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A preliminary study on diversity of butterflies in *Premna serratifolia*, Tamil Nadu, India

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Abstract
The present study was conducted to explain the interaction between the butterflies and nectar plant *Premna serratifolia.* A total of 24 different butterfly species were found nectaring on this plant, belonging to 4 different families. The highest number of species was documented in the Nymphalidae family (11), followed by Papilionidae (8), Pieridae (2), Hesperiidae (2) and Lycaenidae (1). Butterflies were attracted to this plant due to the presence of bioactive constituents in the flowers of *Premna serratifolia*. Qualitative analysis is essential to identify the phytochemical constituents present in the flowers of the *Premna serratifolia.* Alkaloids, Phenols, Flavonoids and Tannins present in these flowers were found to attract butterflies as a source of food. Each of these components has specific biological significance in the life cycle of these butterflies. This study explains the importance of phytochemical constituents present in the flowers of the nectarine plant *Premna serratifolia* and also the butterfly diversity. The presence of butterflies indicates a healthy, biodiverse environment. Hence, it is important for us to protect the butterfly diversity by conserving and planting more nectar plants for butterflies on Campus.

Keywords: Butterfly diversity, *Premna serratifolia*, phytochemicals, Qualitative analysis, Queen Mary's College

Introduction
Butterflies are the most fascinating flying insects depending on plant source for food and to complete their life cycle. Butterflies are significantly important component for our biodiversity. In addition to their crucial role in the ecosystem, they are regarded as biological indicators of the health of terrestrial habitats. (Kunte 2000; Aluri and Rao 2002; Thomas 2005) [4, 32, 46]. Butterflies exhibit wide dispersal and distribution (Padhye *et al*. 2006; Ramesh *et al.* 2010) [36, 39] in the order Lepidoptera. India is the home to about 20% of the world's butterfly species (Kunte 2000; Kumari *et al.* 2016) [32] Kren31n. They play a valuable role as a pollinator (Kunte 2000; Tiple *et al.* 2009) [32, 47] and indicators of environmental health and are also admired for their beauty (Chakravathy *et al.* 1997).

Butterflies rely exclusively on flower nectar, the juice of overripe fruits, honeydew and other liquid substance including minerals. They source these nutrients from their environment by visiting flowers, bird droppings, decaying fruits and mud puddles in suitable locations. Apart from this, their presence Rely on the variety of factors including climate, suitable foods, suitable areas for flight and courtship. They are very active during the day, some species like the Dark evening brown (*Melanitis phedima*, Cramer 1780) from the Nymphalidae family, are also known to fly at night (Adler and Pearson 1982) [3].

Butterflies often serve as a good "Flagship" species for biodiversity inventories (Lawton *et al*. 1998). They exhibit opportunistic behaviours (Courtney and Shapiro 1986) [12] when gathering nectar, but their flower selection is usually not common; they often prefer certain flower species (Gutierrez *et al.*1998) [18]. These insects have a history of co-evolution with both host and nectar plants (Ehrlich and Raven 1964). As herbivorous insects, the availability of larval hosts and nectar plants significantly influences butterfly diversity (Culin 1997; Solman Raju 2004) [13, 43].

Most butterflies derive all their nourishment during the larval stage, while adults primarily replenish their energy through carbohydrates found in nectar. Additionally, nectar and other adult food sources can supply some amino acids to make up for inadequate nutrition during the larval phase (Mevi-Schutz and Erhardt 2003) [35].

In the present study, the objective was to study the species diversity of butterflies nectaring on *Premna serratifolia* in Queen Mary's College Campus, Chennai - 600 004. The phytochemical constituents of the necatrine plant *Premna serratifolia* along with this Fourier Transform Infrared Spectroscopic Analysis (FTIR) was performed to identify the presence of various functional groups that exist in the methanol extract of the flowers of *Premna serratifolia* that attracts these butterflies to this plant.

Materials and methods
Survey and Identification
The field survey was conducted for a period of six months from July 2024 to Dec 2024 at Queen Mary's College Campus. Photographs were taken with the help of Canon EOS 200D II DSLR Camera and Mobile phone *vivo* V27. Identification and classification of butterflies and scientific names was done using the field guide of Kehimkar (2016) [26] and no specimen was collected for the study.

Collection of plant material
Premna serratifolia (family – Lamiaceae) commonly called as Headache tree, in Tamil "Pasumunnai" is commonly used as a medicinal plant to treat various diseases and also attracts butterflies and bees. It flowers and fruits between May and November. During flowering season, it attracts a large number of butterflies and bees.

Fresh flowers of *Premna serratifolia* were collected from the Queen Mary's College Campus, Chennai – 04. The plant was identified taxoomically and authenticated in the Department of Plant Biology and Biotechnology, Queen Mary's College, Chennai, Tamil Nadu, India. The flowers were collected, cleaned, shade dried within a temperature range of 35℃ to 40℃. After drying, electrical grinder was used to crush the dried flowers into a coarse powder.

Fig 1: Nectarine plant *Premna serratifolia*

Preparation of plant extract by Soxhlet method

Extraction refers to separating the desired material by physical or chemical means with the aid of the solvent.

Active substances were extracted from the powdered flowers by methanol extraction method. The extracted powder was re-suspended in the respective organic solvent at a concentration of 10g/100ml in Soxhlet at 50°C for 8+8hrs.

The powdered flowers (10g/100ml) of *Premna serratifolia* were successively extracted using methanol solvent. After extraction, extract was dried by distilling the solvents in a rotary vacuum evaporator (Verma *et al*. 2010) [48]. The yield of methanolic extract was about 0.3g. The extract was dissolved in the respective solvent to get the required concentration of plant extract.

Drying of the extract

Lyophilized in freeze dryer, freeze the product at -80°C, lowering pressure, then the ice was removed by sublimation. Weight of Extract yield: 0.3g % of yield = Dry weight of the extract×100/ weight of dry flowers = 0.3×100/10 = 30%

Phytochemical analysis

Qualitative estimation of secondary metabolites was performed by using Harborne method (1973) [19]. The methanol extracts were subjected to this analysis.

Results

Table 1: List of butterflies nectaring on *Premna serratifolia*

S. No	Scientific name	Common name
	Family: Papilionidae	
1	*Graphium sarpedon* (Linnaeus, 1758)	Blue Bottle
2	*Graphium doson* (C. & R. Felder, 1864)	Common Jay
3	*Graphium agamemnon* (Linnaeus, 1758)	Tailed Jay
4	*Graphium nomius* (Esper, 1793)	Spot Swordtail
5	*Papilio polytes* (Linnaeus, 1758)	Common Mormon
6	*Papilio demoleus* (Linnaeus, 1758)	Lime Swallowtail
7	*Pachliopta aristolochiae* (Fabricius, 1775)	Common Rose
8	*Pachliopta hector* (Linnaeus, 1758)	Crimson Rose
	Family: Nymphalidae	
9	*Euploea core* (Cramer, 1780)	Common Crow
10	*Euploea sylvester* (Fabricius, 1775)	Double Branded Crow
11	*Tirumala limniace* (Cramer, 1775)	Blue Tiger
12	*Tirumala septentrionis* (Butler, 1874)	Dark Blue Tiger
13	*Parantica aglea* (Stoll, 1782)	Glassy Blue Tiger
14	*Danaus chrysippus* (Linnaeus, 1758)	Plain Tiger
15	*Danaus genutia* (Cramer, 1779)	Striped Tiger
16	*Acraea terpsicore* (Linnaeus, 1758)	Tawny Coster
17	*Phalanta phalantha* (Drury, 1773)	Common Leopard
18	*Hypolimnas bolina* (Linnaeus, 1758)	Great Eggfly
19	*Hypolimnas misippus* (Linnaeus, 1758)	Danaid Eggfly
	Family: Pieridae	
20	*Catopsilia pomona* (Fabricius, 1775)	Common Emigrant
21	*Catopsilia pyranthe* (Latreille, 1758)	Mottled Emigrant
	Family: Hesperiidae	
22	*Hasora chromus* (Cramer, 1782)	Common Banded Awl
23	*Parnara bada* (Moore, 1878)	Oriental Straight Swift
	Family: Lycaenidae	
24	*Prosota nora* (R. Felder, 1860)	Tailless Line Blue

Fig 2: 1. Blue Bottle *(Graphium sarpedon*), 2. Common Jay (*Graphium doson*), 3. Tailed Jay (*Graphium agamemnon*), 4. Spot Swordtail (*Graphium nomius*), 5. Common Mormon (*Papilio polytes*), 6. Lime Swallowtail (*Papilio demoleus*), 7. Common Rose (*Pachliopta aristolochiae*), 8. Crimson Rose (*Pachliopta hector*), 9. Common Crow (*Euploea core*), 10. Double Branded Crow (*Euploea sylvester*), 11. Blue Tiger (*Tirumala limniace*), 12. Dark Blue Tiger (*Tirumala septentrionis*), 13. Glassy Blue Tiger (*Parantica aglea*), 14. Plain Tiger (*Danaus chrysippus*), 15. Striped Tiger (*Danaus genutia*), 16. Tawny Coster (*Acraea terpsicore*), 17. Common Leopard (*Acraea terpsicore*), 18. Great Eggfly (*Hypolimnas bolina*), 19. Danaid Eggfly (*Hypolimnas misippus*), 20. Common Emigrant (*Catopsilia pomona*), 21. Mottled Emigrant (*Catopsilia pyranthe*), 22. Common Banded Awl (*Hasora chromus*), 23. Oriental Straight Swift (*Parnara bada*), 24. Tailless Line Blue (*Prosota nora*).

Table 2: Phytochemical analysis of flowers of *Premna serratifolia*

S. No	Phytochemical components	Methanol extract
1.	Carbohydrates	+ ve
2.	Resins	+ ve
3.	Alkaloids	+ ve
4.	Flavonoids	+ ve
5.	Phenols	+ ve
6.	Terpenoids	- ve
7.	Saponins	- ve
8.	Tannins	- ve

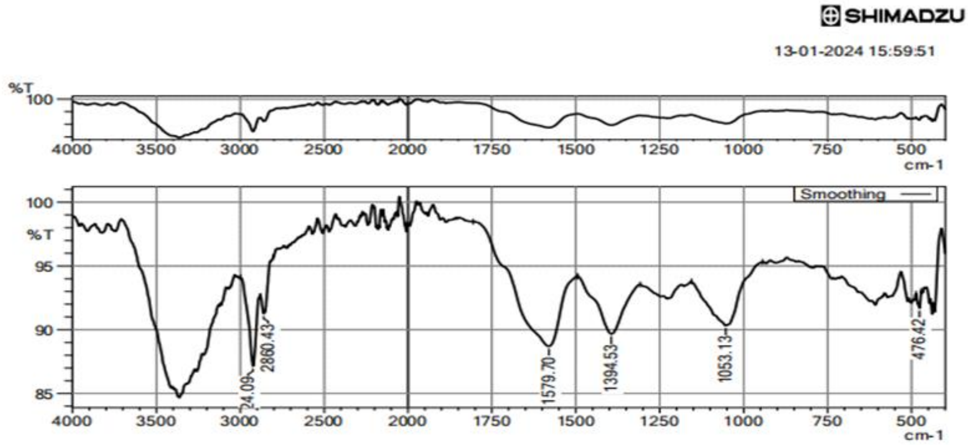
Fourier Transform Infrared Spectroscopic (FTIR) analysis

Fourier Transform Infrared Spectroscopic analysis was performed to identify the presence of various functional groups that exist in the methanol extract of the flowers of *Premna serratifolia*, the observed results were presented in Table 3 and Figure 3. The FTIR spectrum was observed between 400 cm^{-1} and 4000 cm^{-1}. The FTIR spectrum reveals the presence of various functional groups characterized by their stretching and bending vibrations with the bond length.

The results presented in Table 3 reveal the presence of six functional groups in the tested plant extract. A strong broad peak observed at 2924.09 cm^{-1} indicates the presence of carboxylic acid represented by OH bonding, the stretch observed at 2860.43 cm^{-1} represents the functional group alkane (C-H), and a strong peak recorded at 1579.70 cm^{-1} represents the presence of cyclic alkane with C=C bonding. Presence of sulfate ester group was recorded with a strong peak observed at 1394.53 cm^{-1}, the peak observed at 1053.13 cm^{-1} represents the presence of primary alcohol (C-O) and the presence of alkyl halides was recorded with the presence of a median stretch at 476.42 cm^{-1}. The results indicate the presence of various functional groups that exist in the methanol flower extract of *Premna serratifolia*.

Table 3: Fourier Transform Infrared spectroscopic analysis of methanol mediated flower extract of *Premna serratifolia*

Wavenumber cm^{-1}	Functional Group	Absorption Intensity
2924.09	Carboxylic acid (O-H) Bending	Strong
2860.43	Alkane (C-H) Stretching	Medium
1579.70	Cyclic alkane (C=C) Stretching	Medium
1394.53	Sulfate ester (S=O) Bending	Strong
1053.13	Primary alcohol (C-O) Bending	Strong
476.42	Alkyl halides (C-I) Bending	Medium



	Item	Value
	Acquired Date&Time	13-01-2024 15:58:54
	Acquired by	System Administrator
	Filename	E:\NRC-FTIR-DATA\FTIR-2024\JAN 2024\26609-13-01-24\1.ispd
	Measure Parameter File	
	Spectrum name	Smoothing
	Sample name	TEST
	Sample ID	
	Option	
	Comment	
	No. of Scans	30
	Resolution	4 [cm-1]
	Apodization	Happ-Genzel

	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	476.42	91.70	1.29	486.06	470.63	119.935	11.219	
2	1053.13	90.33	4.20	1155.36	943.19	1569.980	415.581	
3	1394.53	89.68	4.17	1494.83	1307.74	1503.332	357.066	
4	1579.70	88.71	6.65	1805.37	1494.83	2132.918	995.565	
5	2860.43	91.27	2.07	2877.79	2802.57	497.564	58.673	
6	2924.09	87.15	6.00	2997.38	2883.58	1037.279	281.824	

Fig 3: Fourier transform infrared spectroscopic analysis report

Discussion

Butterflies maintain a crucial relationship with nectar plants, which significantly influence butterfly populations. Butterflies generally feed on sweet liquids like flower nectar for energy. Most butterflies prefer specific nectar plants. The flowers of *Premna serratifolia* are bisexual, greenish white in terminal corymbose panicled cyme with short corolla tube. In flowers the length of the corolla tube facilitates the butterflies to suck nectar.

A total of 24 species of butterflies belonging to four different families, such as Papilionidae, Nymphalidae, Hesperiidae and Lycaenidae were recorded in the *Premna serratifolia* tree present at Queen Mary's College Campus. The nectaring behaviour of butterflies depends on various factors such as corolla depth, flower clustering and butterfly characteristics including the proboscis length, body mass and wing loading (Porter *et al*. 1992) [38]. Butterflies like *Graphium agamemnon* (Tailed Jay), *Papilio demoleus* (Limeswallow Tail) and *Catopsilia pyranthe* (Mottled Emigrant) are known to be eager visitors of flowers of *Premna serratifolia* for nectaring. Nectar – feeding butterflies display distinctive proboscis movements while interacting with flowers (Krenn 2010) [30]. According to Krenn 1990 Kren28n, the proboscis movement takes on a flat, vertical spiral shape when at rest. It consists of two main parts and works mainly through hydraulic mechanics. The coiling action is accomplished by the contraction of intrinsic galeal muscles along with the elasticity of the proboscis, while the internal stipes muscle contracts to return the proboscis to its resting state.

Kunte 2000 [32], reports that there is a limited knowledge about the feedin g habits and food resources of adult butterflies compared to larvae. In general*Graphium sarpedon* (Blue Bottle) butterflies use chemoreceptors on its appendages to locate flowers with nectar. Butterflies extend the proboscis deep into a flower to sip nectar, they coil when not in use. The *Graphium doson* (Common Jay) is active throughout the day, while feeding it never settle down and keeps its wings flapping.

Premna serratifolia flower has a short corolla, allows both longer and shorter proboscis butterflies to feed on it. Butterflies with shorter proboscis may struggle to feed from flowers that have long corollas. During bright sunshine, *Papilio polytes* (Common Mormon) butterfly is attracted to the flowers, whereas males are found flying above the Inflorescence. This active species flies in a zig-zag pattern and lands only to feed. The *Pachliopta aristolochiae* (Common Rose) butterfly slowly approaches flowers to sip their nectar by diving down with its wings held back for feeding. The *Pachliopta hector* (Crimson Rose) butterfly has a gentle, fluttering flight when it feeds.

Several butterflies, including the *Euploea core* (Common Crow), *Tirumala limniace* (Blue Tiger), *Hypolimnas bolina* (Great Eggfly), *Graphium nomius* (Spot Swordtail) and *Acraea terpsicore* (Common Leopard), move from flower to flower in search of nectar. The *Euploea core* (Common Crow) has a distinctive preference for plant fluids high in alkaloids, while *Tirumala limniace* (Blue Tiger) butterflies move from flower to flower by snapping their wings. *Danaus chrysippus* (Plain Tiger) and *Danaus genutia* (Striped Tiger) butterflies obtain nectar by deeply bending their heads into the flowers. *Hasora chromus* (Common Banded Awl) butterflies specifically nectarine from flowers in the Lamiaceae family, *Premna serratifolia.* Skippers primarily move their proboscis up and down, with a slight side-to-side bend at the tip. Their usual feeding behavior involves lifting the proboscis off the surface before extending or flexing the tip (Krenn 2008) [29]. *Prosota nora*

(Tailless Line Blue) butterflies are generally found in areas abundant with flowering plants like *Premna serratifolia*.

The Role of Phytochemicals in Attracting Butterflies

Phytochemical analysis of *Premna serratifolia* reveals the presence of carbohydrates, resins, alkaloids, flavonoids, phenols, terpenoids, saponins and tannins. Butterflies get attracted to *Premna serratifolia* due to the bioactive components in its flowers, which are vital, for their nourishment and life cycle.

Insects generate a wide range of sex pheromones to draw in mates of the same species. These pheromones are highly specific to each species, characterized by distinct chemical structures and unique combinations produced through specific biosynthetic pathways. Additionally, some insects utilize certain plant secondary metabolites to attract and engage their partners during courtship. *Premna serratifolia* contains alkaloids that attract lepidopterans, particularly butterflies from the Nymphalidae family. Butterflies feed on the nectar of *Premna serratifolia* flowers, which are enriched with alkaloids, to produce their own sex pheromones. The attraction is largely driven by olfactory signals, as the floral scent may include volatile compounds found in decaying plant materials that are particularly appealing to male butterflies. Alkaloids may also serve as a nitrogen-rich nutrient source (Thomas Eisner and Jerrold Meinwald 2003) [45].

In addition to nutritional needs, animals may consume particular phytochemicals for self-medication, chemical defense, or pheromone production. The term pharmacophagy refers to this behavior, which is common across the animal kingdom (Boppre M 1984) [6]. Insects show this behavior in contexts of self-medication (Abbott J 2014) [1], as seen in honey bees (Erler S and Moritz RFA 2016) [15], monarch butterflies (Lefevre T *et al*. 2010) [34], and some moths (Smilanich AM 2011) [44] as well as for defense and pheromone synthesis in various insect orders. Among the extensively studied plant chemicals involved in insect-plant interactions are pyrrolizidine alkaloids (PAs), known for their hepatotoxic properties and significant ecological roles, especially among arctiid moths and certain butterfly subfamilies, such as Danainae and Ithomiinae (Nymphalidae), (Honda K 2008; Boppre M 1990 and Hartmann T 2009) [8, 20, 21]. Field observations have shown that adult males of many danaine butterflies gather around dead or decaying plant parts or flowers containing PAs, primarily from the families Boraginaceae, Asteraceae, and Fabaceae to absorb these alkaloids (Pliske TE, 1975; Boppre M 1981) [5, 37].

Male butterflies have a pronounced attraction to PA sources, likely due to their use in sex pheromone production (Schneider D *et al*. 1975; Honda K *et al*. 2016) [22, 41]. Male danaine butterflies possess two types of androconial organs: eversible abdominal hairbrushes and alar glands (Ackery PR and Vane-Wright RI 1984; Boppre M and Vane-Wright RI 1989) [7]. During courting, males extrude these hairpencils, releasing chemicals that females can detect. Components derived from PAs such as danaidone (DO) and hydroxydanaidal (HD) are present in many danaines, with DO particularly significant in the courtship process of species like Danaus gilippus and Idea leuconoe (Honda *et al*. 2018) [23]. Insects comprise the most diverse animal group on Earth, making up over half of all known organisms. While they typically depend on plants' primary metabolites (carbohydrates, lipids, proteins), plants have developed a wide range of secondary metabolites (alkaloids, terpenoids, phenolics) to defend against herbivory. Insects face ongoing challenges to detoxify or evade plant defenses, yet they have also evolved cooperative relationships with plants through pollination. This intricate ecological interaction has led to the development of highly sensitive chemical detection systems in insects, enabling them to locate host plants and nectar sources, avoid harmful plants, escape predators, and communicate with conspecifics (Huffman MA 2003) [24].

Female butterflies generally exhibit a higher concentration of flavonoids than males, possibly indicating that these compounds play a role in visual mate recognition (Martina Geuder *et al.* 1997) [17]. Flavonoids are found in species of the Papilionidae, Lycaenidae, and Nymphalidae families the reason behind their presence is due to feeding on their nectarine plant. Feeding experiments have demonstrated that the flavonoids come from dietary sources (Wilson 1985; Wiesen *et al*.1994; Burghardt *et al*. 1995) [9, 49, 50]. Ford (1941) [16] proposed that flavonoids could influence wing colouration in diurnal species. Tannins are key plant secondary metabolites that play a significant role in plant defense against herbivorous insects.

Insects that feed on plants with secondary metabolites often gain additional benefits. For instance, plants containing flavonoids allow insects to sequester these compounds for protection against pathogens and predators or to enhance male-attracting features, such as wing colouration (Samanta *et al*. 2011) [40]. Flavonoids, synthesized throughout the plant, contribute to the colour, fragrance, and flavour of fruits, flowers, and seeds, making them appealing to various pollinators and aiding in pollen or seed dispersal. These compounds have been found to influence oviposition and feeding behaviours. For instance, naringenin, hesperetin-7-O-rutinoside, and quercetin-3-O-rutinoside can enhance egg-laying in the swallowtail butterfly *Papilio* that feeds on citrus plants (Justyna Mierziak *et al*. 2014) [25].

Phenols are common in flowering plants and act as biologically active secondary metabolites that help protect against ultraviolet radiation and pathogen attacks. While some studies have linked tannins with beneficial nutritional factors, they primarily serve as feeding deterrents for various herbivorous species. While these compounds may deter non-adapted herbivores, they can stimulate feeding in species that have adapted to them (Schultz 1989) [42]. Recent research has focused on insect feeding behaviour (CardinalAucoin *et al*. 2009) [10], showing that using artificial diets with tannins tends to exaggerate their effects on food preference and consumption (with some exceptions noted in caterpillar studies), (Klocke and Chan 1982) [27]. The bioactive constituents present in the flowers of the *Premna serratifolia* attracts butterflies for it source of food and has considerable biological significance in their life cycle

Conclusion

Butterflies have tremendous relationship with nectar plants. Nectar plants are significantly important for supporting butterfly species population. Food for adult butterflies usually consists of sweet liquids, such as nectar from flowers, that provide energy. Almost all the butterflies select specific nectarine plants for feeding and this study explains as to why butterflies are attracted to *Premna serratifolia.* Thus, conserving and protecting this plant species will enhance the diversity of butterflies which are playing an important role as biological indicators in the environment.

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