Exploring the Pharmacological Potential of *Pandanus tectorius* **Floral Extract: An Investigation of its Therapeutic Activities and Bioactive Compounds**

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ABSTRACT

Background: Recent scientific research has shed light on the potential medicinal benefits of edible and wild floral extracts. Materials and Methods: The antioxidant activity of root, stem, leaf, bract and floral extracts of *P*. *tectorius* was investigated using DPPH, ABTS^{*}, Superoxide, FRAP and Phosphomolybdenum assay. The extract with the highest antioxidant activity was further subjected to antibacterial, anti-inflammatory and alpha-amylase inhibition assays. Results: *P. tectorius* floral extract had the highest TPC (346.65±0.30 mg/g GAE), TFC (143.29±0.22 mg/g QE) and antioxidant activity when compared to root, leaf, bract and stem extracts. Amongst all tested bacterial strains, the highest antibacterial activity was observed against *Micrococcus luteus*, *Proteus vulgaris* and *Staphylococcus aureus*. The inhibitory effects on alpha-amylase (IC₅₀=15.12) μ g/mL) and inflammation (IC₅₀=70.19 μ g/mL) were observed to increase dose-dependently. GC-MS analysis of *P. tectorius* floral extract showed the presence of sesquiterpenoid, flavonoid, diterpene, phenolic compounds, piperazine, yohimbine and oleic acid. Conclusion: The results of this research hold promise for improving the progress and integration of *P. tectorius* flowers in the cosmetics and pharmaceutical industries.

Keywords: Antibacterial, Antioxidant, Floral Extracts, GCMS, *Pandanus tectorius*.

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INTRODUCTION

Since antiquity, medicinal plants have been utilized in treating different diseases and disorders. Secondary metabolites produced by medicinal plants are responsible for health-promoting properties.[1] Market trends reveal a significant surge in the demand for natural plantbased products derived from various plant parts such as seeds, fruits, roots, rhizomes and flowers. Amongst

them, floral extracts are widely recognized for their abundance of phytochemicals, which have significant role in the food, cosmetics and pharmaceutical industries.[2] Furthermore, it has been reported in the Ayurveda and Siddha systems that certain flowers exhibit unique medicinal properties.^[3]

Pandanus, a member of the Pandanaceae family and closely related to pine trees, boasts an impressive diversity of approximately 600 species. India is the natural habitat of thirty to forty species of Pandanus. Pandanus includes several major species including *Pandanus tectorius, Pandanus spiralis*, *Pandanus amaryllifolius*, *Pandanus utilis*, *Pandanus odoratissimus*, *Pandanus conoideus*, *Pandanus furcatus*, *Pandanus candelabrum*, and *Pandanus pygmaeus*. [4] *P. tectorius*, commonly referred to as the hala tree or screw pine, is a tropical plant that has been traditionally utilized across various cultures for its medicinal properties. This species is widely distributed along the east and west coasts of India, particularly in the states of Andhra Pradesh, Kerala, Orissa and Tamil Nadu.[5] The plant has been employed in folk medicine due to its anti-inflammatory, antioxidant, antimicrobial and wound-healing properties. The leaves, fruits and roots of *P. tectorius* contains a wide range of bioactive compounds, including flavonoids, tannins and phenolic acids, which are believed to contribute to its therapeutic effects.[6] Furthermore, *P. tectorius* is dioecious, with the inflorescences of the male plants serving as a vital raw material for the local perfume industry. Essential oils are also extracted from these highly fragrant male inflorescences.[7]

Despite the numerous reported medicinal properties of *P. tectorius*, there has been limited scientific evaluation of its potential benefits of *P. tectorius* flowers. To address this gap, the present study aims to investigate the potential antioxidant, antibacterial, anti-inflammatory and antidiabetic properties of *P. tectorius* floral extract.

MATERIALS AND METHODS

Extraction

P. tectorius plant was collected from Nellore district Andhra Pradesh. The plant was identified and authenticated by a plant taxonomist, Chennai (Voucher no: PERC/2023/4521). The root, stem, leaf, bract and flower were thoroughly cleaned with distilled water, shade-dried and pulverized using an electric blender. For extraction, 20 g of each plant part was soaked in 200 mL of methanol for 48 hr and filtered using Whatman filter paper. The filtrate was concentrated using a hot plate condenser. The resulting residue was used for subsequent analysis.

Estimation of TPC

Briefly, 100 µL of different parts of *P. tectorius* was combined with 0.9 mL of MeOH and 1 mL of diluted FCR (1:10). The mixture was then allowed to stand for 10 min before adding 1 mL of 20% $\text{Na}_2\text{Co}_3(\text{w/v})$. After 30 min, the OD was measured at 765 nm. TPC is reported as mg/g GAE.^[8]

Estimation of TFC

In brief, 500 µL of different parts of *P. tectorius* were mixed with 0.5 mL of MeOH, 5% NaNo₂ (w/v) and 10% $\mathrm{Al}_2\mathrm{Cl}_3(w/v)$, followed by incubation for 10 min. The solution was then neutralized with 2 mL of 1M NaOH and the volume was adjusted to 10 mL with

de-ionized water. The OD was measured at 510 nm. TFC is reported as mg/g QE.^[9]

DPPH (2, 2 diphenyl-1-picrylhydrazyl) radical scavenging assay

Varying concentrations of different parts of *P. tectorius* were mixed with 1000 µL of freshly prepared 0.2 mM DPPH solution in MeOH. The reaction mixture was kept in the dark for 30 min at room temperature and the OD was measured at 517 nm.^[10] Results are expressed as % inhibition of DPPH radical.

% inhibition of DPPH free radical $= \left| \frac{A_c - A}{A_c} \right|$ $C \quad$ ¹s C $(A_c$ l $\overline{}$ ö ø \vert × é $\overline{\mathsf{L}}$ $\mathsf L$ 100 ù û ú ú

 A_c corresponds to control absorbance. A_s corresponds to sample absorbance.

ABTS•+ (2, 2-azino-bis (3-ethylbenzothiazoline-6 sulfonic acid) radical scavenging assay

Fresh ABTS^{*+} solution was used for this assay. For this, 7 mM ABTS and 2.45 mM $K_2S_2O_8$ were mixed and allowed to stand for 16 hr in the dark at room temperature. To this solution, phosphate-buffer saline (5 mM; pH 7.4) was added until an OD of 0.70 was obtained at 734 nm. Varying concentrations of different parts of *P. tectorius* were mixed with 1 mL of freshly prepared ABTS^{•+} solution and incubated for 10-15 min. Finally, OD was measured at 734 nm.^[11] Results are expressed as % inhibition of ABTS●+ radical.

% inhibition of ABTS^{**} free radical =
$$
\left[\left(\frac{A_c - A_s}{A_c} \right) \times 100 \right]
$$

 A_c corresponds to control absorbance. A_s corresponds to sample absorbance.

Superoxide (O₂-) radical scavenging assay

Varying concentrations of different parts of *P. tectorius* were mixed with a solution of 0.9 mL of 50 mM phosphate buffer (pH 7.4), 0.3 mL of 20 mM PMS, 150 mM NADH and 50 mM of NBT. The mixture was incubated for 15 min, after which OD was measured at 590 nm. Results are expressed as % inhibition of superoxide anion (O_2^-) radical.^[12]

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% inhibition of superoxide
free radical =
$$
\left[\left(\frac{A_c - A_s}{A_c} \right) \right]
$$

 A_c corresponds to control absorbance.

A_s corresponds to sample absorbance.

Fe3+ reducing power assay

Test tubes containing varying concentrations of different parts of *P. tectorius* were mixed with 1 mL of

PBS (0.2 M; pH 6.6) and 1000 μ L of 1 % $\mathrm{K_3[Fe}$ (CN) $_{\mathrm{6}}]$ (w/v) . The test tubes were kept in a thermostatic bath for 25 min and the temperature was maintained at 50ºC. After cooling, 1 mL of 10% TCA (w/v) and 0.5 mL of 0.1% freshly prepared FeCl₃ (w/v) were added. The OD was measured at 700 nm.^[13] Results are expressed as % reduction of Fe^{3+} to Fe^{2+} ions.

% reduction of Fe³⁺ to Fe²⁺ ions =
$$
\left[\left(\frac{A_s - A_c}{A_s} \right) \times 100 \right]
$$

 A_c corresponds to control absorbance.

 A_s corresponds to sample absorbance.

Phosphomolybdenum reducing assay

Varying concentrations of different parts of *P. tectorius* were mixed with 1000 µL of freshly prepared reagent solution [0.6 M H_2SO_4 , 28 mM Na_3PO_4 and 4 mM $(\mathrm{NH}_4)_{2}\mathrm{MoO}_4$. The test tubes were incubated for 90 min in a thermostatic bath at 95ºC. After cooling, the OD was measured at 695 nm.^[14] Results are expressed as a percentage reduction of Mo (VI) to Mo (V).

% reduction of Mo (VI) to Mo (V) =
$$
\left[\left(\frac{A_s - A_c}{A_s} \right) \times 100 \right]
$$

 A_c corresponds to control absorbance.

 A_s corresponds to sample absorbance.

Antibacterial assay

The antibacterial activity was evaluated using the agar well contact technique against gram-positive (*Bacillus subtilis, Micrococcus luteus,* and *Staphylococcus aureus*) and gram-negative (*Escherichia coli, Proteus vulgaris,* and *Shigella flexneri*) bacterial strains. The bacterial suspension was uniformly streaked on separate petri plates using a cotton swab. Five wells (8 mm) were made in each plate using a corrosion-resistant steel cork borer. Varying concentrations of *P. tectorius* FE were loaded to the wells and incubated for 24 hr at 37ºC. Results are expressed as the zone of inhibition (mm).^[15]

Alpha amylase inhibition assay

Varying concentrations of *P. tectorius* FE were added to 0.7 mL of SPB containing 6 mM NaCl (0.02 M; pH 6.9) and 15 µL of alpha-amylase solution. The mixture was then incubated for 10 min. Later, 0.6 mL of 1% starch solution (w/v) was added and incubated for 1 hr. To stop the reaction, 0.1 mL of diluted HCl was introduced and 0.2 mL of iodine solution was added. The OD was measured at 565 nm.^[16] Results are expressed as % inhibition of alpha-amylase.

% inhibition of alpha-amylase =
$$
\left[\left(\frac{A_c - A_s}{A_c} \right) \times 100 \right]
$$

 A_c corresponds to control absorbance. A_c corresponds to sample absorbance.

Anti-inflammatory assay-Membrane stabilization assay

Varying concentrations of *P. tectorius* FE were mixed with 0.2 mL 10% RBCs suspension (freshly prepared). The final volume was adjusted to 1 mL using freshly prepared saline solution. The solutions were incubated at 56ºC for 30 min. The solutions were centrifuged after cooling at 2500 rpm for 5 min. The OD was measured at 560 nm.[17] Results are expressed as % inhibition of haemolysis.

% inhibition of hemolysis =
$$
\left[\left(\frac{A_c - A_s}{A_c} \right) \times 100 \right]
$$

 A_c corresponds to control absorbance. A_s corresponds to sample absorbance.

GCMS analysis

GC-MS analysis was done using an Agilent 6890N JEOL GC Mate II combined gas chromatography system and mass spectrophotometer, equipped with an HP-5 column (30 m \times 0.25 mm ID with 0.25 µm film thickness). Helium gas served as the carrier gas flowing at a rate of 1 mL/min. The ionization voltage was set at 70 eV and the ion source and interface temperatures were maintained at 250ºC. The column oven temperature was programmed to increase from 50 to 250ºC at a rate of 10ºC/min. The National Institute Standard and Technology (NIST) database, which includes over 62,000 patterns, was used for interpretation.^[18]

RESULTS

Table 1 provides data on the spectrophotometric analysis of TPC and TFC of different parts of *P. tectorius*. Upon quantification, it is evident that the FE had the highest TPC (346.65±0.30 mg/g GAE) and TFC (143.29±0.22 mg/g QE). On the other hand, the leaf extract displayed the lowest TPC (186.12**±**0.16 mg/g GAE) and TFC (23.93**±**0.36 mg/g QE) respectively.

Free radical scavenging activity by different parts of *P. tectorius* is presented in Table 2. Ascorbic acid was used as the standard. FE displayed the highest ability to scavenge free radicals when compared to the root and other aerial parts of *P. tectorius*. Based on the IC_{50} value, FE was able to inhibit the action of DPPH radical $(10.98 \,\mu g/mL)$ effectively followed by superoxide $(15.99$ μ g/mL) and ABTS^{•+} free radical (36.15 μ g/mL).

Values are the mean of three independent observations.

TPC: Total Phenol Content. TFC: Total Flavonoid Content.

The reducing power of various parts of *P. tectorius* and ascorbic acid demonstrates a proportional increase with a rise in concentrations. Among the extracts tested, the FE demonstrated the highest activity, with an IC_{50} value of 13.94 µg/mL and 13.72 µg/mL for FRAP and Phosphomolybdenum assay (Table 3). This observation may be due to higher TPC and TFC in FE when compared to other extracts.

The antibacterial effect of *P. tectorius* FE was assessed by measuring the zone of inhibition against gram-negative and gram-positive bacteria. The maximum inhibitory

effect was observed against *S. aureus* (28±0.35 mm), followed by *M. luteus* (27±0.18 mm) and *S. flexneri* $(23\pm0.38 \text{ mm})$ at 500 μ g/mL (Table 4).

P. tectorius FE showed promising results in preventing the lysis of erythrocyte membranes and inhibiting alpha-amylase activity in a concentration-dependent manner (Table 5). FE displayed 77.25% α-amylase inhibitory activity at a concentration of 120 μg/mL with an IC₅₀ value of 15.12 μ g/mL, compared to Acarbose, which showed 87.29% inhibition with an IC_{50} value of 13.65 μg/mL.

Values are the mean of three independent observations.

DPPH: 2, 2 diphenyl-1-picrylhydrazyl.

ABTS*: 2, 2-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid.

Values are the mean of three independent observations.

Values are the mean of three independent observations.

Spectral analysis of methanolic extract of *P. tectorius* FE showed the presence of sesquiterpenoid, flavonoid, diterpene, phenolic compounds, piperazine, yohimbine and monounsaturated fatty acid. The retention time, name, structure, molecular weight and formula of the compounds are presented in Table 6 and the GCMS chromatogram is shown in Figure 1.

DISCUSSION

Plants produce phytochemicals as a natural defense against pathogens. These compounds have been utilized tremendously for their potential therapeutic benefits in managing metabolic dysfunctions, immunological disorders and neurological impairments.^[19] According to

Values are the mean of three independent observations

Figure 1: GCMS chromatogram of *P. tectorius* flower extract.

Andersen and Markham (2006), photosynthesis leads to the accumulation of secondary metabolites, especially phenols and flavonoids, in leaves more than other plant parts. However, contrary to this notion, recent studies have suggested that flowers are also a rich source of secondary metabolites. The results of this study indicate that the TPC and TFC of FE were greater than those of other plant parts. In line with these findings, Zheng *et al*. [21] quantified the TPC and TFC in different parts of *Prinsepia utilis*. Results showed that the maximum amount of TPC $(24. \pm 0.23 \text{ mg/g } GAE)$, was detected in flower, succeeded by leaf $(23.48\pm1.54 \text{ mg/g } GAE)$ and stem $(21.49\pm0.83 \text{ mg/g } GAE)$ extracts. Similarly, the TFC was found to be highest in flower (117.86±2.92 mg/g GAE), followed by leaf $(91.36 \pm 1.44 \text{ mg} \text{ GAE/g})$ and stem extracts (89.77 \pm 4.53 mg/g GAE).

When the generation of free radicals surpasses the body's ability to manage them, oxidative stress arises. Antioxidants play a crucial role in counteracting oxidative stress.[22] The FE demonstrated a notably stronger free radical scavenging activity when compared to other parts. Omodamiro and Ikekamma (2016)^[23] investigated the antioxidant properties of *P. tectorius* leaf extract, employing DPPH and nitric oxide assays. The DPPH spectrophotometric assay showed percentage inhibition at varying extract concentrations: 25.81% at 62.5 mg/ mL, 59.26% at 125 mg/mL, 81.39% at 250 mg/mL and

95.01% at 500 mg/mL. In the nitric oxide assay, the percentage inhibition was recorded as 45.52% at 62.5 mg/mL, 78.07% at 125 mg/mL, 88.15% at 250 mg/mL and 98.77% at 500 mg/mL.

Medicinal plants, particularly their flower extracts, have gained attention due to their potent antibacterial activity.[24] For example, the benzene floral extract of *Saraca asoca* displayed antimicrobial properties against *Klebsiella pneumoniae* and the ethanolic floral extract of *Pavetta indica* exhibited inhibition against *S. aureus* and Salmonella typhi.^[25] In India, Ayurvedic practitioners have long employed fresh juices from the flowers of *Catharanthus roseus* to treat skin conditions like acne, dermatitis and eczema.[26] Suvetha *et al*. [27] investigated the antibacterial properties of *P. odoratissimus* FE and found that the acetone and methanolic extracts displayed effective growth inhibition of *E. coli* at 100 µg/mL, producing zone of inhibition of $(16±4$ mm) and $(12±3)$ mm), respectively. Thus, this study's results and previous research suggest that floral extracts possess promising antibacterial properties and could serve as a basis for a novel, broad-spectrum antimicrobial formulation.

The rising focus on addressing inflammation to enhance the prevention and management of metabolic disorders has resulted in heightened research activity in this domain. Tsalamandris *et al*. [28] have shed light on the potential of inflammation-targeting therapies. GCMS

analysis showed the presence of sesquiterpenoid, flavonoid, diterpene, phenolic compounds, piperazine, yohimbine and monounsaturated fatty acid. Flavones in particular demonstrate antioxidant, anti-proliferative, anti-microbial, estrogenic, acetylcholinesterase and antiinflammatory activities.[29] Phytol, a diterpene, is reported to have anti-nociceptive effects, inflammation-reducing properties, immune system-regulating properties and antimicrobial effects.[30] Yohimbine, which belongs to the monoterpenoid indole alkaloid class inhibits the activity of the α 2-adrenergic receptor, which is useful in treating conditions such as impotence, cardiac impairment, inflammatory disorders and cancer.^[31] Furthermore, 2, 6-bis (1, 1-dimethyl ethyl) phenol derivatives are responsible for anti-inflammatory activity. Patchoulane also contributes to health-promoting properties. Patchoulane extracted from *Malva neglecta* exhibits antimicrobial, antioxidant, anti-inflammatory, hepato-protective and neuroprotective properties.^[32] These active compounds in *P. odoratissimus* flowers could contribute to different therapeutic properties.

SUMMARY AND CONCLUSION

Results demonstrate the antioxidant, antibacterial, antidiabetic and anti-inflammatory properties of *P. tectorius* flowers, as confirmed by *in vitro* assays. These flowers hold promising potential for developing pharmaceutical products to prevent chronic diseases. Nonetheless, it is crucial to conduct further research using *in vivo* studies to establish the safety and effectiveness of these plant species before using them for medicinal purposes.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

TPC: Total phenol content; **TFC:** Total Flavonoid content; **GAE:** Gallic acid equivalent; **QE:** Quercetin equivalent; **FE:** Flower extract; **PBS:** Phosphate buffer solution; **OD:** Optical Density; **PMS:** Phenazine methosulfate.

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