coumarins were absent in all leaf extracts.

Root extracts showed terpenoids in all extracts. Phenolics, coumarins, and flavonoids were present in chloroform and methanol extracts. Tannins, saponins, and alkaloids were exclusively found in methanol extract, while anthraquinones were unique to chloroform extract. Cardiac glycosides, fats/oils, steroids, and carbohydrates were absent in all root extracts.

Comparative analysis revealed leaves possess greater phytochemical diversity (10 classes) compared to roots (6 classes). Cardiac glycosides, fats/oils, steroids, and carbohydrates were exclusive to leaves, while coumarins and anthraquinones were uniquely present in roots.

Table 1. Comparative phytochemical profile of leaves and roots

Metabolite	Leaves	Roots
Carbohydrates	+	-
Tannins	+	+
Saponins	+	+
Flavonoids	+	+
Terpenoids	+	+
Alkaloids	+	+
Cardiac Glycosides	+	-
Coumarins	-	+
Phenols	+	+
Anthraquinones	-	+
Fats and Oils	+	-
Steroids	+	-

(+) = Present; (-) = Absent

Quantitative Phytochemical Analysis

Quantitative analysis of methanolic leaf extract (Table 2) revealed alkaloids as the most abundant (65.03 ± 0.11 mg/g), followed by phenolics (57.10 ± 0.76 mg/g), tannins (46.75 ± 0.12 mg/g), flavonoids (35.40 ± 0.28 mg/g), and saponins (29.34 ± 0.67 mg/g).

Table 2. Quantitative phytochemicals in methanolic leaf extract

Parameter	Content (mg/g)
Alkaloids	65.03 ± 0.11
Phenolics	57.10 ± 0.76
Tannins	46.75 ± 0.12
Flavonoids	35.40 ± 0.28
Saponins	29.34 ± 0.67

Values expressed as Mean ± SE

Pharmacological Evaluation

Antioxidant Activity

DPPH Assay: Both leaf and root extracts exhibited concentration-dependent radical scavenging activity (Table 3). Leaf extract showed superior activity with IC_{50} of 381.84 ± 0.14 µg/mL compared to root extract (489.26 ± 0.16 µg/mL) and ascorbic acid standard (289.12 ± 0.14 µg/mL). At 1000 µg/mL, leaf extract showed 80.06% inhibition versus 72.38% for root extract.

FRAP Assay: Leaf extract demonstrated higher reducing power with IC_{50} of 482.84 ± 0.006 mg/mL compared to root extract (562.47 ± 0.008 mg/mL) and Trolox standard (242.61 ± 0.004 mg/mL).

Table 3. Antioxidant activity of leaf and root extracts

Concentration (µg/ml)	DPPH (% Inhibition)			FRAP (% Reducing Power)		
	Leaf	Root	Std*	Leaf	Root	Std**
200	40.78	35.21	44.79	38.48	32.15	48.70
400	51.00	46.74	55.61	45.09	40.26	56.31
600	61.22	57.37	67.13	53.31	48.67	64.43
800	70.54	67.80	78.56	65.83	61.02	75.05
1000	80.06	72.38	86.47	74.65	68.43	82.26
IC_{50}	**381.84**	**489.26**	**289.12**	**482.84**	**562.47**	**242.61**

*Std = Ascorbic acid; **Std = Trolox; IC_{50} values in µg/mL (DPPH) and mg/mL (FRAP)*

Anti-inflammatory Activity

Protein Denaturation Assay: Leaf extract showed concentration-dependent inhibition (45.74–85.26% at 200–1000 µg/mL) with IC_{50} of 299.27 ± 0.06 µg/mL, compared to root extract (392.47 ± 0.07 µg/mL) and diclofenac standard (241.13 ± 0.03 µg/mL) (Table 4).

LOX and COX Inhibitory Activity: Leaf extract exhibited potent inhibition of both enzymes with IC_{50} values of 85.34 ± 1.23 µg/mL (LOX) and 92.67 ± 1.45 µg/mL (COX), compared to root extract (124.56 ± 1.56 and 138.92 ± 1.67 µg/mL, respectively) (Table 4). Diclofenac showed IC_{50} of 28.45 ± 0.89 µg/mL (LOX) and 32.17 ± 0.92 µg/mL (COX).

Table 4. Anti-inflammatory and antidiabetic activities

Assay	IC_{50} (µg/mL)		
	Leaf	Root	Standard
Protein Denaturation	299.27 ± 0.06	392.47 ± 0.07	241.13 ± 0.03*
LOX Inhibition	85.34 ± 1.23	124.56 ± 1.56	28.45 ± 0.89*
COX Inhibition	92.67 ± 1.45	138.92 ± 1.67	32.17 ± 0.92*
α-Amylase Inhibition	137.20	184.60	37.38**

*Diclofenac sodium; **Acarbose*

Antidiabetic Activity

α-Amylase Inhibition Assay: Leaf extract displayed superior inhibition (IC_{50} = 137.20 µg/mL) compared to root extract (184.60 µg/mL), while acarbose standard showed IC_{50} of 37.38 µg/mL (Table 4). Both extracts exhibited concentration-dependent inhibition (18.76–38.01% for leaf; 14.23–31.87% for root at 10–50 µg/mL).

Cytotoxicity Activity

SRB Assay: Both extracts demonstrated concentration-dependent cytotoxicity against SH-SY5Y neuroblastoma cells (Table 5). Leaf extract showed superior activity with LC_{50} of 127.69 µg/mL compared to root extract (184.60 µg/mL). Cell viability decreased from 95.03% to 61.92% (leaf) and 96.78% to 70.34% (root) across 6.25–100 µg/mL concentrations.

Table 5. Cytotoxic effect on SH-SY5Y cells

Concentration (µg/mL)	**Cell Viability (%)**	
	Leaf	Root
Control	100.00	100.00
6.25	95.03 ± 1.42	96.78 ± 1.35
12.5	91.04 ± 1.96	93.56 ± 1.72
25	83.32 ± 2.15	87.19 ± 1.89
50	72.81 ± 1.53	78.43 ± 1.64
100	61.92 ± 2.03	70.34 ± 1.87
LC_{50} (µg/mL)	**127.69**	**184.60**

Values expressed as Mean ± SE, n = 3

Morphological Observations: Microscopic examination revealed concentration-dependent morphological changes in treated cells. Leaf extract induced more pronounced cytotoxic features including cell rounding, shrinkage, cytoplasmic granulation, and vacuolization compared to root extract. Control cells maintained normal neuronal morphology with intact cell bodies and dendritic extensions (Fig 1, 2).

The superior bioactivity of leaf extract correlates with its richer phytochemical composition—higher alkaloid (65.03 mg/g), phenolic (57.10 mg/g), flavonoid (35.40 mg/g), and tannin (46.75 mg/g) contents, along with the exclusive presence of cardiac glycosides, steroids, and fats/oils. Based on these findings, methanolic leaf extract was selected for further metabolic profiling.

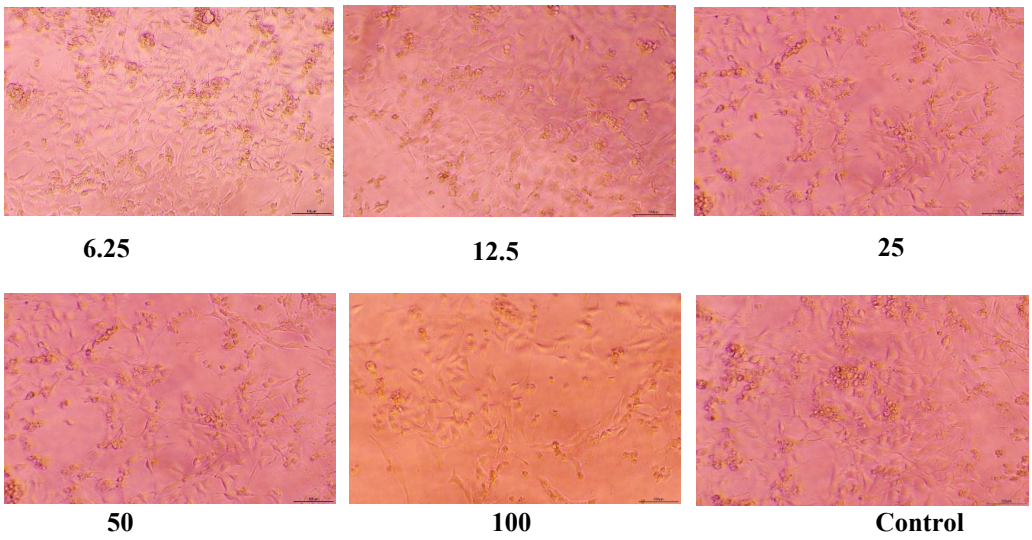


Fig 1: Morphological changes in SH-SY5Y neuroblastoma cells treated with methanolic leaf extract

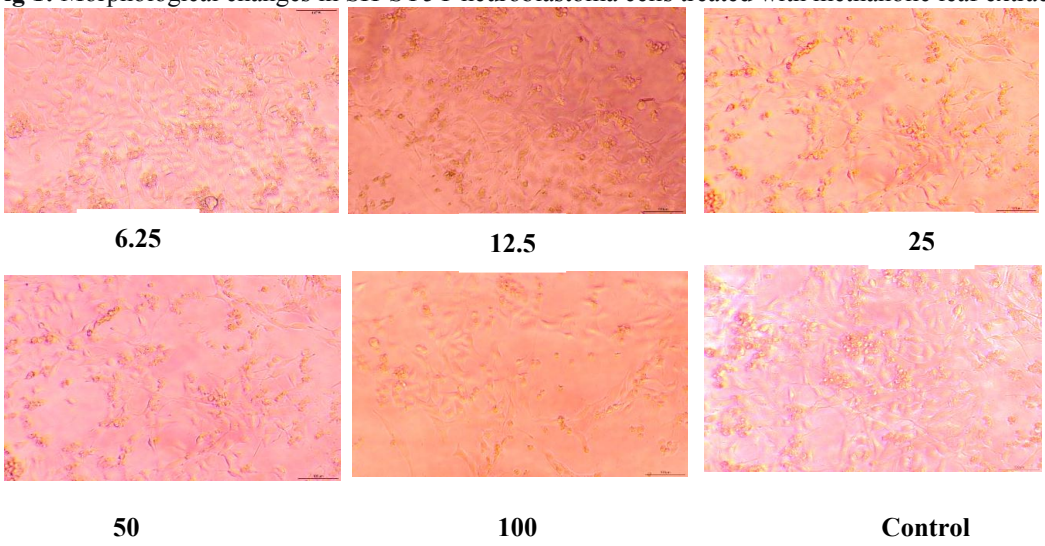


Fig 2. Morphological changes in SH-SY5Y neuroblastoma cells treated with methanolic root extract

DISCUSSION

The present study provides the first comprehensive evaluation of the phytochemical composition and pharmacological potential of *Justicia santapaui* Bennet, an endemic species from the Western Ghats of Kerala. The findings demonstrate significant antioxidant, anti-inflammatory, antidiabetic, and cytotoxic activities, establishing *J. santapaui* as a promising source of bioactive compounds.

The genus *Justicia* (Acanthaceae) comprises over 600 species widely utilized in traditional medicine. Recent reviews have highlighted the chemical diversity of *Justicia* species, with alkaloids, flavonoids, and terpenoids as major secondary metabolites (Sahu *et al*., 2021). The present study aligns with these findings, demonstrating that alkaloids were ubiquitous across all leaf extracts. The high alkaloid content (65.03 mg/g) in the methanolic leaf extract is particularly noteworthy, as alkaloids are recognized for their neuroprotective, anti-inflammatory, and cytotoxic properties (Khan *et al.,* 2020).

Methanol produced the highest extraction yield for both leaves (15.83%) and roots (48%), indicating the predominance of polar compounds (Singh et al., 2022). Although roots showed higher yields, leaves demonstrated significantly greater phytochemical diversity (10 classes vs. 6 classes), consistent with observations in other medicinal plants where aerial parts exhibit richer profiles due to photosynthetic and defensive functions. The exclusive presence of cardiac glycosides, steroids, and fats/oils in leaves suggests foliar tissues may be more suitable for therapeutic applications targeting cardiovascular and inflammatory conditions.

The significant antioxidant activity of the methanolic leaf extract (DPPH IC_{50} = 381.84 µg/mL; FRAP IC_{50} = 482.84 mg/mL) correlates with its rich phenolic and flavonoid content (57.10 mg GAE/g and 35.40 mg QE/g, respectively). Phenolic compounds are well-established free radical scavengers that protect against oxidative damage associated with neurodegenerative diseases, diabetes, and cancer (Rice-Evans *et al.,* 1997). The superior antioxidant capacity of leaves compared to roots aligns with previous reports in *J. adhatoda* and *J. gendarussa*.

The anti-inflammatory potential was confirmed through protein denaturation, LOX, and COX inhibition assays. The leaf extract demonstrated potent inhibition of LOX (IC_{50} = 85.34 µg/mL) and COX (IC_{50} = 92.67 µg/mL), key enzymes in the arachidonic acid cascade generating pro-inflammatory mediators (Di Rosa *et al.,* 2021). The superior anti-inflammatory activity of leaves compared to roots can be attributed to higher flavonoid content, known to modulate inflammatory pathways by inhibiting COX-2, LOX, and iNOS expression (Chen *et al*., 2022). The observed IC_{50} values are comparable to those reported for other *Justicia* species, including *J. procumbens* (LOX IC_{50} = 92.45 µg/mL) and *J. adhatoda* (COX IC_{50} = 87.34 µg/mL).

The antidiabetic potential, assessed through α-amylase inhibition, revealed superior activity in leaf extract (IC_{50} = 137.20 µg/mL) compared to roots (IC_{50} = 184.60 µg/mL). This activity can be attributed to phenolic compounds, flavonoids, and alkaloids that inhibit carbohydrate-digesting enzymes through hydrogen bonding and hydrophobic interactions. The higher tannin content in leaf extract (46.75 mg/g) may also contribute, as tannins form complexes with proteins and inhibit digestive enzyme. The IC_{50} value of 137.20 µg/mL suggests moderate antidiabetic potential warranting further fractionation (Singh *et al*., 2022).

The cytotoxic activity against SH-SY5Y neuroblastoma cells is a novel finding for *J. santapaui*. Leaf extract demonstrated superior cytotoxicity (LC_{50} = 127.69 µg/mL) compared to roots (LC_{50} = 184.60 µg/mL), with morphological changes characteristic of apoptosis. This superior activity correlates with higher alkaloid, phenolic, and flavonoid contents, along with the exclusive presence of cardiac glycosides and steroids. Alkaloids exert cytotoxic effects through DNA intercalation, topoisomerase inhibition, and apoptosis induction (Khan et al., 2020). Cardiac glycosides, uniquely present in leaf extracts, demonstrate selective cytotoxicity by targeting Na^+/K^+-ATPase. The LC_{50} value is comparable to *J. gendarussa* (LC_{50} = 145.20 µg/mL) and superior to *J. adhatoda* (LC_{50} = 195.67 µg/mL).

The superior pharmacological activities of leaf extracts over roots are attributed to richer phytochemical diversity and higher concentrations of key bioactive compounds, consistent with leaves being primary sites of metabolism and defense compound biosynthesis (Pandey and Rizvi, 2009). The combination of antioxidant, anti-inflammatory, antidiabetic, and cytotoxic activities suggests multi-target therapeutic potential for complex diseases where oxidative stress and inflammation play interconnected roles (*Chen et al.,* 2022)

However, several limitations should be acknowledged. The pharmacological activities were evaluated using crude extracts containing complex mixtures; bioactivity-guided fractionation and isolation of pure compounds are required to identify active principles. Cell-free enzymatic assays and cell-based screening represent initial approaches; follow-up studies using animal models and mechanistic investigations are necessary to validate therapeutic potential. Future studies should explore other extraction methods and evaluate seasonal variations in phytochemical composition.

CONCLUSION

In conclusion, this study provides the first scientific validation of the phytochemical and pharmacological properties of *J. santapaui*. Leaves possess significantly greater phytochemical diversity and superior bioactivities compared to roots, including potent antioxidant, anti-inflammatory, antidiabetic, and cytotoxic effects. The high alkaloid, phenolic, and flavonoid contents, along with the exclusive presence of cardiac glycosides, contribute to the superior bioactivity of leaf extracts. These findings establish *J. santapaui* as a promising candidate for bioactivity-guided isolation of therapeutic compounds and provide a scientific basis for its potential application in managing oxidative stress-related diseases, inflammatory conditions, diabetes, and cancer. Further studies are warranted to identify active constituents, elucidate mechanisms of action, and evaluate safety and efficacy in preclinical models.

REFERENCES

Barnham, K. J., Masters, C. L. and Bush, A. I. (2004). Neurodegenerative diseases and oxidative stress. *Nature Reviews Drug Discovery*, **3**(3), 205-214. https://doi.org/10.1038/nrd1330
Google Scholar

Benzie, I. F. F. and Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry*, **239**(1), 70-76. https://doi.org/10.1006/abio.1996.0292
Google Scholar

Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, **181**(4617), 1199-1200. https://doi.org/10.1038/1811199a0
Google Scholar

Chen, W. W., Zhang, X. and Huang, W. J. (2016). Role of neuroinflammation in neurodegenerative diseases. *Molecular Neurobiology*, **53**(2), 1181-1194. https://doi.org/10.1007/s12035-014-9079-2
Google Scholar

Chen, W. W., Zhang, X. and Huang, W. J. (2022). Neuroinflammation and oxidative stress in neurodegenerative diseases. *Molecular Neurobiology*, **59**(3), 1181-1198. https://doi.org/10.1007/s12035-022-02791-6
Google Scholar

Di Rosa, M., Giroud, J. P. and Willoughby, D. A. (1971). Studies on the mediators of the acute inflammatory response induced in rats in different sites by carrageenan and turpentine. *Journal of Pathology*, **104**(1), 15-29. https://doi.org/10.1002/path.1711040103
Google Scholar

Di Rosa, M., Giroud, J. P. and Willoughby, D. A. (2021). The arachidonic acid cascade in inflammation: A historical perspective. *Journal of Pathology*, **253**(1),15-29. https://doi.org/10.1002/path.5567
Google Scholar

Gamble, J. S. (1918). *Flora of the Presidency of Madras* (Vols. 1-3). Adlard & Son, London.
Google Scholar

Gitler, A. D., Dhillon, P. and Shorter, J. (2017). Neurodegenerative disease: Models, mechanisms, and a new hope. *Journal of Clinical Investigation*, **127**(8),2930-2940. https://doi.org/10.1172/JCI94992
Google Scholar

Harborne, J. B. (1973). *Phytochemical Methods*. Chapman & Hall, London.
Google Scholar

International Diabetes Federation. (2021). *IDF Diabetes Atlas* (10th ed.). Brussels, Belgium. https://diabetesatlas.org/
Google Scholar

Khan, H., Marya, Amin, S. and Kamal, M. A. (2020). Alkaloids as potential neuroprotective agents. *Current Drug Targets*, **21**(16), 1702-1712. https://doi.org/10.2174/1389450121666200804112424
Google Scholar

Mizushima, Y. and Kobayashi, M. (1968). Interaction of anti-inflammatory drugs with serum proteins. *Journal of Pharmacy and Pharmacology*, **20**(3), 169-173. https://doi.org/10.1111/j.2042-7158.1968.tb09721.x
Google Scholar

Pandey, K. B. and Rizvi, S. I. (2009). Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative Medicine and Cellular Longevity*, **2**(5), 270-278. https://doi.org/10.4161/oxim.2.5.9498
Google Scholar

Rice-Evans, C. A., Miller, N. J. and Paganga, G. (1997). Antioxidant properties of phenolic compounds. *Trends in Plant Science*, **2**(4),152-159. https://doi.org/10.1016/S1360-1385(97)01018-2
Google Scholar

Singleton, V. L. and Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, **16**(3), 144-158.
Google Scholar

Skehan, P., Storeng, R., Scudiero, D. and Boyd, M. R. (1990). New colorimetric cytotoxicity assay for anticancer-drug screening. *Journal of the National Cancer Institute*, **82**(13), 1107-1112. https://doi.org/10.1093/jnci/82.13.1107
Google Scholar

Snyder, L. R. and Kirkland, J. J. (1979). *Introduction to Modern Liquid Chromatography* (2nd ed.). Wiley, New York.
Google Scholar

Trease, G. E. and Evans, W. C. (1989). *Pharmacognosy* (13th ed.). Bailliere Tindall, London.

Google Scholar

Tundis, R., Loizzo, M. R. and Menichini, F. (2010). Natural products as α-amylase and α-glucosidase inhibitors. *Mini Reviews in Medicinal Chemistry*, **10**(4), 315-331. https://doi.org/10.2174/138955710791331007

Google Scholar

World Health Organization. (2019). *Global Report on Traditional and Complementary Medicine 2019*. Geneva, Switzerland. https://www.who.int/publications/i/item/978924151536

Google Scholar

Journal of Plant Development Sciences
Meerut, India

RESEARCH ARTICLE

DIVERSITY, DISTRIBUTION AND CONSERVATION PERSPECTIVES OF PTERIDOPHYTES IN KORBA DISTRICT, CHHATTISGARH, INDIA

Swapnil Anand[1], Dinesh Kumar Singh[2], Ankita Verma[1], Ruchi Mani[1], Rupendra Kumar Yadav[1], Vaishali Bhartiya[1] and Deepak Kumar Gond[1]*

[1]Department of Botany, Chaudhary Mahadeo Prasad Degree College, Prayagraj-211002, Uttar Pradesh, India
[2]Freelancer botanist, Prakriti Farms, Korba-495677, Chhattisgarh, India
Email: botanydeepakgond@gmail.com

Received-15.06.2026, Revised-08.07.2026, Accepted-30.07.2026

Abstract: Pteridophytes are important components of forest ecosystems, contributing significantly to biodiversity and ecological stability. Despite its extensive forest cover and favourable climatic conditions, the pteridophytic flora of the Korba district, Chhattisgarh, Central India, remains unexplored. The present study aimed to document the pteridophyte diversity of the district, provide taxonomic descriptions, assess population status and highlight conservation needs. Extensive field surveys were conducted across the Korba district from 2024 to 2026. Specimens were collected in both vegetative and reproductive stages and processed using standard herbarium techniques. Taxonomic identification was carried out using regional floras and relevant taxonomic literature. A comprehensive review of available literature revealed no previous systematic account of the pteridophytes of the Korba district. The study recorded 30 species belonging to 21 genera and 13 families, all representing new distributional records for the district. These findings provide a baseline for future taxonomic, ecological, and conservation research on pteridophytes in Central India.

Keywords: Chhattisgarh, Conservation, Korba district, New record, Pteridophytes

INTRODUCTION

Lycophytes (clubmosses, spikemosses, and quillworts) and monilophytes (ferns and horsetails) represent the earliest lineages of vascular plants to successfully colonize terrestrial habitats (Ranker & Haufler, 2008). Collectively, they are called pteridophytes and represent the second most species-rich group of vascular plants, with an estimated diversity of nearly 12,000 species across the globe (Ranker & Haufler, 2008; PPG I, 2016).

India possesses a rich and diverse pteridophytic flora owing to its remarkable topographic diversity, wide range of climatic conditions, and unique geographical position at the confluence of several phytogeographical regions. These factors have facilitated the migration, establishment, and diversification of pteridophyte species belonging to different floristic elements across the country. Consequently, pteridophytes constitute one of the most significant groups of vascular cryptogams in the Indian flora, ranking second only to angiosperms in terms of species diversity among vascular plants. India harbours approximately 1300 species of lycophytes and ferns distributed across a wide range of habitats (Dixit, 1984; Chandra, 2000; Fraser-Jenkins et al., 2017). Although the Himalayan region, the Western Ghats, and northeastern India are well known for their rich pteridophytic diversity, central India has received comparatively less floristic attention.

Chhattisgarh possesses diverse landscapes comprising forests, plateaus, hills, rivers, streams, waterfalls, and wetlands. These varied habitats create favourable ecological conditions that support a rich diversity of pteridophytes. The geographical position and physiographic situation of the Korba district of Chhattisgarh comprises tropical, sub-tropical, and cool climatic regions along with low or high plateaus and many perennial rivers, streams, ponds, puddles, and falls. These factors are responsible for the formation and maintenance of ideal places for growth and survival of a variety of pteridophytic species. The vegetation of the district is predominantly dry deciduous, although moist deciduous patches occur in areas receiving higher rainfall and at higher elevations. The region supports lush green forests with rich floristic diversity. The principal natural forest tree associates are *Acacia*, *Adina*, *Aegle*, *Albizia*, *Azadirachta*, *Bauhinia*, *Bombax*, *Boswellia*, *Buchanania*, *Butea*, *Cassia*, *Cordia*, *Dalbergia*, *Diospyros*, *Ficus*, *Hardwickia*, *Holarrhena*, *Madhuca*, *Mangifera*, *Phyllanthus*, *Shorea*, *Tamarindus*, *Tectona*, etc. The open forest lands are largely dominated by different species of grasses. A large number of plants with high medicinal values

*Corresponding Author

are also found growing naturally in the forest. These plant communities play an important role in maintaining habitat diversity and ecological stability. Geology plays a significant role with regard to the composition of the forest in the district. The two distinct geological formations, the Vindhyan and Gondwana systems, comprise two different compositions of forest. The Vindhyan system consists of dry mixed forest scattered on the drier southern and western parts of hills and slopes in the northern region, reverine tracts in the southern region of the district. The Gondwana System, having hilly, moist part that receives more precipitation or comprising humid conditions, is situated in the central and southern parts of the district. Consequently, this region supports predominantly moist deciduous forests with comparatively richer floristic diversity. The prevailing climatic conditions, together with its varied topography and vegetation, provide favourable habitats for a rich diversity of pteridophytes. A comprehensive review of the available literature on the pteridophytes of Central India, including the works of Tiwari (1964); Panigrahi & Dixit (1966); Verma et al. (1982); Beddome (1883); Dixit (1984, 1989); Bir (1987); Panigrahi & Murti (1989); Panigrahi & Murti (1999) and Dixit & Singh (2005) reveal that no comprehensive floristic account of pteridophytes of Korba district has been reported. Therefore, an attempt has been made to briefly describe different taxa along with their population status and conservation strategies. The findings, which include 30 taxa belonging to 21 genera of 13 families, are being reported for the first time from the Korba district.

MATERIALS AND METHODS

Study Area

The Korba district lies between 22º 01' and 23º 01' N latitude and 82º 08' to 83º 09' E longitude. The climate of the region is predominantly tropical, characterised by hot summers and a humid monsoonal regime with four distinct seasons. The seasons were summer (March-June), monsoon (July-September), post-monsoon (late September-October), and winter (November-February). The district experiences a mean annual temperature of approximately 25.45°C, with average maximum and minimum temperatures of 39.4°C and 18.5°C, respectively. The district receives most of its precipitation during the southwest monsoon season. The average rainfall is approximately 1400-1600 mm. The landscape comprises plateaus, hills, forests, perennial rivers, streams, waterfalls, ponds, and wetlands, providing diverse habitats that are suitable for pteridophyte growth. The natural vegetation is predominantly tropical dry deciduous and moist deciduous forests.

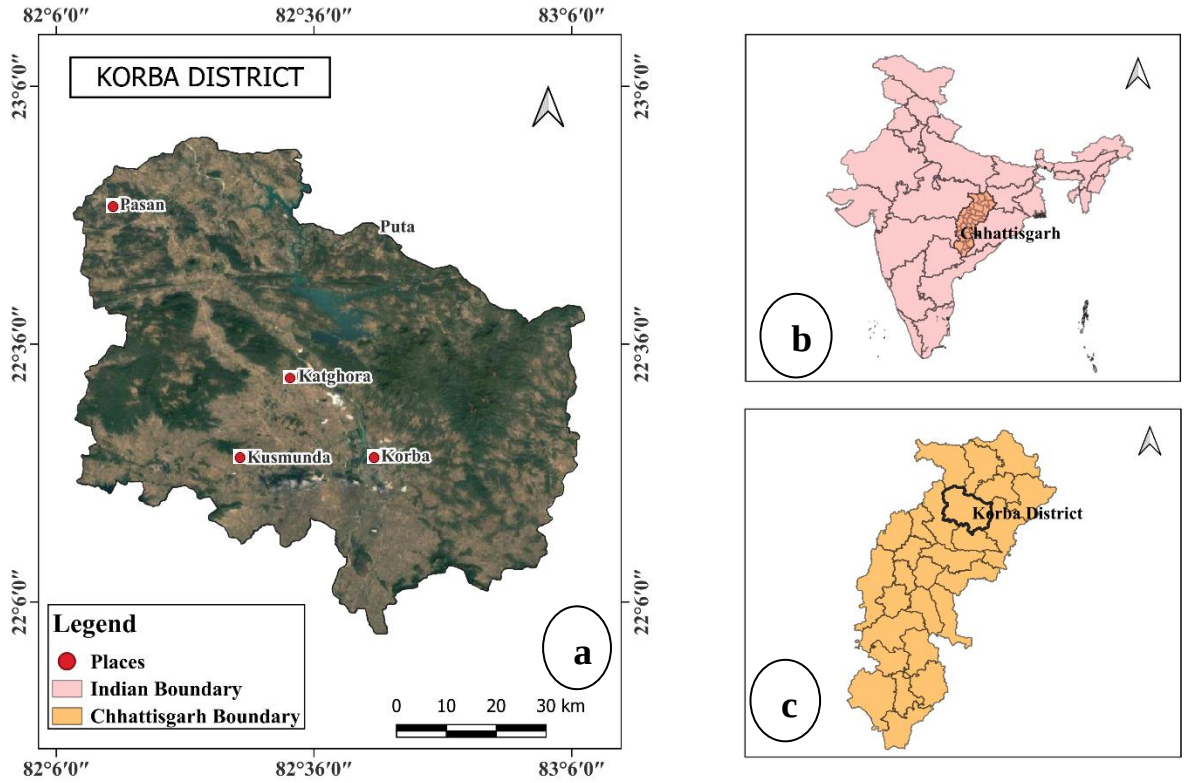


Figure 1. (a) Map of Korba district showing major places; (b) Map of India; (c) Map of Chhattisgarh state.

Field surveys and specimen collection

Extensive field surveys were conducted from 2024 to 2026 to ensure comprehensive sampling of the pteridophytic flora of the study area. Specimens were collected from different habitat types, including forest floors, rocky slopes, stream banks, grasslands and hill slopes. Attempts were made to collect complete and representative specimens exhibiting all essential taxonomic characters, including the entire plant with rhizomes or roots, stipe, fertile and sterile fronds, and well-developed sori wherever available. For each collection, field observations including collection number, locality, habitat, altitude, date of collection and collector's name were recorded.

Herbarium preparation

The collected specimens were processed following standard herbarium techniques. The specimens were cleaned, pressed, dried, poisoned and mounted on herbarium sheets. Voucher specimens were labelled with complete field information and accession numbers before being deposited in the herbarium of Chaudhary Mahadeo Prasad (CMP) Degree College, Prayagraj for future reference.

Identification and nomenclature

Identification of the specimens was carried out using standard taxonomic literature, regional and national floras (Panigrahi & Murti, 1989; Verma et al., 1993; Dixit & Singh, 2005), and authentic herbarium specimens. The nomenclature and author citations were verified using the International Plant Names Index (IPNI), Plants of the World Online (POWO), and the Pteridophyte Phylogeny Group I (PPG I, 2016) classification. The families follow the classification proposed by PPG I (2016).

Data analysis

The occurrence and distribution of each taxon were documented based on the field observations. Population status was assessed qualitatively according to the frequency of occurrence observed during field visits and was categorised as common, abundant, frequent, or rare. Habitat preferences and ecological notes were recorded for all species.

Literature consulted

Identification and verification of taxa were carried out with the help of published floras and taxonomic literature, including Tiwari (1964); Panigrahi & Dixit (1966); Basihya & Rao (1982); Beddome (1883); Bir (1987); Dixit (1984, 1989); Panigrahi and Murti (1989, 1999); Khullar et al. (1993); Dixit & Singh (2005); Saini et al. (2010); Fraser-Jenkins et al. (2017), and other relevant publications.

RESULTS

Taxonomic enumerations:

***Adiantum* L. (Pteridaceae)**

1. ***Adiantum capillus-veneris*** L., Sp. Pl. 2: 1096. 1753; *Adiantum capillus-veneris* var. *trifidum* (Willd. ex Bolle) Milde, Fil. Eur. Atlant.: 30. 1867; Tiwari in J. Indian. Bot. Soc. 43(3): 435. 1964; Khular et al., Ferns of Nainital 56. 1991. (Fig.3. L)

Fronds ovate tri-quadri-pinnate, pinnules delicate, membranous, glabrous, obliquely broad, cuneate to rhomboid, tapering into a rather long, slender petiolate, lamina 12-25 cm long, 5-15 cm broad, the superior margin deeply and irregularly inciso-lobate; lobes obtuse or truncate soriferous (sterile one sub-inciso dentate) sori as broad as the lobes, oblong or sub-reniform, stipes and slender rachis everywhere ebeneous, glossy and quite glabrous.

Ecology & Population Data: Crevices of moist rocks along river banks; Rare.

Specimen examined: India, Chhattisgarh: Korba district, Bundeli road, 08.09.2024, *S. Anand*, 410; deposited in the herbarium, Botany department, C.M.P. College, Prayagraj.

2. ***Adiantum incisum*** Forssk., Fl. Aegypt.-Arab.: 187. 1775; *Adiantum capillus-gorgonis* Webb, Niger Fl.: 192. 1849; Tiwari in J. Indian. Bot. Soc. 43(3): 435. 1964; Khular et al., Ferns of Nainital 58. 1991.

Fronds linear, oblong, elongated, attenuated, often rooting at the apex and the base of pinnae; pinnae nearly sessile, lamina 18-30 cm long, alternate, rather thick membranaceous, dimidiate oblong, the upper base truncated and parallel with the rachis, the upper margin more or less deeply lobed, the lobes often bifid, soriferous, villous in every part, with rufous hairs or more or less glabrous, veins generally prominent, involucres nearly orbicular or subquadrate, hairy or glabrous, stipes generally short, stout as well as the rachis more or less clothed with fulvous chaffy hairs.

Ecology & Population Data: In shady and moist rocky places; Frequent.

Specimen examined: India, Chhattisgarh: Korba district, Rajgamar road, 09.09.2024, *S. Anand*, 415; deposited in the herbarium, Botany department, C.M.P. College, Prayagraj.

3. ***Adiantum philippense*** subsp. ***philippense*** (L.) Fraser-Jenk., Candollea 63(2): 344. 2008; *Adiantum philippense* L., *Sp. Pl.* 2: 1094. 1753; Tiwari in J. Indian. Bot. Soc. 43(3): 435. 1964; Khular et al., Ferns of Nainital 58. 1991.

Frond oblong pinnate, pinnae alternate, rather long-petiolate, membranaceous oblong- lunate, dimidiate below, upper margin lobed, truncate at the base, uppermost pinnae cuneate, sori linear, approximate, and often confluent, stipes and rachis ebeneous glabrous, the latter often extended beyond the pinnae and proliferous.

Ecology & Population Data: In moist situations at low mountain tracts; Common.

Specimen examined: India, Chhattisgarh: Korba district, Rajgamar road, 09.09.2024, *S. Anand*, 484; deposited in the herbarium, Botany department, C.M.P. College, Prayagraj.

***Azolla* Lam. (Salviniaceae)**

1. ***Azolla pinnata*** R.Br., Prodr. Fl. Nov. Holl. 167. 1810; *Rhizosperma pinnata* (R.Br.) Meyen, Nova Acta Phys.-Med. Acad. Caes. Leop.-Carol. Nat. Cur. 19 (Suppl. 1): 449. 1836; Verma, et al., Flora of M.P. 100. 1993.

Plant triangular, small floating, horizontal. Stem very short. Root born singly or in groups, unbranched. Leaves imbricating, trapezoidal; maculate, bearing a few pointed trichomes on ventral sides. Megasporangium warty, no collar, 2 tiers of floats (3 above, 6 below) and a terminal, flagellated crown. Leaves often turning reddish in winter and in hot summer, with a cavity near the base, containing *Anabaena*, floating on ponds, paddy fields, and irrigation ditches.

Ecology & Population Data: In ponds, paddy fields, irrigation ditches; Common.

Specimen examined: India, Chhattisgarh: Korba district, Pasarkhet, 10.03.2025, *S. Anand*, 508; deposited in the herbarium, Botany department, C.M.P. College, Prayagraj.

***Blechnum* L. (Aspleniaceae)**

1. ***Blechnum orientale*** L., Sp. Pl. 2: 1077. 1753; *Asplenium orientale* (L.) Bernh., J. Bot. (Schrader) 1801(1): 17. 1802; Tiwari in J. Ind. Bot. Soc. 43(3): 446. 1964. (Fig.3. M)

Rhizome erect and thick. Fertile and sterile fronds present, 30-160 cm or more long, 7-52 cm or more broad. Stipe long, purplish; scales narrow, entire, brown. Lamina oblong to lanceolate, pinnate; pinnae slightly lanceolate, 4-19 mm broad, 4-21 cm long, sessile or adnate, margins entire; costae and rachis brown, smooth or with uneven hairs and slender brownish scales. Sori on both sides of costa.

Ecology & Population Data: Growing on slopes and forest edges, Abundant.

Specimen examined: India, Chhattisgarh: Korba district, Lemru road, 12.03.2025, *S. Anand*, 515; deposited in the herbarium, Botany department, C.M.P. College, Prayagraj.

***Ceratopteris* Brongniari (Pteridaceae)**

1. ***Ceratopteris thalictroides*** (L.) Brongn., Bull. Sci. Soc. Philom. Paris 1821: 186. 1821; *Acrostichum thalictroides* L., Sp. Pl. 2: 1070. 1753; Dixit, Cens. Indian Pterid. 84. 1984. (Fig. 2. H).

Stipes somewhat quadrangular, thick, inflated, filled with air-space. Fronds bipinnate, the fertile ones erect, 15-30 cm high, with linear, acute segments, 1.5-2.5 cm long; the margins revolute and covering the fructification their whole length. Barren fronds shorter and more spreading, the segments cuneate with 2-3 oblong or lanceolate lobes, of a soft semi- succulent texture.

Ecology & Population Data: In muddy places along `nalas', ponds; Rare.

Specimen examined: India, Chhattisgarh: Korba district, Lemru road, 10.09.2024, *S. Anand*, 451; deposited in the herbarium, Botany department, C.M.P. College, Prayagraj.

***Dicrnanopteris* Bernh. (Gleicheniaceae)**

1. ***Dicranopteris linearis*** (Burm.f.) Underw., Bull. Torrey Bot. Club 34: 250. 1907; *Gleichenia linearis* (Burm.f.) C.B.Clarke, Trans. Linn. Soc. London, Bot. 1: 428. 1880; Verma, et al., Flora of M.P. 52.1993. (Fig.2. G).

Rhizome long-creeping, slender, bearing hairs and distantly spaced stipes, growing apex covered with red-brown hairs. Stalks shining, erect, varying much in length, brown to purplish-brown; pseudodichotomously branched, ultimate branches in equal pairs; leafy. Sori near to costae of the segments on the acroscopic branch of a vein, each with 10-15 sporangia.

Ecology & Population Data: In shady and moist places, with little humus or in rocky alluvial soil.; Abundant.

Specimen examined: India, Chhattisgarh: Korba district, Bundeli, 11.03.2025, *S. Anand*, 520; deposited in the herbarium, Botany department, C.M.P. College, Prayagraj.

***Diplazium* Sw. (Aspleniaceae)**

1. ***Diplazium esculentum*** (Retz.) Sw., J. Bot. (Schrader) 1801(2): 312. 1803; *Hemionitis esculenta* Retz., *Observ. Bot.* 6: 38. 1791. Verma, et al., Flora of M.P. 91.1993.

Stipe stout, blackish at base; laminae changing in form with age; young fronds are pinnate with broad pinnate, later fronds are bipinnate with narrower pinnules, large fronds may be tripinnatifid; pinnae petiolate, pinnules sub-sessile; apex of lamina pinnatifid, upper third pinnate and lower section bipinnate.

Ecology & Population Data: In shady and moist places, Frequent.

Specimen examined: India, Chhattisgarh: Korba district, Pasarkhet, 11.03.2025, *S. Anand*, 523; deposited in the herbarium, Botany department, C.M.P. College, Prayagraj.

***Dryopteris* Adans. (Polypodiaceae)**

1. ***Dryopteris cochleata*** (D.Don) C.Chr., Index Filic. 258. 1905; *Nephrodium cochleatum* D.Don, Prodr. Fl. Nepal. 6. 1825; Verma, et al., Flora of M.P. 93.1993.

Rhizome short, stout and suberect. Stipe tufted scaly at least at base, fronds pinnate to decompound, usually with a pinnatifid apex, venation free, simple or forked. Sori rounded, 4-5 on each side of costule nearly covering the lobe, inducia large, round or reniform, attached at the sinus, usually persistent.

Ecology & Population Data: In moist localities in Sal forests; Frequent.

Specimen examined: India, Chhattisgarh: Korba district, Dhaurabhata, 22.03.2026, *S. Anand*, 546; deposited in the herbarium, Botany department, C.M.P. College, Prayagraj.

***Equisetum* L. (Equisetaceae)**

1. ***Equisetum arvense*** L., Sp. Pl. 2: 1061. 1753; *Equisetum campestre* Schleich. ex F.Weber & D.Mohr, *Bot. Taschenb.* 1807; Saini, et al.

Biodiversity of aquatic and semi-aquatic plants of Uttar Pradesh, 403. 2010.

Stem with 2-3 branches per node or unbranched, 1-7 mm in diameter. Sheath longer, lower sheath brown and upper ones green; teeth 3-9 mm long, Strobili apiculate.

Ecology & Population Data: Along riverbanks; Frequent.

Specimen examined: India, Chhattisgarh: Korba district, Devpahri road, 22.03.2026, *S. Anand*, 542; deposited in the herbarium, Botany department, C.M.P. College, Prayagraj.

2. ***Equisetum ramosissimum*** Desf. var. ***huegelii*** (Milde) Christenh. & Husby, Bot. J. Linn. Soc. 189(4): 343. 2019; *Equisetum huegelii* Milde, Verh. Zool.-Bot. Ges. Wien 11: 439. 1861; Dixit, Cens. Indian Pterid. 19. 1984. (Fig.2. E).

Rush like perennial plant with a creeping rhizome. Stem green, grooved, jointed, 30 cm to 1 m tall, with 1-4 branches per node; stomata in double lines in the grooves; sheath green, cylindrical, 4-12 mm long, teeth thin, brown to white, drying and deciduous. Strobili terminal on main stem, apiculate; sporangia born on the back of stalked sporangiophores. Spores rounded, green with 4 elaters. Gametophyte green, lobed.

Ecology & Population Data: Growing along streams and paths; Abundant.

Specimen examined: India, Chhattisgarh: Korba district, Bundeli, 10.03.2025, *S. Anand*, 532; deposited in the herbarium, Botany department, C.M.P. College, Prayagraj.

Hemionitis **L. (Pteridaceae)**

1. Hemionitis tenuifolia (Burm.f.) Christenh., Global Fl. 4: 22. 2018; *Cheilanthes tenuifolia* (Burm.f.) Sw., Syn. Fil.: 129, 332. 1806. (Fig.3. O)

Rhizome short, creeping, bearing scales. Fronds distant, stipes 5-20 cm, scaly at base, naked above, purple-black; lamina 10-45 x 5-15 cm, lanceolate, glabrous; segments of the primary and secondary divisions the largest, pinnatifid, segments of various shapes, margins crenate. Sori marginal, confluent; indusia elongated, denticulate, wrinkled.

Ecology & Population Data: Along roadside and in rock crevices; Abundant.

Specimen examined: India, Chhattisgarh: Korba district, Lemru road, 08.09.2024, *S. Anand*, 548; deposited in the herbarium, Botany department, C.M.P. College, Prayagraj.

Lindsaea **Dryand. ex Sm. (Lindsaeaceae)**

1. Lindsaea ensifolia Sw., J. Bot. (Schrader) 1800(2): 77. 1801; *Schizoloma ensifolia* (Sw.) J. Sm. In Hook. J. Bot.3:414:1841; Fraser-Jenk., Rev. Indian Pterid. 2008; PPG I, J. Syst. Evol. 54: 563–603. 2016. (Fig.3. K)

Rhizome short-creeping, scales reddish brown. Fronds close, pinnate, stipes 10-25 cm, dark brown; ascending, evenly narrowed from the base to apex, sub-acute to acuminate, dark green to olivaceous-green when dry; margins entire, forming 2-3 series of areoles. Sori continuous, indusium entire, strongly reflexed and concealed at maturity.

Ecology & Population Data: In open areas and forest undergrowth; Frequent.

Specimen examined: India, Chhattisgarh: Korba district, Devpahri road, 23.03.2026, *S. Anand*, 576; deposited in the herbarium, Botany department, C.M.P. College, Prayagraj.

Lycopodiella **Holub (Lycopodiaceae)**

1. ***Lycopodiella cernua*** (L.) Pic.Serm., Webbia 23: 166. 1968. *Lycopodium cernuum* L., Sp. Pl. 2: 1103. 1753, Baishya and Rao, Ferns and Ferns-allies of Meghalaya State, India 21. 1982. (Fig.2. D).

Main stem stout, erect, much branched when growing in thickets or among tall grasses; weak and prostrate when growing on exposed hillside. Leaves spirally arranged, narrowly linear, 2-5 mm long, incurved, entire. Strobili solitary at the end of short branches, oblong, 4-9 mm long, sessile; sporophylls ovoid, pale yellowish, irregularly serrate, ciliate at the margins.

Ecology & Population Data: On hillsides in exposed moist places; Rare.

Specimen examined: India, Chhattisgarh: Korba district, Dhaurabhata, 22.03.2026, *S. Anand*, 579; deposited in the herbarium, Botany department, C.M.P. College, Prayagraj.

Lygodium **Swartz (Schizaeaceae)**

1. ***Lygodium flexuosum*** (L.) Sw.; J. Bot. (Schrader) 1800(2): 106. 1801. *Ophioglossum flexuosum* L., Sp. Pl. 2: 1063. 1753. Khullar et al, Ferns of Nainital 54. 1991. (Fig. 1.i). Syn. *L. japonicum* (Thbg.) Sw. (Fig.2. I)

Stem climbing, slender, glabrous or pubes- cent, fronds bipinnate, glabrous or slightly hairy, pairs of fronds stipitate-pinnate with the pinnules again pinnate or variously lobed or sub -palmate; segments short, serrulate, the fertile ones much contracted, terminal segment linear- lanceolate, serrulate, lamina 15-30 cm broad. Sori occupying the whole of the undersurface or protruding from the margin.

Ecology & Population Data: In forest under moist situations along river banks; Rare.

Specimen examined: India, Chhattisgarh: Korba district, Lemru road, 30.06.2026, *S. Anand*, 597; deposited in the herbarium, Botany department, C.M.P. College, Prayagraj.

2. ***Lygodium microphyllum*** (Cav.) R.Br., Prodr. Fl. Nov. Holl. 162. 1810 Dixit, Cens. Indian Pterid. 60. 1984, Syn. *L. scandens* Sw.

Rachis twining, glabrous, bearing very short primary branches, 2-4 mm long, dormant bud covered with stiff brown hairs; secondary branches opposite, 4-10 cm long; sterile pinnules 2-3 cm long, about 1 cm broad, ovate, often slightly lobed at the base, glabrous, crenulate, articulate at base, when deciduous, leaving the petiole on the secondary branches; upper prinnules fertile. Sporangia born in 2-rows on the finger-like projecting lobes, covered with scale-like indusia.

Ecology & Population Data: In the moist areas along sloppy hills; Rare.

Specimen examined: India, Chhattisgarh: Korba district, Lemru road, 30.06.2026, *S. Anand*, 609; deposited in the herbarium, Botany department, C.M.P. College, Prayagraj.

***Marsilea* L. (Marsileaceae)**

1. ***Marsilea minuta*** L., Mant. Pl. Altera: 308. 1771. *Marsilea tetraphylla* Thunb., Fl. Jap.: 340. 1784.Verma, et al., Flora of M.P. 70. 1993. (Fig.3. P)

A creeping aquatic herb, Rhizome slender, branched, creeping on or just below the surface of the soil, growing tip hairy. Leaves borne on the nodes and arranged alternately in two rows, one on each side of the median line, variable in size, lamina divided into 4 small, usually equal pinnae, petiole long. Sporocarp 0.3cm long, ellipsoid.

Ecology & Population Data: In small ditches, ponds and other reservoirs along road-sides; Common.

Specimen examined: India, Chhattisgarh: Korba district, Pasarkhet, 11.03.2025, *S. Anand*, 513; deposited in the herbarium, Botany department, C.M.P. College, Prayagraj.

***Odontosoria* (C.Presl.) Fée (Lindsaeaceae)**

1. ***Odontosoria chinensis*** (L.) J.Sm., Bot. Voy. Herald: 430. 1857. *Trichomanes chinense* L., *Sp. Pl.* 2: 1099. 1753; Verma, et al., Flora of M.P. 44.1993. (Fig.2. F).

Rhizome short creeping to semierect, densely covered with paleae. Paleae hair-like, 1- celled broad, ferruginous. Fronds bi-or tripinnate, lanceolate, up to 7-50x3-15 cm, glabrous; pinnae much dissected, rather dichotomously forked in to 4-8 pinnules; pinnules rhomboidal, each again forked in to 2-4 lobes, lobes subulate. Sori marginal, rather submarginal inside the marginal flaps, terminal on free veinlets. Indusium attached at base and sides, opens outward. Annulus 18-cellrd. Spores bean-shaped with one longitudinal dark slit, light brown, hyaline.

Ecology & Population Data: Growing on hill slope; Abundant.

Specimen examined: India, Chhattisgarh: Korba district, Lemru road, 09.09.2024, *S. Anand*, 498; deposited in the herbarium, Botany department, C.M.P. College, Prayagraj.

***Ophioglossum* L. (Ophioglossaceae)**

1. ***Ophioglossum reticulatum*** L., *Sp. Pl.* 2: 1063. 1753; *Ophioglossum vulgatum* var. *reticulatum* (L.) D.C.Eaton, *Mem. Amer. Acad. Arts*, n.s., 8: 218. 1861; Khullar et al., Ferns of Nainital 49. 1991.

Stem subterranean with adventitious roots and tuberous, cylindrical rhizome and every year produce an aerial complex consisting of a long stipe bearing a single sterile chordate-ovate, acute or obtuse fronds or laminar portion, and a long fertile spike (contracted frond), fronds 3-5 x 2.5 cm, simple with reticulate venation.

Ecology & Population Data: In Sal Forest under moist situations along streams where organic matter is rich; Frequent.

Specimen examined: India, Chhattisgarh: Korba district, Lemru road, 22.03.2026, *S. Anand*, 560; deposited in the herbarium, Botany department, C.M.P. College, Prayagraj.

2. ***Ophioglossum vulgatum*** L., Sp. Pl. 2: 1062. 1753.; *Ophioglossum ovatum* Sw. ex Opiz, *Kratos* 1(4): 12. 1819, nom. illeg. superfl.; Khullar et al., Ferns of Nainital 50. 1991. (Fig.2. B)

Rhizome fibrous. Common stalk 6.5-7.5 cm long, blade sterile, nearly orbicular or broadly ovate, cordate at base, rounded at apex about 3.5 cm long and 3 cm wide, areolae with included veinlets. Fertile segment arising from near base of sterile blade. Spores roughly reticulate.

Ecology & Population Data: In moist area of sal forests, marshy localities; Rare.

Specimen examined: India, Chhattisgarh: Korba district, Lemru road, 22.03.2026, *S. Anand*, 578; deposited in the herbarium, Botany department, C.M.P. College, Prayagraj.

***Psilotum* Sw. (Psilotaceae)**

1. ***Psilotum nudum*** (L.) P.Beauv., Prodr. Aethéogam.: 112. 1805; *Lycopodium nudum* L., *Sp. Pl.* **2**: 1100. 1753Verma, et al., Flora of M.P. 44. 1993. (Fig.2. A)

Rhizome bearing rhizoid. Aerial stem 10-30 cm long, simple below, repeatedly dichotomously branched above, erect or pendent. Leaves small awl shaped, 1-2mm long, scale like, scattered, veinlets, lacking midrib. Sporangia 3-lobed, born on adaxial side in the axil of minute. Leaves eusporangiate, homosporous; spores bilateral.

Ecology & Population Data: In crevices of moist rocks; Rare.

Specimen examined: India, Chhattisgarh: Korba district, Lemru road, 11.03.2025, *S. Anand*, 535; deposited in the herbarium, Botany department, C.M.P. College, Prayagraj.

***Pteris* L. (Pteridaceae)**

1. ***Pteris biaurita*** L., Sp. Pl. 2: 1076. 1753; *Pteris flavicaulis* Hayat, J. Coll. Sci. Imp. Univ. Tokyo, 30(1): 443-444, 1911; R. D. Dixit, Census Indian Pterid., 69. 1984; Khullar, III. Fern Fl. West Himalaya, 1:260. t.92. 1994.

Rhizome short, erect, scaly. Scales long, bicolorous, linear, apex acuminate. Stipes long, 25-55 cm long, glabrous above. Lamina pinnate, 30-60 cm long, 15-20 cm wide, herbaceous, green, lanceolate. Rachis, costa and costule glabrous. Sori linear, marginal, extending from sinus, not reaching apices, continuous, indusiate. Indusium 0.5-1 cm long, 0.1 cm wide, transparent, membranaceous, persistent, curls out at maturity. Sporangia round, globose, 15-17-celled annulus.

Ecology & Population Data: Along streams and forest edges; Rare.

Specimen examined: India, Chhattisgarh: Korba district, Lemru road, 10.09.2024, *S. Anand*, 437; deposited in the herbarium, Botany department, C.M.P. College, Prayagraj.

2. ***Pteris cretica*** L., Mant. Pl. 13. 1767; *Pteris nervosa* Thunb., Fl. Japan. 332. 1784; Moony in Indian For. Rec. 3(7): 248. 1942; R. D. Dixit, Census Indian Pterid., 69. 1984; Khullar, III. Fern Fl. West Himalaya, 1:260. t.93. 1994.

Rhizome short, creeping, densely covered with scales; scales 0.3-0.5 cm long, brown, linear, lanceolate, acuminate, margin smooth. Stipes 25-40 cm long, yellow, glabrous above, scaly ata base. Fronds dimorphic. Lamina15-25 cm long, fertile lamina longer than sterile ones; lamina shorter than stipe. Sori at the margin of fertile pinnae except at base and apex, linear, indusiate. Indusium 0.5-1 mm broad. Sporangia with 16-celled annulus, stalked.

Ecology & Population Data: In open places and forest edges; Abundant.

Specimen examined: India, Chhattisgarh: Korba district, Bundeli road, 11.03.2025, *S. Anand, 525*; deposited in the herbarium, Botany department, C.M.P. College, Prayagraj.

3. ***Pteris multifida*** Poir., Encycl. (Lamarck) 5: 714. 1804; *Pteris serrulata* L.f., *Suppl. Pl.*: 445. 1782, nom. illeg.; Singh J. Forestry 82. 1989. (Fig.2. J)

Rhizome short, creeping, thick, scaly. Scales small up to 2.5 mm long, entire, apex acute. Fronds dimorphic, thin, fertile fronds 25-32 cm in height; sterile fronds 13-20 cm in height, green, ovate. Pinnae 2-3 pairs, pinnate, opposite, lanceolate, green, terminal pinnae larger than lateral pinnae, terminal pinnae 15.0-16.0 cm long, and 0.6-0.8 cm broad; lateral pinnae 6.0- 9.0cm long and 0.4-0.5 cm broad; margin wavy, apex acute, serrate, glabrous, thin, papyraceous, pinna decurrent to form a winged rachis; lower pinnae multifid; venation free, forking, numerous. Sori brown along the margin. Indusium white. Sporangium globose, 220-240 µm long; stalk 250-300 µm, 2-celled. Annulus 17-18-celled, Spores brown 40-45 µm, trilete-tetrahedron.

Ecology & Population Data: In moist and shady places; Frequent.

Specimen examined: India, Chhattisgarh: Korba district, Dhengurdih, 09.09.2024, *S. Anand*, 506; deposited in the herbarium, Botany department, C.M.P. College, Prayagraj.

4. ***Pteris vittata*** L., Sp. Pl. 2: 1074. 1753; *Pycnodoria vittata* (L.) Small, Ferns Florida: 89. 1932; Khular et. al., Ferns of Nainital 80. 1991.

The plant is generally 30-60 cm in height. Rhizome is erect, sub erect or short creeping, depending on age, unbranched, densely scaly at apex.; scales lanceolate, entire, acuminate. Stipes bent at base, grooved above, densely scaly towards the base, sparsely upwards. Fronds simple, pinnate, gradually narrowing towards the base, terminated by a larger pinna than the lateral ones. Pinnae opposite or sub opposite, sessile, ascending, lanceolate with acute apex. Margin of soriferous pinna entire and finely serrulate, veins forked, continuous along of the margin from above the base.

Ecology & Population Data: Along `nalas' in the crevices of walls and rocks; Common.

Specimen examined: India, Chhattisgarh: Korba district, Rajgamar road, 08.09.2024, *S. Anand*, 493; deposited in the herbarium, Botany department, C.M.P. College, Prayagraj.

***Salvinia* Ség. (Salviniaceae)**

1. ***Salvinia natans*** (L.) All., Fl. Pedem. 2: 289. 1785; *Salvinia vulgaris* Rupr., Distr. Crypt. Vasc. Ross.: 27. 1845; Saini et al., Biodiversity of aquatic and semi-aquatic plants of Uttar Pradesh:411.2010.

A small floating herb, stem horizontal, roots absent. Leaves of two types; floating green leaves in pairs, oval-oblong, 10-15 mm long, midrib distinct, the upper surface covered with papillae, the under-surface covered with straight, multicellular hairs, with 4 free hairs on tips of each papilla; submerged leaves finely dissected, root like. Sporocarps borne in cluster on a branch of the root like leaf, globular or ovoid, covered with brown multicellular hair, some containing megasporangia, and other microsporangia.

Ecology & Population Data: Floating in stagnant water in ponds, ditches and paddy fields; Frequent. Vegetative reproduction rapid by fragmentation.

Specimen examined: India, Chhattisgarh: Korba district, Kerakachhar, 23.03.2026, *S. Anand*, 564; deposited in the herbarium, Botany department, C.M.P. College, Prayagraj.

***Selaginella* P.Beauv. (Selaginellaceae)**

1. ***Selaginella ciliaris*** (Retz.) Spring, Bull. Acad. Roy. Sci. Bruxelles 10: 231. 1843; *Lycopodium ciliare* Retz., Observ. Bot. 5: 32. 1789; Dixit, Cens. Indian Pterid. 12. 1984.

Main stems creeping, 3-10 cm long, stramineous, glabrous, angular, rooting at intervals but mostly near base. Leaves dimorphic; lateral leaves unequal sided, ovate lanceolate, ciliate, acute or obtuse at apex, pointing outwards; median leaves nearly equal sided, ovate with prominent midrib, ciliate, cuspidate at apex, pointing upwards; axillary leaves equal sided, ovate, acute at apex, ciliate at base. Strobili terminal, 5-10 mm long; sporophylls dimorphic, those of the upper side unequal sided, lanceolate, ciliate, acuminate at apex; those of the lower side, ovate, keeled, acuminate at apex, the margins with long conspicuous cilia.

Ecology & Population Data: Growing on exposed rocks; Abundant.

Specimen examined: India, Chhattisgarh: Korba district, Lemru road, 30.06.2026, *S. Anand*, 410; deposited in the herbarium, Botany department, C.M.P. College, Prayagraj.

***Tectaria* Cav. (Polypodiaceae)**

1. ***Tectaria coadunata*** (J.Sm.) C.Chr., Contr. U.S. Natl. Herb. 26: 331. 1931; *Sagenia coadunata* J.Sm.,

J. Bot. (Hooker) 4: 184. 1841; Dixit, Cens. Indian Pterid. 142. 1984. (Fig.2. C).

Rhizome short creeping, stout. Stipes about 20 cm long, not winged, with lanceolate, puberulent, brown scales at base; fronds ovate-deltoid, bipinnatifid, with 1-3 pairs of free lateral pinnae below the pinnatifid apical portion; texture thin; stipes and rachis stramineous, covered with small ctenitis hairs, the costae veins on both surfaces and margins also bearing ctenitis hairs; veins anastomosing with included veinlets; veins along the costa forming one row of costal arches. Sori usually borne on the end of included veinlets, sometimes on a plexus of veinlets; indusia thin, brown, reniform, puberulent.

Ecology & Population Data: In forests at low lands; Rare.

Specimen examined: India, Chhattisgarh: Korba district, Labed, 30.06.2026, *S. Anand*, 620; deposited in the herbarium, Botany department, C.M.P. College, Prayagraj.

***Thelypteris* Schmidel (Aspleniaceae)**

1. Thelypteris dentata (Forssk.) E.P.St.John, Amer. Fern J. 26(2): 44. 1936; *Christella dentata* (Forssk.) Brownsey & Jermy, Brit. Fern Gaz. 10: 338. 1973. (Fig.3. N)

Rhizome suberect, short, scaly; scales upto 1 cm long, margin entire. Stipe 12-20 cm long, brownish, scaly and hairy. Lamina unipennate, 40-55 cm long, lanceolate, hairy, apex acuminate; pinnae 15-20 pairs, subopposite to alternate, lanceolate. Rachis hairy. Venation veins pinnate, alternate, parallel, reaching margin. Sori small, brown, round, in two rows. Indusium reniform brown, shortly hairy.

Ecology & Population Data: On stream banks; Rare.

Specimen examined: India, Chhattisgarh: Korba district, Rajgamar road, 30.06.2026, *S. Anand*, 624; deposited in the herbarium, Botany department, C.M.P. College, Prayagraj.

2. Thelypteris prolifera (Retz.) C.F.Reed, Phytologia 17: 306. 1968; *Hemionitis prolifera* Retz., Observ. Bot. 6: 38. 1791; Verma, et al., Flora of M.P. 80. 1993.

Rhizome creeping, scaly; scales ovate- subtriangular, hairy on margins. Stipes 30-60 cm long fronds pinnate, 1-2 mm apart, buds producing long secondary fronds, common in the axil of the pinnae; laminae of two kinds, one with a stalked terminal pinnae, the other growing indefinitely with small, lateral pinnae; pinnnae almost sessile except for the terminal one, the lower distant, up to 20 cm long and 2 cm wide, the upper more closely placed and smaller, 4-9 cm long, 1-1.5 cm wide, truncate to cordate at base, acuminate at apex, texture thin but firm, usually glabrous, veinlets 7-8 pairs, close, 4-6 pairs, uniting alternately to form an intermediate excurrent vein. Sori oblong, or elongate, along conjugated veinlets, ex-indusiate, but with capitate, orange paraphyses.

Ecology & Population Data: In shady wet places along ditches in lowlands, common.

Specimen examined: India, Chhattisgarh: Korba district, Labed, 30.06.2026, *S. Anand*, 617; deposited in the herbarium, Botany department, C.M.P. College, Prayagraj.

CONCLUSION

The present study provides the first systematic documentation of pteridophyte flora from the Korba district, Chhattisgarh. A total of 30 species belonging to 21 genera and 13 families were recorded from the study area. The most species-rich family was Pteridaceae (9 species), followed by Aspleniaceae (4), while Salviniaceae, Polypodiaceae, Equisetaceae, Lindsaeaceae, Schizaeaceae, and Ophioglossaceae were each represented by two species. The rest of the families were represented by single genus and single species in each family. The result highlights that most of the species are categorized as Least Concern (LC), and several remain Not Evaluated (NE). Notably, *Psilotum nudum* is listed as Critically Endangered (CR), highlighting the conservation significance of the district and the need for the targeted protection of its habitats. The district has diverse physiographic features ranging from Vindhyan dry mixed to Gondwana moist deciduous forests, along with perennial rivers that provide the ideal microhabitats for the survival and proliferation of pteridophytes. However, increasing anthropogenic disturbances, including habitat degradation and forest alteration, pose significant threats to these species by reducing the availability of suitable habitats.

This study addresses a significant gap in the floristic knowledge of central India, a region that has historically received considerably less attention than the Himalayas, Western Ghats and Northeast India. The findings presented here provide a valuable baseline for future ecological, taxonomic, biogeographical, and conservation studies. Furthermore, the documentation of both common and threatened taxa underscores the importance of long-term ecological monitoring, habitat protection, and conservation planning to ensure the persistence of this ancient lineage of vascular plants. Overall, the findings establish Korba district as a significant repository of pteridophytic diversity and emphasize its significance for future research and biodiversity conservation initiatives.

Table 1. Pteridophytes recorded from the Korba district in Chhattisgarh

S.N.	Botanical Name	Common Name	Family	IUCN Status	Habitat
1	*Adiantum capillus-veneris* L.	Southern maidenhair	Pteridaceae	LC	Crevices of moist rocks along river banks
2	*Adiantum incisum* Forssk.	Northern maidenhair	Pteridaceae	NE	In shady and moist rocky places
3	*Adiantum philippense* subsp. *Philippense*	Black maidenhair	Pteridaceae	NE	In moist situation at low mountain tracts
4	*Azolla pinnata* R. Br.	Mosquito fern	Salviniaceae	LC	In ponds, paddy fields, irrigation ditches
5	*Blechnum orientale* L.	Centipede fern	Aspleniaceae	NE	On open slopes and forest edges
6	*Ceratopteris thalictroides* (L.) Brongn.	Water sprite	Pteridaceae	LC	In muddy places along `nalas', ponds
7	*Dicranopteris linearis* (Burm.f.) Underw.	False staghorn fern	Gleicheniaceae	LC	In shady and moist places, with little humus or in rocky alluvial soil
8	*Diplazium esculentum* (Retz.) Sw.	Vegetable fern	Aspleniaceae	LC	In shady and moist places
9	*Dryopteris cochleata* (D.Don) C.Chr.	Indian male fern	Polypodiaceae	NE	In moist localities in Sal forests
10	*Equisetum arvense* L.	Horsetail	Equisetaceae	LC	Along river-banks
11	*Equisetum ramosissimum* var. *huegelii* (Milde) Christenh. & Husby	Branched horsetail	Equisetaceae	LC	Growing along streams and paths
12	*Hemionitis tenuifolia* (Burm.f.) Christenh.	Poison fern	Pteridaceae	LC	Along roadside and in rock crevices
13	*Lindsaea ensifolia* Sw.	Graceful necklace fern	Lindsaeaceae	LC	In open areas
14	*Lycopodiella cernua* (L.) Pic.Serm.	Staghorn clubmoss	Lycopodiaceae	LC	On hillsides on exposed moist places
15	*Lygodium flexuosum* (L.) Sw.	Maidenhair creeper	Schizaeaceae	NE	In forest under moist situations along river banks
16	*Lygodium microphyllum* (Cav.) R.Br.	Old World climbing fern	Schizaeaceae	LC	In the moist areas along sloppy hills
17	*Marsilea minuta* L.	Water clover	Marsileaceae	LC	In small ditches, ponds and other reservoir along road-sides
18	*Odontosoria chinensis* (L.) J.Sm.	Lace fern	Lindsaeaceae	NE	Growing on hill slope

19	*Ophioglossum reticulatum* L.	Netted Adder's tongue	Ophioglossaceae	LC	In Sal Forest under moist situation along streams where organic matter is rich
20	*Ophioglossum vulgatum* L.	Adder's tongue	Ophioglossaceae	LC	In moist area of sal forests, marshy localities
21	*Psilotum nudum* (L.) P.Beauv.	Whisk fern	Psilotaceae	CR	In crevices of moist rocks
22	*Pteris biaurita* L.	Thinleaf brake	Pteridaceae	NE	Along streams and forest edges
23	*Pteris biaurita* L.	Cretan brake	Pteridaceae	NE	In open places and forest edges
24	*Pteris multifida* Poir.	Spider fern	Pteridaceae	NE	In moist and shady place
25	*Pteris vittata* L.	Chinese brake fern	Pteridaceae	LC	Along `nalas' in the crevices of walls and rocks
26	*Salvinia natans* (L.) All.	Floating fern	Salviniaceae	LC	Floating in stagnant water in ponds, ditches and paddy fields
27	*Selaginella ciliaris* (Retz.) Spring	Fringed spikemoss	Selaginellaceae	NE	Growing on exposed rocks
28	*Tectaria coadunata* (J.Sm.) C.Chr.	Halberd fern	Polypodiaceae	NE	In forests at low lands
29	*Thelypteris dentata* (Forssk.)E.P.St. John	Downy maiden fern	Aspleniaceae	LC	On stream bank
30	*Thelypteris prolifera* (Retz.) C.F.Reed	Riverine scrambler	Aspleniaceae	NE	In shady wet places along ditches in low lands

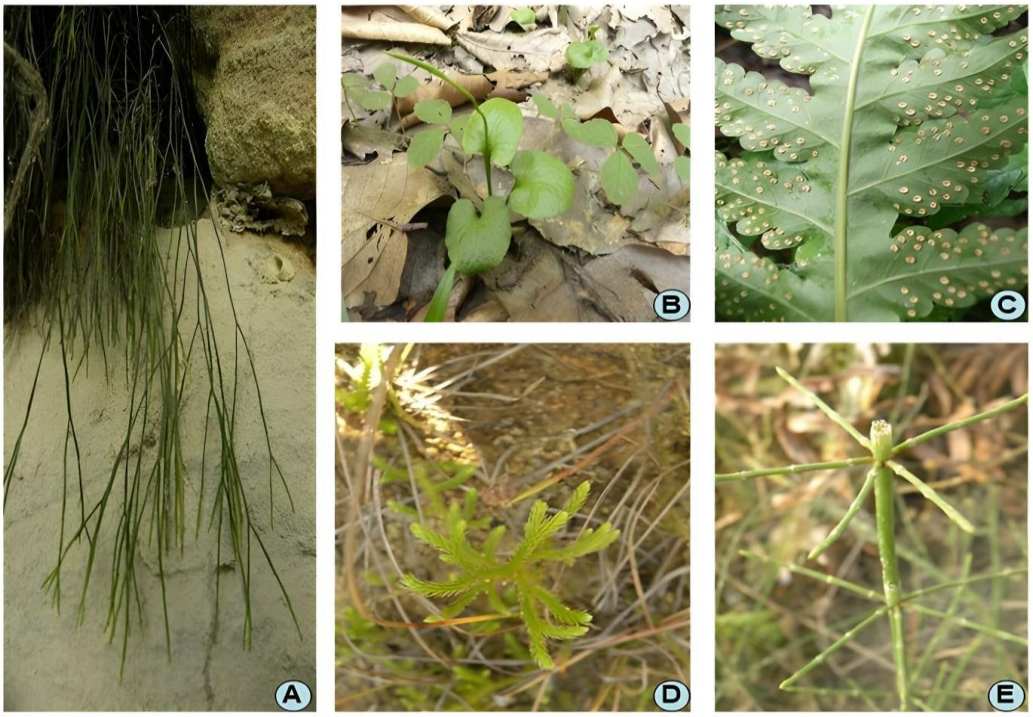


Figure 2. Representation of some species collected during the field study. (A) *Psilotum nudum* (L.) P.Beauv.; (B) *Ophioglossum vulgatum* L.; (C) *Tectaria coadunata* (J.Sm.) C.Chr.; (D) *Lycopodiella cernua* (L.) Pic.Serm.; (E) *Equisetum ramosissimum* var. *huegelii* (Milde) Christenh. & Husby; (F) *Odontosoria chinensis* (L.) J.Sm.; (G) *Dicranopteris linearis* (Burm.f.) Underw.; (H) *Ceratopteris thalictroides* (L.) Brongn.; (I) *Lygodium flexuosum* (L.) Sw.; (J) *Pteris multifida* Poir

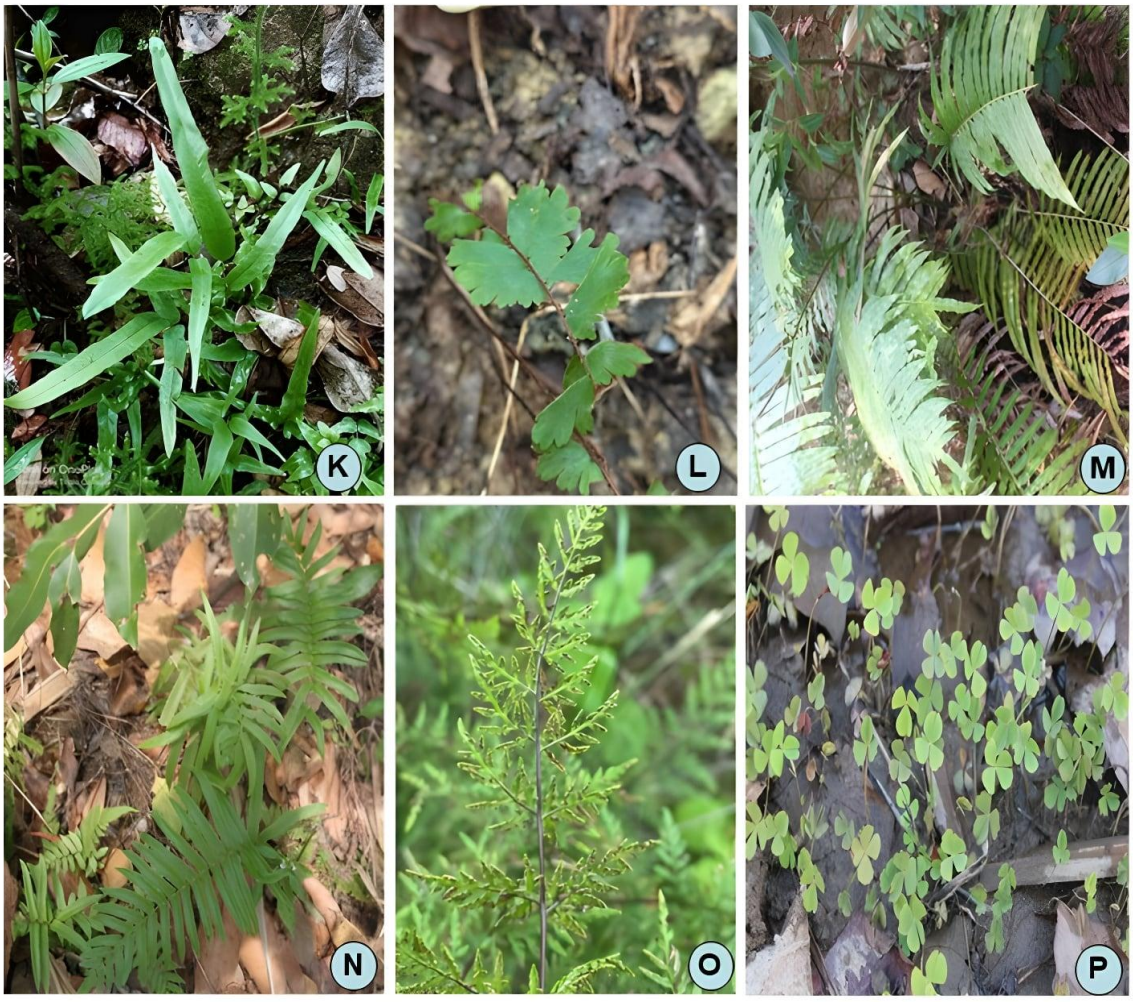


Figure 3. Representation of some species collected during the field study. (K) *Lindsaea ensifolia* Sw.; (L) *Adiantum capillus-veneris* L.; (M) *Blechnum orientale* L.; (N) *Thelypteris dentata* (Forssk.) E.P.St.John; (O) *Hemionitis tenuifolia* (Burm.f.) Christenh.; (P) *Marsilea minuta* L.

ACKNOWLEDGEMENT

The authors are thankful to the Principal and Head of the Department of Botany, CMP Degree College, Prayagraj, for providing laboratory facilities.

REFERENCES

Basihya, A.K and Rao, R.R. (1982). *Ferns and Fern-allies of Meghalaya State, India*. Scientific Publishers, Jodhpur, India.

Google Scholar

Beddome, R.H. (1883). *A Handbook to the Ferns of British India, Ceylon and the Malaya Peninsula*. Thacker Spink & Co., Calcutta.

Google Scholar

Bir, S.S. (1987). Pteridophytic flora of India: Rare and endangered elements and their conservation. *Indian Fern J.* **4**: 95-101.

Google Scholar

Chandra, S. (2000). *The Ferns of India (Enumeration, Synonyms and Distribution).* International Book Distributors, Dehradun.

Google Scholar

Dixit, R.D. (1984). *A census of the Indian Pteridophytes, Flora of India Ser. IV*. Botanical Survey of India, Department of Environment, Government of India, New Delhi.

Google Scholar

Dixit, R.D. (1989). Ecology and Taxonomy of Pteridophytes of Madhya Pradesh. *Indian Fern J.* **6**:140-159.

Google Scholar

Dixit, R.D. and Singh, S. (2005). Diversity of Pteridophytes in Chhattisgarh and their conservation. *Journal of Economic and Taxonomic Botany*, 29(2), p.251.

Google Scholar

Fraser-Jenkins, C.R., Gandhi, K.N. and Kholia, B.S. (2017). *An Annotated Checklist of Indian Pteridophytes. Part I (Lycopodiaceae to Thelypteridaceae).* Bishen Singh Mahendra Pal Singh, Dehradun.

Google Scholar

Garg, A., Sinha, G.P. and Mao, A.A. (2025). *Flora of Chhattisgarh* (Vol. 3: *Hydrocharitaceae–Poaceae*). Botanical Survey of India, Calcutta.

Google Scholar

Khullar, S.P., Pangtey, Y.P.S., Samant, S.S., Rawal, R.S. and Singh, P. (1991). *Ferns of Nanital*. Bishen Singh Mahendra Pal Singh Dehradun (India).

Google Scholar

Panigrahi, G.R. and Dixit, D. (1966). New Records for ferns from Madhya Pradesh. *Proc. Nat. Acad. Sci. India Sec. B*. **36**: 135-144.

Google Scholar

Panigrahi, G. and Murti, S.K. (1989). *Flora of Bilaspur District, Madhya Pradesh* (Vol. 1: Ranunculaceae–Convolvulaceae). Botanical Survey of India, Calcutta.

Google Scholar

Panigrahi, G. and Murti, S.K. (1999). *Flora of Bilaspur District, Madhya Pradesh* (Vol. 2: Solanaceae–Poaceae). Botanical Survey of India, Calcutta.

Google Scholar

Pteridophyte Phylogeny Group (PPG I). (2016). A community-derived classification for extant lycophytes and ferns. *Journal of Systematics and Evolution*, 54(6), 563–603. https://doi.org/10.1111/jse.12229

Google Scholar

Ranker, T.A. and Haufler, C.H. (Eds.) (2008). *Biology and Evolution of Ferns and Lycophytes*. Cambridge University Press, Cambridge.

Google Scholar

Saini, D.C., Singh, S.K. and Rai, K. (2010). *Biodiversity of aquatic and semi-aquatic plants of Uttar Pradesh (with reference to eastern Uttar Pradesh)*. Uttar Pradesh State Biodiversity Board Publisher, Lucknow (U.P.).

Google Scholar

Tiwari, S.D.N. (1964). Ferns of Madhya Pradesh. *J. Indian. Bot. Soc.* **43** (3): 431-452.

Google Scholar

Verma, D.M., Balakrishnan, N.P. and Dixit, R.D. (1993). *Flora of Madhya Pradesh (Vol. 1)*. Botanical Survey of India, Calcutta.

Google Scholar

Journal of Plant Development Sciences
Meerut, India

RESEARCH ARTICLE

EFFECT OF STORAGE CONDITIONS AND PACKAGING MATERIALS IN MAINTAINING QUALITY OF *CENTELLA ASIATICA* (L.) URB. RAW DRUG

C. Beena*

AICRP on MAP & B, Kerala Agricultural University, Vellanikkara, Kerala-680656, India
Email: beenac2@gmail.com

Received-20.06.2026, Revised-12.07.2026, Accepted-29.07.2026

Abstract: *Centella asiatica* (L.) Urb. is an important medicinal herb valued for its pentacyclic triterpenoid constituents, particularly asiaticoside, madecassoside, asiatic acid and madecassic acid. However, the raw drug remains vulnerable to quality deterioration during storage. The present study evaluated the influence of storage temperature and packaging material on the retention of important marker compounds and the physical and microbiological quality of *C. asiatica* raw drug over six months. The raw drug was stored under refrigerated (4 °C; M1) and ambient (M2) conditions using six packaging systems: glass jar (S1), plastic jar (S2), vacuum pack (S3), low-density polyethylene (LDPE) bag (S4), aluminium pouch (S5) and cloth bag (S6). Samples were assessed after three and six months for asiaticoside and asiatic acid contents. After three months, refrigerated vacuum packaging (M1S3) retained the highest asiaticoside (0.978%) and asiatic acid (0.097%) contents, while LDPE and cloth-bag samples under ambient storage had spoiled. After six months, the refrigerated vacuum-packed treatment retained 0.86% asiaticoside, equivalent to 88% of the initial value (0.98%), and had the lowest reported total viable count among the surviving treatments (10×10^7 CFU/g compared with 16×10^7 CFU/g initially). Refrigerated aluminium-pouch and cloth-bag samples retained 70% and 74% of the initial asiaticoside content, respectively, whereas the ambient vacuum-packed sample retained 62%. Most other treatments were recorded as spoiled by six months. Overall, the results indicated that combining refrigeration with vacuum packaging was the most effective of the tested storage strategies for maintaining the chemical and sensory quality of *C. asiatica* raw drug for upto six months. The study provides practical information for post-harvest handling and storage of *C. asiatica* intended for medicinal and value-added applications.

Keywords: *Centella asiatica*, Raw drug, Storage stability, Vacuum packaging, Asiatic acid

INTRODUCTION

Centella asiatica (L.) Urb., commonly known as gotu kola or mandukaparni, is a small, creeping herb of the family Apiaceae (Umbelliferae) that has a long history of use in traditional systems of medicine. Its pharmacological and therapeutic interest is largely associated with a group of pentacyclic triterpenoids, asiaticoside, madecassoside, asiatic acid and madecassic acid. These compounds have been investigated for wound-healing, anti-inflammatory, neuroprotective and other biological activities, making *C. asiatica* an important medicinal raw material for pharmaceutical, nutraceutical and cosmeceutical applications (Tomar 2008; Gohil *et al*., 2010).

Maintaining the quality of medicinal plant material after harvest is essential because the chemical composition and microbiological status of the raw drug can change during drying, packaging and storage. Moisture, temperature, oxygen exposure and inadequate protection from environmental conditions may contribute to loss of chemical markers and deterioration of physical quality. For this reason, the World Health Organization recommends systematic quality control of medicinal plant materials, including appropriate assessment of identity, purity, microbial quality and other factors relevant to their intended use (WHO, 1998; WHO, 2011). Stability investigations are particularly important for herbal materials because changes in marker compounds may occur even when the material remains visually acceptable (Kaur *et al.*, 2016).

Storage temperature and packaging are therefore important components of post-harvest management. Temperature influences the rate of chemical and biological reactions, whereas packaging determines the movement of moisture and gases between the material and its surroundings. Vacuum packaging can reduce the amount of oxygen in the package and may also limit moisture exchange when an appropriate barrier film is used. The effectiveness of such systems, however, depends on the characteristics of the raw material, package barrier properties, storage temperature and duration. Evidence from dried food systems shows that microbial survival and growth are strongly affected by temperature, relative humidity and packaging conditions (Hyun *et al*., 2018). Similar principles are relevant to dried medicinal plant materials also, although direct evidence for *C. asiatica* raw drug remains limited.

*Corresponding Author

Stability studies on *C. asiatica* extracts have demonstrated that marker compounds can decline during storage and that the extent of loss depends on extract composition and storage conditions. Kaur *et al*. (2016) reported reductions in chemical markers and biological activities during six months of accelerated stability testing of dried *C. asiatica* extracts. Puttarak *et al*. (2016) also demonstrated the stability characteristics of a standardized pentacyclic triterpene-enriched *C. asiatica* extract, while Monton *et al*. (2018) showed that the stability of asiaticoside and madecassoside can vary substantially with formulation and storage temperature. These findings support the need to investigate storage conditions at the raw-drug level rather than assuming that stability data generated for extracts or finished formulations will directly apply to raw drug material.

Accordingly, the present study was undertaken to compare two storage temperatures and six packaging systems for maintaining the bioactive quality and overall acceptability of *C. asiatica* raw drug during six months of storage. Particular attention was given to asiaticoside as chemical quality marker, together with sensory observations and, at the end of storage, total viable microbial count of selected best samples.

MATERIALS AND METHODS

Plant material and preparation of raw drug

Fresh, disease-free *C. asiatica* plants were harvested at physiological maturity from the herbal garden of the AICRP on Medicinal and Aromatic Plants & Betelvine (MAP & B), Kerala Agricultural University, Vellanikkara, Thrissur, Kerala, India. The plant material was authenticated by qualified botanist. The harvested material was cleaned and processed under hygienic conditions to obtain a uniform raw drug. The material was shade-dried for 24 hours and then used for the storage experiment.

Experimental design and storage conditions

The storage experiment comprised two storage conditions and six packaging materials, with three replications. The treatments were as follows:

Factor	Treatment
Storage condition (Factor A)	M1 – Refrigerated storage (4 °C) M2 – Ambient storage (room temperature)
Packaging material (Factor B)	S1 – Glass jar S2 – Plastic jar S3 – Vacuum pack S4 – Low-density polyethylene (LDPE) bag S5 – Aluminium pouch S6 – Cloth bag

A known quantity (1 kg) of raw drug was packed in each packaging system and stored under the respective temperature condition. Samples were evaluated at 0 (initial), 3 and 6 months. Because the sampling was destructive, separate packages were used for each scheduled sampling point.

Estimation of asiaticoside and asiatic acid

Asiaticoside and asiatic acid were quantified by high-performance thin-layer chromatography (HPTLC) (Abhishek *et al.*, 2014). Samples were analysed on silica gel 60 F254 plates using mobile phase of toluene: ethyl acetate: formic acid (5:5:1) for asiatic acid and n-butanol: ethyl acetate: water (4:1:5) for asiaticoside. Methanolic solution of samples and standard compounds were applied to the silica plates. Detection was performed using anisaldehyde–sulphuric acid derivatization and densitometric scanning on a CAMAG TLC Scanner. Asiaticoside and asiatic acid reference standards were obtained from Natural Remedies Pvt. Ltd. and Sisco Research Laboratories, respectively. Results were expressed as percentage content (% w/w) on a dry-weight basis.

The percentage retention of asiaticoside was calculated as follows:

Retention (%) = (Asiaticoside content at a given storage interval / Initial asiaticoside content) × 100.

Total viable microbial count

Microbial load of selected samples was assessed as total viable count (TVC) using a standard plate-count dilution method and expressed as colony-forming units per gram (CFU/g) of raw drug (WHO, 1998; WHO, 2011).

Sensory and physical quality assessment

Stored samples were examined for colour, odour and general appearance by a semi-trained panel. Samples showing visible mould growth, caking, pronounced off-odour or marked discoloration were classified as spoiled. Once a sample was classified as spoiled, it was not used for subsequent chemical or microbiological evaluation. Quantitative data were analysed using analysis of variance appropriate to the experimental design, and treatment means were compared at the 5% probability level using the critical difference (CD) wherever applicable.

RESULTS AND DISCUSSION

Effect of storage condition and packaging on asiaticoside and asiatic acid after three months

After three months, both asiaticoside and asiatic acid contents were lower in most stored samples than in the initial raw drug, although the magnitude of change differed among storage treatments (Table 1).

The initial asiaticoside and asiatic acid contents were 0.980% and 0.110%, respectively. Among the refrigerated treatments, vacuum packaging (M1S3) showed the highest recorded asiaticoside content (0.978%) and asiatic acid content (0.097%). The refrigerated cloth-bag treatment (M1S6) also retained a high asiaticoside content (0.973%). Refrigerated LDPE packaging (M1S4) recorded the lowest asiaticoside content among the refrigerated treatments (0.790%).

Under ambient storage, the retention of the marker compounds was generally lower than under refrigeration. The LDPE-bag (M2S4) and cloth-bag (M2S6) treatments were recorded as spoiled by three months and were therefore unavailable for chemical analysis. This pattern suggests that storage temperature and package characteristics jointly influenced the preservation of the raw drug. The superior performance of vacuum packaging under refrigeration is consistent with the broader principle that temperature and moisture/oxygen management are important determinants of stability in dried biological materials (Hyun et *al*., 2018).

Table 1. Effect of three-month storage on asiaticoside and asiatic acid contents of C. asiatica raw drug.

Storage condition	Packaging material	Asiaticoside (%)	Asiatic acid (%)
Initial	—	0.980	0.110
M1 (Refrigerated)	S1 – Glass jar	0.932	0.077
	S2 – Plastic jar	0.890	0.080
	S3 – Vacuum pack	0.978[a]	0.097[a]
	S4 – LDPE bag	0.790	0.083
	S5 – Aluminium pouch	0.950	0.087
	S6 – Cloth bag	0.973	0.093
M2 (Ambient)	S1 – Glass jar	0.893	0.073
	S2 – Plastic jar	0.873	0.073
	S3 – Vacuum pack	0.947	0.083
	S4 – LDPE bag	Spoiled	Spoiled
	S5 – Aluminium pouch	0.830	0.080
	S6 – Cloth bag	Spoiled	Spoiled
CD (0.05)		0.018	NS

Effect of storage on quality after six months

By six months, differences among the storage treatments had become more pronounced (Table 2). The refrigerated vacuum-packed treatment (M1S3) was the best-performing treatment in the experiment, retaining 0.86% asiaticoside, equivalent to 88% of the initial concentration. It also recorded the TVC 10 × 10^7 CFU/g, compared with 16 × 10^7 CFU/g in the initial material. Refrigerated cloth-bag (M1S6) and aluminium-pouch (M1S5) samples retained 74% and 70% of the initial asiaticoside content, respectively, and were observed as in usable condition at the end of six months.

The vacuum-packed storage under ambient conditions, (M2S3) was the only ambient treatment that remained unspoiled after six months. However, asiaticoside content had declined to 0.61%, corresponding to 62% retention. All other ambient treatments were observed as spoiled. Results indicated that vacuum packaging alone did not provide the same level of chemical preservation as the combination of vacuum packaging and refrigeration.

Decline in asiaticoside content during storage is in consistent with earlier stability studies on *C. asiatica* derived materials. Kaur *et al*., (2016) reported reductions in chemical markers during six months of accelerated stability testing of dried *C. asiatica* extracts. Puttarak *et al*., (2016) reported stability changes in a standardized pentacyclic triterpene-enriched extract, while Monton *et al.*, (2018) demonstrated that the stability of asiaticoside and madecassoside can depend strongly on the storage temperature and formulation matrix. These studies were performed on extracts or formulations rather than raw drug, but they support the interpretation that the triterpenoid profile of *C. asiatica* is sensitive to storage conditions.

The present findings also agree with the importance of maintaining suitable environmental conditions for medicinal plant materials. WHO quality-control guidance emphasizes the need to control contamination and deterioration in medicinal plant materials, while studies on dried biological products have shown that temperature, humidity and packaging can influence microbial behaviour during storage (WHO, 1998; WHO, 2011; Hyun *et al*., 2018). In the present study, the combination of refrigeration and vacuum packaging was associated with the longest observed period of acceptable quality among the tested treatments.

Table 2. Effect of six-month storage on asiaticoside retention and total viable count of *C. asiatica* raw drug.

Storage condition	Packaging material	Asiaticoside (%)	Retention (%)	TVC (CFU/g)
Initial	—	0.98	100	16×10^7
M1 (Refrigerated)	S1 – Glass jar	Spoiled	Spoiled	Spoiled
	S2 – Plastic jar	Spoiled	Spoiled	Spoiled
	S3 – Vacuum pack	0.86[a]	88	10×10^7
	S4 – LDPE bag	Spoiled	Spoiled	Spoiled
	S5 – Aluminium pouch	0.68[c]	70	
	S6 – Cloth bag	0.73[b]	74	
M2 (Ambient)	S1 – Glass jar	Spoiled	Spoiled	Spoiled
	S2 – Plastic jar	Spoiled	Spoiled	Spoiled
	S3 – Vacuum pack	0.61[d]	62	—
	S4 – LDPE bag	Spoiled	Spoiled	Spoiled
	S5 – Aluminium pouch	Spoiled	Spoiled	Spoiled
	S6 – Cloth bag	Spoiled	Spoiled	Spoiled
CD (0.05)		0.025		

Combined influence of temperature and packaging on shelf-life extension

The overall pattern of results suggests that the refrigerated vacuum-packed treatment provided the most favourable environment for preserving *C. asiatica* raw drug. Refrigeration slows down many chemical processes, whereas vacuum packing reduces the oxygen in the package and, depending on the material used, it will provide a barrier against the moisture exchange. The apt material and vacuum in combined form thus give protection to materials stored.

Vacuum packing under ambient conditions preserved the sample avoiding visible spoilage for six months, but the marker-compound retention was lower than the sample stored under refrigerated vacuum condition. Refrigeration alone did not guarantee chemical preservation, as some refrigerated packages were noted as spoiled.

The decline in asiaticoside observed in this study even in the best treatment agrees with the reported literature on *C. asiatica*. Puttarak *et al*., (2016) reported stability changes in a standardized triterpene-enriched extract, and Monton *et al*., (2018) found temperature-dependent changes in asiaticoside and madecassoside in a film-forming formulation. Kaur *et al*., (2016) likewise demonstrated measurable losses of chemical markers during accelerated stability testing of dried extracts. These observations support the present finding that refrigeration can reduce, though not completely prevent, chemical changes during prolonged storage.

From a practical perspective, the findings are relevant to medicinal plant growers, processors and raw-material suppliers who need to maintain quality between harvest and downstream processing. The refrigerated vacuum-pack system identified here may be particularly useful where the raw drug is intended for longer-term storage or transport.

CONCLUSION

The present study demonstrates that storage temperature and packaging material are important determinants of the quality of *C. asiatica* raw drug during extended storage. Among the combinations evaluated, refrigerated storage at 4 °C together with vacuum packaging provided the best overall preservation of the raw drug for six months. This treatment retained 88% of the initial asiaticoside content. The findings indicated that combining temperature control with suitable packaging is more effective than relying on either measure alone.

ACKNOWLEDGEMENT

The author gratefully acknowledges ICAR DMAPR as well as Kerala Agricultural University for providing the necessary facilities and funds to carry out this work.

REFERENCES

Abhishek, G., Shikhar, V., Pradeep., K Sharad, S. and Rawat, AKS. (2014). *Indian Journal of Pharmaceutical Education and Research*, **48**(3),75-79.

Google Scholar

Gohil, K.J., Patel, J.A. and Gajjar, A.K. (2010). Pharmacological Review on *Centella asiatica*: A Potential Herbal Cure-all. *Indian J. Pharm. Sci*.**72**(5):546-56. doi: 10.4103/0250-474X.78519. PMID: 21694984; PMCID: PMC3116297.

Google Scholar

Hyun, J.E., Lee, S. and Park, H.J. (2018). Evaluation of microbial quality of dried foods stored at different relative humidity and temperature, and effect of packaging methods. *Journal of Food Safety*, **38**, e12433. https://doi.org/10.1111/jfs.12433

Google Scholar

Kaur, I., Suthar, N., Kaur, J., Bansal, Y. and Bansal, G. (2016). Accelerated stability studies on dried extracts of Centella asiatica through chemical, HPLC, HPTLC, and biological activity analyses. *Journal of Evidence-Based Complementary & Alternative Medicine*, **21**(4), 261–271. https://doi.org/10.1177/2156587216661468

Google Scholar

Monton, C., Luprasong, C., Suksaeree, J. and Songsak, T. (2018). Validated high-performance liquid chromatography method for simultaneous determination of stability of madecassoside and asiaticoside in film-forming polymeric dispersions. *Revista Brasileira de Farmacognosia*, **28**(3), 289–293.

Google Scholar

Tomar, A. (2008). Folk medicinal plants in Muzaffarnagar district of Western Uttar Pradesh, India. *Journal of Indian Botanical Society*, **87** (3 & 4): 200-208.

Google Scholar

WHO (World Health Organization). (1998). Quality control methods for medicinal plant materials. Geneva: World Health Organization. ISBN 92-4-154510-0.

Google Scholar

WHO (World Health Organization). (2011). Quality control methods for herbal materials. Geneva: World Health Organization. ISBN 978-92-4-150073-9.

Google Scholar