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PHYTOCHEMICAL COMPOSITION, ANTIOXIDANT ACTIVITY AND POLLINATOR VISITATION IN SELECTED MEDICINAL PLANTS FROM KALWAN REGION, NASHIK, MAHARASHTRA, INDIA

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ABSTRACT

Medicinal plants are valuable sources of bioactive compounds and also play an important role in maintaining ecological interactions by supporting pollinator diversity. The present study was conducted during 2023–2024 in the Kalwan region of Nashik district, Maharashtra, to examine phytochemical composition, antioxidant activity, and pollinator visitation in five medicinal plant species: Ocimum sanctum, Azadirachta indica, Hibiscus rosa-sinensis, Vitex negundo, and Calotropis gigantea. Phytochemical screening confirmed the presence of phenolic compounds and saponins in all species. Antioxidant activity, evaluated using the DPPH assay, showed clear variation among plants, with Ocimum sanctum exhibited the highest antioxidant activity (71.8%), followed by Azadirachta indica (65.2%), Calotropis gigantea (63.5%), Vitex negundo (61.8%), and Hibiscus rosa-sinensis (56.2%). Field observations indicated that bees were the dominant pollinators, while butterflies and flies were recorded in lower numbers. A consistent trend was observed in which plants with higher flavonoid content attracted greater pollinator visits. Overall, the study demonstrates a clear association between phytochemical composition and pollinator attraction, highlighting the ecological importance of medicinal plants in semi-arid regions.

KEYWORDS: *Phytochemicals, Antioxidant activity, Pollinators, Medicinal plants, DPPH, Kalwan*

INTRODUCTION

Medicinal plants have long been an important part of traditional healthcare systems because they contain a wide range of bioactive compounds such as alkaloids, flavonoids, tannins, and phenolic substances (Harborne, 1998; Trease and Evans, 2009). These phytochemicals are responsible for many therapeutic properties and also function as antioxidants, helping to reduce damage caused by free radicals in biological systems (Brand-Williams *et al.,* 1995; Sharma *et al.,* 2022). Compared to synthetic alternatives, plant-based antioxidants are generally considered safer and are widely preferred due to their lower side effects (Patil *et al.,* 2023).

The concentration of these compounds is not constant and may vary depending on environmental factors such as temperature, light intensity, and soil conditions (Jadhav *et al.,* 2022). Such variations can influence not only the medicinal value of plants but also their ecological interactions. Floral characteristics like colour, scent, and nectar production, which are controlled by plant chemicals, play a major role in attracting pollinators (Shinde *et al.,* 2024). Pollinators including bees, butterflies, flies, and ants are essential for successful plant reproduction, as they assist in pollination and seed formation (Suryanarayana *et al.,* 2023). These insects are guided by both visual and chemical signals, and compounds such as flavonoids contribute to flower colour and ultraviolet (UV) patterns that are visible to them (Glover, 2014; Rao *et al.,* 2021). Despite the importance of both phytochemicals and pollinators, studies combining these two aspects in Indian medicinal plants are still limited. The Kalwan region of Nashik district, with its rich plant diversity and suitable environmental conditions, provides an ideal location for such investigations. Therefore, the present study was undertaken to analyze phytochemical composition, antioxidant activity, and pollinator visitation in selected medicinal plants and to understand their interrelationship.

MATERIALS AND METHODS

Study Area

The investigation was carried out in the Kalwan region of Nashik district, Maharashtra, India, during the period 2023–2024. The study area is characterized by a semi-arid climate with moderate rainfall, as reported by the Indian Meteorological Department (IMD, 2023).

Plant Selection

Five medicinal plant species commonly distributed in the study area were selected for analysis. These included *Ocimum sanctum* L. (Tulsi), *Azadirachta indica* A. Juss. (Neem),

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Hibiscus rosa-sinensis L. (Hibiscus), *Vitex negundo* L. (Nirgudi), and *Calotropis gigantea* (L.) Dryand. (Milkweed).

Preparation of Plant Extracts

Fresh plant materials were collected from the field, thoroughly washed with water to remove impurities, and shade-dried for a period of two to three weeks. The dried samples were then powdered using a grinder. For extraction, 10 g of each powdered sample was soaked in 100 mL of methanol and kept undisturbed for 48 hours. The mixture was subsequently filtered, and the filtrate was collected for further analysis (Harborne, 1998).

Phytochemical Screening

Qualitative phytochemical analysis was performed using standard protocols (Trease and Evans, 2009). Alkaloids were detected using Mayer's test, flavonoids by the Shinoda test, tannins by the ferric chloride test, saponins by the foam test, and phenols by the lead acetate test. The results were recorded as present (+), absent (−), or strongly present (++).

Antioxidant Activity (DPPH Assay)

Antioxidant activity was evaluated using the DPPH radical scavenging method (Brand-Williams *et al.,* 1995). A 0.1 mM solution of DPPH was prepared in methanol. For each sample, 2 mL of DPPH solution was mixed with 2 mL of plant extract at a concentration of 100 µg/mL. The reaction mixture was kept in the dark for 30 minutes, after which absorbance was measured at 517 nm using a spectrophotometer. All experiments were carried out in triplicate. The percentage inhibition was calculated using the following formula:

% Inhibition = [(A control – Ensample) / A control] × 100

Pollinator Observation

Pollinator activity was recorded during peak flowering hours between 8:00 AM and 11:00 AM (Kunte, 2023). For each plant species, five individual plants were selected, and observations were conducted for one hour per plant. Visiting insects were counted and categorized into bees, butterflies, flies, and ants based on their taxonomic groups.

Statistical Analysis

All data were expressed as mean ± standard deviation (SD). Basic descriptive statistics and trend analysis were used to evaluate variation among plant species.

RESULTS

Phytochemical Composition

Phytochemical analysis showed that phenolic compounds and saponins were present in all five plant species. *Ocimum sanctum*, *Azadirachta indica*, *Vitex negundo*, and *Calotropis gigantea* exhibited the presence of all tested phytochemicals, whereas *Hibiscus rosa-sinensis* lacked alkaloids. Flavonoids were detected in all species, with comparatively higher levels recorded in four plants **(Table 1).** Tannins were present in all studied species, while phenols showed strong presence in *O. sanctum*, *A. indica*, *V. negundo*, and *C. gigantea*. Saponins were consistently observed across all plants.

Table 1: Phytochemical Composition of Selected Medicinal Plants

Plant	Alkaloids	Flavonoids	Tannins	Phenols	Saponins
Tulsi (*O. sanctum*)	+	++	+	++	+
Neem (*A. indica*)	+	++	+	++	+
Hibiscus (*H. rosa-sinensis*)	–	+	+	+	+
Nirgudi (*V. negundo*)	+	++	+	++	+
Milkweed (*C. gigantea*)	+	++	+	++	+

(+ = present, ++ = strongly present, – = absent)

Antioxidant Activity

The DPPH assay indicated clear differences in antioxidant activity among the selected plant species. *Ocimum sanctum* exhibited the highest activity, followed by *Azadirachta indica*, *Calotropis gigantea*, and *Vitex negundo*. *Hibiscus rosa-sinensis* showed comparatively lower activity. These variations are presented in **Table 2.** The variation in antioxidant activity among the five plant species is illustrated in **Fig. 1.**

Table 2: Antioxidant Activity of Plant Extracts

Plant	% Inhibition (Mean ± SD)
Tulsi (*O. sanctum*)	71.8 ± 1.2
Neem (*A. indica*)	65.2 ± 1.8
Milkweed (*C. gigantea*)	63.5 ± 1.4
Nirgudi (*V. negundo*)	61.8 ± 1.6
Hibiscus (*H. rosa-sinensis*)	56.2 ± 1.5

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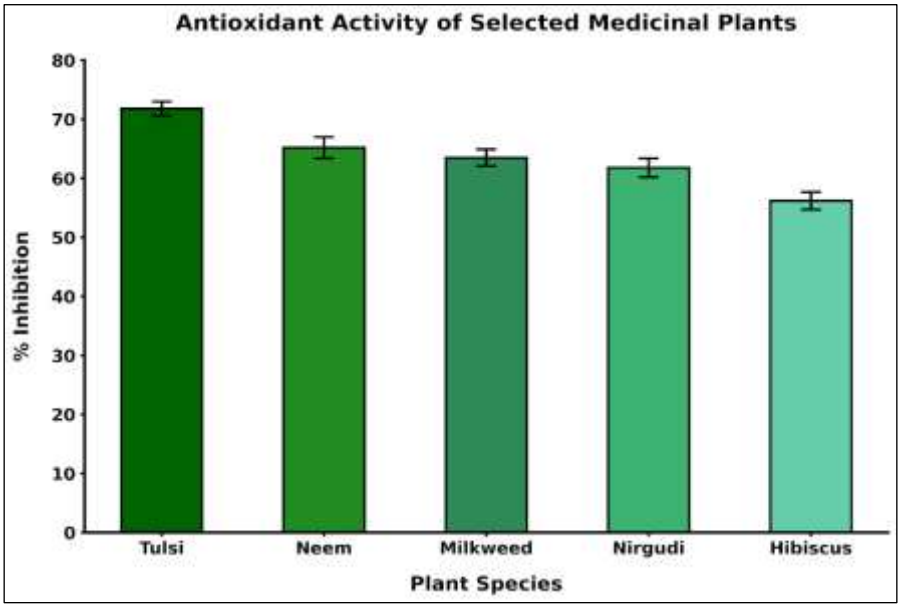


Fig. 1. Pollinator visitation patterns across plant species showing visits per hour by different pollinator groups. Bees dominated visitation across all species, with comparatively lower contributions from flies and ants.

Pollinator Visitation

Pollinator observations indicated that bees were the dominant visitors across all plant species, contributing the highest proportion of total visits. Among the studied plants, *Ocimum sanctum* received the highest number of visits (46.2 per hour), followed by *Calotropis gigantea* (44.8), *Azadirachta indica* (42.4), *Vitex negundo* (40.2), and *Hibiscus rosa-sinensis* (37.0). Butterflies showed a greater preference for *Hibiscus rosa-sinensis*, whereas ants were more frequently observed on *Azadirachta indica*. Flies were recorded on all plants with relatively uniform visitation patterns **(Table 3).** The distribution of different pollinator groups across the five plant species is presented in **Fig. 2.**

Table 3: Pollinator Visits per Hour (Mean ± SD)

Plant	Bees	Butterflies	Flies	Ants	Total
Tulsi (*O. sanctum*)	24.2 ± 2.1	11.2 ± 1.6	5.2 ± 1.1	6.0 ± 1.2	46.2 ± 3.1
Neem (*A. indica*)	20.2 ± 1.9	7.2 ± 1.3	6.0 ± 1.2	9.2 ± 1.5	42.4 ± 2.7
Hibiscus (*H. rosa-sinensis*)	15.0 ± 1.5	13.8 ± 1.7	5.0 ± 1.0	3.2 ± 0.8	37.0 ± 2.4
Nirgudi (*V. negundo*)	21.2 ± 1.8	9.2 ± 1.4	5.2 ± 1.1	5.0 ± 1.0	40.2 ± 2.6
Milkweed (*C. gigantea*)	22.2 ± 2.0	10.0 ± 1.5	5.8 ± 1.2	7.0 ± 1.3	44.8 ± 2.9

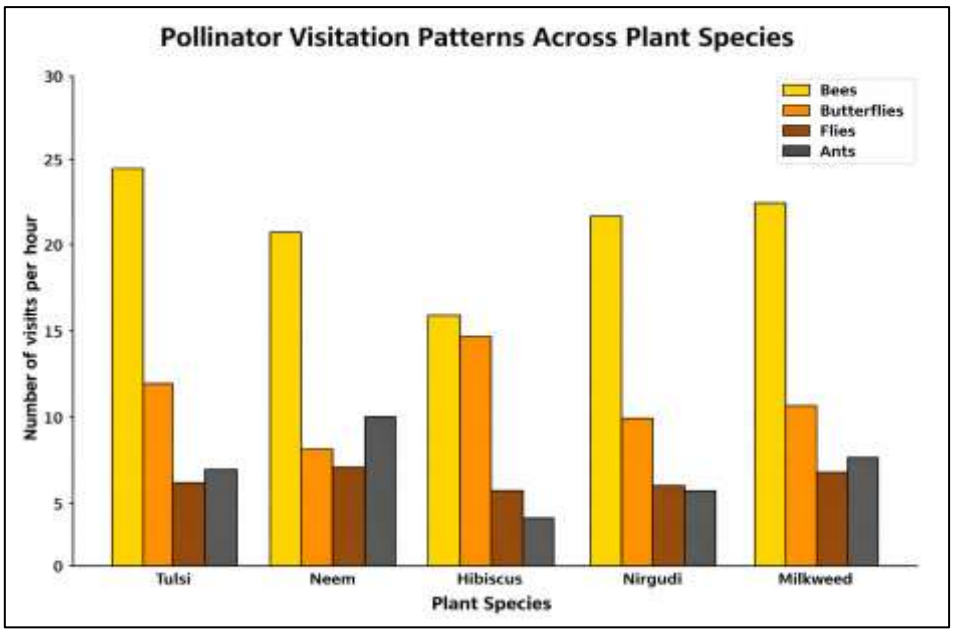


Fig. 2. Pollinator visitation patterns across plant species showing visits per hour by different pollinator groups. Bees dominated visitation across all species, with comparatively lower contributions from flies and ants.

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SUMMARY OF FINDINGS

A comparative evaluation of phytochemical composition, antioxidant activity, and pollinator visitation showed a clear and consistent trend. Plant species with higher flavonoid levels, including *Ocimum sanctum*, *Azadirachta indica*, *Vitex negundo*, and *Calotropis gigantea*, recorded greater pollinator visits than *Hibiscus rosa-sinensis*, which had moderate flavonoid content. A similar pattern was observed for antioxidant activity, where species with higher phenolic content exhibited stronger activity **(Table 4).** The positive correlation between flavonoid content and pollinator visitation is shown in **Fig. 3.**

Table 4: Summary of Key Findings

Plant	Flavonoid Level	Total Pollinator Visits	Antioxidant Activity (%)
Tulsi (*O. sanctum*)	High	46.2	71.8
Neem (*A. indica*)	High	42.4	65.2
Milkweed (*C. gigantea*)	High	44.8	63.5
Nirgudi (*V. negundo*)	High	40.2	61.8
Hibiscus (*H. rosa-sinensis*)	Moderate	37.0	56.2

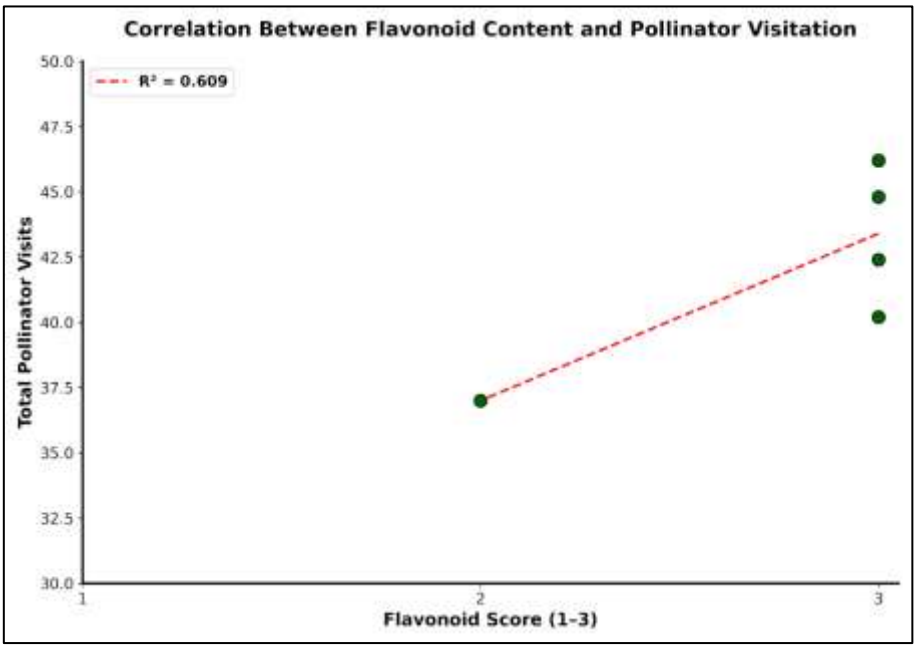


Fig. 3. Correlation between flavonoid content and total pollinator visits across plant species. A positive relationship ($R^2 \approx 0.61$) indicates that higher flavonoid levels are associated with increased pollinator visitation.

DISCUSSION

The present study evaluated the phytochemical composition, antioxidant activity, and pollinator visitation of five commonly occurring medicinal plants from the Kalwan region. The results revealed noticeable variation among the species across all these parameters.

All five plants were found to contain phenolic compounds and saponins, which are known to play a role in plant defense against pathogens and herbivores (Harborne, 1998). Among the studied species, Tulsi, Neem, Nirgudi, and Milkweed showed the presence of all tested phytochemicals, supporting their traditional medicinal importance (Patil *et al.,* 2023). In contrast, Hibiscus lacked alkaloids, a feature commonly reported for members of the Malvaceae family (Trease and Evans, 2009). The antioxidant assay indicated that Tulsi exhibited the highest free radical scavenging activity, which is consistent with earlier findings highlighting its strong antioxidant potential (Sharma *et al.,* 2022). This enhanced activity may be attributed to its higher phenolic content. Nirgudi displayed moderate antioxidant activity, aligning with previous reports (Patil *et al.,* 2023), while Hibiscus showed comparatively lower activity, which falls within the expected range for its methanolic extracts (Rao *et al.,* 2021). Pollinator observations indicated that bees were the dominant pollinators in the study area. They visited all plant species, with a higher preference for Tulsi, Milkweed, and Neem, which is in agreement with earlier studies conducted in tropical regions (Suryanarayana *et al.,* 2023). Nirgudi also attracted a considerable number of bees, likely due to its floral characteristics such as colour and structure (Kunte, 2023). Butterflies were more frequently observed on Hibiscus, possibly because of its large and brightly coloured flowers (Rao *et al.,* 2021). A key outcome of this study is the positive association between flavonoid content and pollinator visitation. Flavonoids contribute to flower coloration and ultraviolet patterns that are visible to insects, thereby enhancing their

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attraction (Shinde *et al.*, 2024). This explains the higher pollinator visits observed in Tulsi, Neem, Nirgudi, and Milkweed, which showed strong flavonoid presence. The relationship between antioxidant activity and pollinator visitation was moderate, suggesting that while these two parameters may be influenced by overlapping metabolic pathways, they are not directly functionally linked. This may be due to the fact that antioxidant activity is mainly related to plant defense mechanisms, whereas pollinator attraction depends on visual and chemical signals (Jadhav *et al.*, 2022). However, the study has certain limitations. The analysis was restricted to only five plant species, which may limit the general applicability of the findings. Additionally, phytochemical assessment was qualitative in nature. Future research should focus on quantitative estimation of phytochemicals and include a wider range of plant species for better understanding.

CONCLUSION

This study shows that medicinal plants from the Kalwan region contain valuable phytochemicals that contribute to their antioxidant properties. Tulsi showed the highest antioxidant activity. Bees were the most common pollinators, with Tulsi and Milkweed attracting the most visitors. Plants with higher flavonoid content attracted more pollinators, suggesting that these chemicals play a role in both medicine and ecology. This study provides one of the first integrative assessments in the Kalwan region and highlights the importance of protecting both medicinal plants and their pollinators.

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