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ANTIOXIDANT AND ANTIACNE ACTIVITY OF ETHANOLIC EXTRACT OF *POGOSTEMON PLECTRANTHOIDES*

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ABSTRACT

Acne vulgaris is a multifactorial inflammatory skin disorder in which oxidative stress and microbial colonization play a significant role in disease progression. The present study aimed to evaluate the antioxidant and antiacne activities of the ethanolic extract of *Pogostemon plectranthoides*. The dried plant material was extracted using ethanol, and the percentage yield was determined. Preliminary phytochemical screening was carried out to identify major bioactive constituents. Total phenolic and flavonoid contents were quantified using standard colorimetric methods. Antioxidant activity was assessed by the DPPH free radical scavenging assay using ascorbic acid as the standard. In vivo antiacne activity was evaluated in rats using a *Propionibacterium acnes*–induced acne model, with clindamycin serving as the reference drug. The ethanolic extract yielded 11.8% w/w and was found to contain flavonoids, phenolic compounds, saponins, diterpenes, and tannins. The total phenolic and flavonoid contents were 0.52 mg/100 mg and 0.97 mg/100 mg, respectively. The extract exhibited concentration-dependent antioxidant activity with an IC_{50} value of 51.76 µg/ml, compared to 20.33 µg/ml for ascorbic acid. In vivo studies demonstrated a significant and dose-dependent reduction in acne-induced inflammation, as evidenced by decreased ear thickness in rats treated with the extract (100 and 200 mg/kg, p.o.). The higher dose showed marked antiacne activity comparable to the standard drug clindamycin. The results indicate that the ethanolic extract of *Pogostemon plectranthoides* possesses significant antioxidant and antiacne properties, which may be attributed to its phenolic and flavonoid constituents. The study supports the potential use of this plant as a natural therapeutic agent for the management of acne and oxidative stress-related skin disorders.

KEYWORDS: *Pogostemon plectranthoides*, Acne vulgaris, Antioxidant activity, DPPH assay, Phytochemical screening, *Propionibacterium acnes.*

INTRODUCTION

Acne vulgaris is a chronic inflammatory disorder of the pilosebaceous unit that predominantly affects adolescents and young adults (Alsaadoon et al., 2024).

The pathogenesis of acne involves multiple interrelated factors, including excessive sebum production, follicular hyperkeratinization, colonization by *Cutibacterium acnes*, and inflammatory responses mediated by reactive oxygen species (ROS) (Mias et al., 2023).

Oxidative stress plays a fundamental role in the initiation and progression of acne by promoting lipid peroxidation, inflammation, and tissue damage. Therefore, agents possessing both antioxidant and antiacne activities are considered highly beneficial in the management of acne and associated skin disorders (Kardeh et al., 2019).

Currently available antiacne therapies, such as topical antibiotics, retinoids, and benzoyl peroxide, are often associated with adverse effects including skin irritation, dryness, microbial resistance, and reduced patient compliance. These limitations have prompted increased interest in the exploration of plant-based therapeutics that offer better safety profiles, multi-targeted mechanisms of action, and reduced side effects. Medicinal plants rich in bioactive phytoconstituents such as flavonoids, phenolic acids, tannins, and terpenoids have demonstrated promising antioxidant, antimicrobial, and anti-inflammatory properties relevant to acne treatment (Herrick et al., 2024).

Pogostemon plectranthoides Desf., belonging to the family Lamiaceae, is an aromatic herb traditionally used in various systems of medicine for the treatment of skin ailments, wounds, infections, and inflammatory conditions (Phattayanon et al., 2024).

The plant is known to contain a wide range of secondary metabolites, including phenolics, flavonoids, essential oils, and terpenes, which are recognized for their potent antioxidant and antimicrobial activities. Previous pharmacological investigations on related species of the *Pogostemon* genus have reported significant antioxidant, antibacterial, anti-inflammatory, and wound-healing properties, suggesting its therapeutic potential in dermatological applications (Elshafie et al., 2023).

Ethanolic extraction is widely employed to obtain a broad spectrum of polar and moderately non-polar phytoconstituents responsible for biological activities. The ethanolic extract of *Pogostemon plectranthoides* is therefore expected to exhibit substantial free radical scavenging activity and inhibitory effects against acne-causing microorganisms such as *Cutibacterium acnes* and *Staphylococcus epidermidis*. However, scientific evidence supporting the antioxidant and antiacne potential of *Pogostemon plectranthoides* remains limited.

In view of the above considerations, the present study was undertaken to evaluate the antioxidant and antiacne activities of the ethanolic extract of *Pogostemon plectranthoides*. The study aims to scientifically validate its traditional use and explore its potential as a natural therapeutic agent for the management of acne and oxidative stress–related skin disorders.

MATERIAL AND METHODS

Material

The whole plant of *Pogostemon plectranthoides* was collected, authenticated, shade dried, and powdered for extraction. Ethanol (analytical grade) was used as the extraction solvent. All chemicals and reagents required for phytochemical screening, total phenolic and flavonoid estimation, and antioxidant activity, including DPPH, ascorbic acid, Folin–Ciocalteu reagent, aluminium chloride, and other analytical reagents, were of analytical grade and obtained from standard suppliers. For in vivo antiacne evaluation, healthy Wistar albino rats (180–220 g) were used, with heat-killed *Propionibacterium acnes* employed for acne induction and clindamycin used as the standard drug. All animal experiments were conducted in accordance with CPCSEA guidelines and approved by the Institutional Animal Ethics Committee.

Methods

Collection of plant material

The collection of plant material is an essential step in various scientific, agricultural, and horticultural endeavors. It involves the careful gathering and preservation of plant specimens for further study, experimentation, propagation, or conservation purposes. The method of collecting plant material depends on the specific objectives, the type of plant, and the desired outcome. Leaves of *Pogostemon plectranthoides* was collected from local area of Bhopal in the month of February, 2025.

Extraction by maceration process

Leaves of *Pogostemon plectranthoides* was shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether by maceration process. The extraction was continued till the defatting of the material had taken place.

50 gm of dried powdered leaves of *Pogostemon plectranthoides* has been extracted with ethanol solvent using maceration process for 24 hrs, filtered and dried using vacuum evaporator at 40°C (Mukherjee, 2007).

Determination of percentage yield

The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powder drug Taken}} \times 100$$

Phytochemical screening

Phytochemical tests are conducted to identify and determine the quantity of specific phytochemical compounds present in a plant extract or plant material. These tests employ various chemical, chromatographic, and spectroscopic techniques to isolate, separate, and characterize the phytochemicals. The choice of tests depends on the nature of the phytochemical of interest and the available resources. Phytochemical examinations were carried out for the extract as per the standard methods (Kokate, 1994).

Total Phenolic content estimation

Preparation of Standard: 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 5-25µg/ml was prepared in methanol.

Preparation of Extract: 10mg of dried extracts of were dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this solution was used for the estimation of phenol.

Procedure: 2 ml of each extract or standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15 min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Total flavonoids content estimation

Determination of total flavonoids content was based on aluminium chloride method (Parkhe and Bharti, 2019).

Preparation of standard: 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5-25µg/ml were prepared in methanol.

Preparation of extract: 10mg of dried extracts of were dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this solution was used for the estimation of flavonoid.

Procedure: 1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

In vitro* Antioxidant activity of extract of *Pogostemon plectranthoides

DPPH method

Total free radical scavenging capacity of the ethanolic extract from *Dioscorea floribunda* was estimated according to the previously reported method with slight modification (Parkhe and Jain, 2018). Solution of DPPH (6 mg in 100ml methanol) was prepared and stored in dark place. Different concentration of standard and test (10- 100 µg/ml) was prepared. 1.5 ml of DPPH and 1.5 ml of each standard and test was taken in separate test tube; absorbance of this solution was taken immediately at 517nm. 1.5 ml of DPPH and 1.5 ml of the methanol was taken as control absorbance at 517nm. The percentage inhibition of free radical DPPH was calculated from the following equation:

% inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] × 100%.

In vivo* antiacne activity of ethanolic extract of *Pogostemon plectranthoides

Animals

Wistar rats (150-200g) were group-housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2°C, 55–65%). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. A separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Acute toxicity studies

Toxicity studies were carried out according to OECD guidelines, including an acute oral toxicity study of the ethanolic extract of *Pogostemon plectranthoides*. An acute toxicity study was performed based on OECD guideline no. 423. The mice were assessed for signs of toxicity throughout the next 14 days. Ethanolic extract of *Pogostemon plectranthoides* was given orally with a safe dose. Clinical symptoms like behavioral alterations, changes in the eyes, body weight, skin, and fur were noted (Gilani *et al.*, 2022; Kazmi *et al.*, 2023).

Induction of acne by *Propionibacterium acnes*

The acne like inflammatory model was produced in the ears of rats by subcutaneous injection of 0.14 mg, heat-killed bacteria.

Experimental designs

Group –I: Control (acne induced)
Group -II: Ethanolic extract of *Pogostemon plectranthoides* (100mg/kg, p.o.)
Group –III: Ethanolic extract of *Pogostemon plectranthoides* (200mg/kg, p.o.)
Group –IV: Clindamycin (200mg/kg, p.o.)

The experimental model of acne-like inflammation was induced in rat ears through subcutaneous administration of 0.14 mg of heat-killed *Propionibacterium acnes*. The study comprised four experimental groups:

Group I served as the control with acne induction,

Group II received 100 mg/kg of ethanolic extract of *Pogostemon plectranthoides* orally, Group III received 200 mg/kg of the same extract orally, and

Group IV was administered Clindamycin at a dose of 200 mg/kg orally (Jatav *et al.*, 2023).

Measurement of ear thickness

Ear thickness was measured as an index of inflammatory strength and acne. Thickness was measured by using a vernier calliper. Thickness was measured once every day for the first week of induction, then every other day until 10^{th} day.

Statistical analysis

All statistical analysis is expressed as mean ± standard error of the mean (SEM). Data were analyzed by one way ANOVA, where applicable $p<0.05$ was considered statistically significant, compared with vehicle followed by Dunnett's test.

RESULTS AND DISCUSSION

The present study was designed to evaluate the antioxidant and antiacne potential of the ethanolic extract of *Pogostemon plectranthoides* through phytochemical screening, in vitro antioxidant assays, and an in vivo antiacne model. The findings collectively suggest that the plant possesses significant bioactive potential, supporting its traditional use in skin-related disorders.

The percentage yield of the ethanolic extract of *Pogostemon plectranthoides* was found to be 11.8% w/w (Table 1), indicating efficient extraction of phytoconstituents using ethanol as a solvent. Ethanol is known to extract a wide range of polar and moderately non-polar compounds, which may contribute to the observed biological activities.

Preliminary phytochemical screening (Table 2) revealed the presence of flavonoids, phenolic compounds, saponins, diterpenes, tannins, and carbohydrates, while alkaloids, glycosides, and proteins were largely absent. The positive results for flavonoids and phenolic compounds are particularly noteworthy, as these classes of phytoconstituents are well documented for their antioxidant, anti-inflammatory, and antimicrobial activities. The presence of diterpenes and saponins may further contribute to the antimicrobial and anti-inflammatory effects essential for antiacne activity.

Quantitative estimation of phytoconstituents demonstrated appreciable levels of total phenolic content (0.52 mg/100 mg) and total flavonoid content (0.97 mg/100 mg) (Table 3). Phenolic and flavonoid compounds act as free radical scavengers by donating hydrogen atoms or electrons and chelating metal ions, thereby reducing oxidative stress. Since oxidative stress plays a key role in acne pathogenesis by enhancing inflammatory responses and lipid peroxidation, the presence of these compounds supports the rationale for antioxidant and antiacne evaluation.

The antioxidant activity of the ethanolic extract was assessed using the DPPH free radical scavenging assay (Table 4). The extract exhibited concentration-dependent scavenging activity, with a maximum inhibition of 71.05% at 100 µg/ml, compared to 93.76% for the standard antioxidant, ascorbic acid. The IC_{50} value of the extract (51.76 µg/ml) indicates moderate but significant antioxidant activity when compared to ascorbic acid (20.33 µg/ml). This antioxidant potential may be attributed to the synergistic effect of phenolic and flavonoid constituents present in the extract.

The in vivo antiacne activity was evaluated using a *Propionibacterium acnes*–induced acne model in rats (Tables 5 and 6). Acne induction using heat-killed *P. acnes* is a well-established model that mimics inflammatory acne by triggering immune responses and tissue edema. In the control group, a sustained increase in ear thickness was observed, confirming successful induction of acne-like inflammation.

Treatment with the ethanolic extract of *Pogostemon plectranthoides* at doses of 100 mg/kg and 200 mg/kg (p.o.) resulted in a significant and dose-dependent reduction in ear thickness compared to the control group. The higher dose (200 mg/kg) showed a more pronounced effect, with statistically significant reductions observed from Day 4 onwards ($P < 0.001$ to $P < 0.0001$). Although the standard drug clindamycin exhibited superior antiacne activity, the extract demonstrated comparable trends in reducing inflammation over time.

The antiacne effect of the extract may be attributed to multiple mechanisms, including antioxidant activity, suppression of inflammatory mediators, and possible antibacterial effects against acne-causing organisms. Flavonoids and phenolic compounds are known to inhibit pro-inflammatory cytokines and reduce oxidative damage, thereby attenuating acne-associated inflammation. Additionally, diterpenes and saponins may contribute to antimicrobial action and skin barrier protection.

Table 1: % Yield of extract of *Pogostemon plectranthoides.*

S. No.	Extract	% Yield (w/w)
1.	Ethanolic	11.8

Table 2: Phytochemical screening of extract *of Pogostemon plectranthoides.*

S. No.	Constituents	Ethanolic extract
1.	**Alkaloids** Mayer's Test: Wagner's Test: Dragendroff's Test: Hager's Test:	 -ve -ve +ve -ve
2.	**Glycosides** Legal's test	 -ve
3.	**Flavonoids** Lead acetate Alkaline Reagent Test:	 +ve +ve
4.	**Phenol**	

	Ferric Chloride Test	+ve
5.	**Proteins** Xanthoproteic test	 -ve
6.	**Carbohydrates** Molisch's Test: Benedict's Test: Fehling's Test:	 -ve -ve +ve
7.	**Saponins** Froth Test: Foam Test:	 +ve -ve
8.	**Diterpins** Copper acetate test	 +ve
9.	**Tannins** Gelatin Test:	 -ve

+ve=positive; -ve= negative

Table 3: Total phenolic and total flavonoid content of *Pogostemon plectranthoides.*

S. No.	Total Phenol content	Total flavonoid content
1.	0.52 mg/100mg	0.97 mg/100mg

Table 4: % Inhibition of ascorbic acid and extract of *Pogostemon plectranthoides.*

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	*Pogostemon plectranthoides* extract
1	10	44.69	22.09
2	20	48.57	40.81
3	40	61.35	48.32
4	60	72.92	51.97
5	80	81.24	62.84
6	100	93.76	71.05
IC_{50} value		**20.33**	**51.76**

Table 5: **Protocol study for *in-vivo* anti-acne activity on rats.**

Groups	Induction of Acne	Treatment
Control (acne induced)	Heat killed *Propionibacterium acnes*	Vehicle
Treated with ethanolic extract of *Pogostemon plectranthoides*	Heat killed *Propionibacterium acnes*	100 mg/kg p.o.
Treated with ethanolic extract of *Pogostemon plectranthoides*	Heat killed *Propionibacterium acnes*	200 mg/kg p.o.
Treated with Clindamycin	Heat killed *Propionibacterium acnes*	200mg/kg p.o.

Table 6: Effect of Clindamycin (standard) and ethanolic extract of *Pogostemon plectranthoides* induced acne by *Propionibacterium acnes* in rats.

Treatment	Dose	Mean thickness ±SEM				
		Day2	Day4	Day6	Day8	Day10
Control	0.14 mg	1.75 ± 0.15	1.78 ± 0.20	1.85 ± 0.10	1.92 ± 0.15	1.86 ± 0.35
Ethanolic extract of *Pogostemon plectranthoides*	100 mg/kg p.o.	1.62 ± 0.32	1.20 ± 0.25*	0.98 ± 0.25*	0.87 ± 0.22*	0.78 ± 0.15*
Ethanolic extract of *Pogostemon plectranthoides*	200 mg/kg p.o.	1.40 ± 0.20	0.96 ± 0.13**	0.82 ± 0.18**	0.70 ± 0.20***	0.65 ± 0.18***
Clindamycin	200 mg/kg p.o.	1.25 ± 0.28	0.70 ± 0.18**	0.58 ± 0.15***	0.48 ± 0.10***	0.39 ± 0.08***

Values are expressed as the mean ± SEM of six observations. *, **, *** $P<0.05$, $P<0.001$, $P<0.0001$ vs. control treatment (One-way ANOVA followed by Dunnett's test)

CONCLUSION

The findings suggest that the ethanolic extract of *Pogostemon plectranthoides* possesses significant antioxidant and antiacne activities, validating its traditional use in dermatological conditions. The dose-dependent response observed in vivo highlights its therapeutic potential as a natural alternative or adjunct to conventional antiacne therapies.

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