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STUDY OF PHYTOCHEMICALS AND ANTI ACNE ACTIVITY OF *NOTONIA GRANDIFLORA* LEAVES EXTRACT

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ABSTRACT

Acne vulgaris is a multifactorial inflammatory skin disorder often associated with *Propionibacterium acnes* colonization, excess sebum production, and inflammatory responses. Plant-based remedies have gained attention as alternative treatments due to their antimicrobial and anti-inflammatory properties. The present study aimed to evaluate the phytochemical profile, antibacterial activity, and in vivo anti-acne potential of the hydroalcoholic extract of *Notonia grandiflora* leaves. Leaves were subjected to hydroalcoholic extraction, and percentage yield was calculated. Preliminary phytochemical screening was carried out to identify major secondary metabolites, followed by quantitative estimation of total flavonoid and alkaloid contents. The antibacterial activity of the extract was assessed in vitro against *Staphylococcus aureus* and *Propionibacterium acnes* using the agar well diffusion method. In vivo anti-acne activity was evaluated in *P. acnes*-induced acne in rats by measuring lesion thickness over 10 days and comparing with the standard drug clindamycin. The hydroalcoholic extract showed a yield of 10.4% w/w and tested positive for alkaloids, flavonoids, saponins, and diterpenes. Total flavonoid and alkaloid contents were 0.87 mg/100 mg and 0.63 mg/100 mg of dried extract, respectively. Antibacterial studies revealed a concentration-dependent zone of inhibition, with maximum inhibition observed at 100 mg/ml against *P. acnes* (16 ± 0.47 mm). In vivo studies demonstrated a significant, dose-dependent reduction in acne lesion thickness, with 200 mg/kg extract showing near-complete resolution by Day 10, comparable to clindamycin treatment ($P < 0.001$).The findings suggest that *Notonia grandiflora* leaves possess potent anti-acne activity, likely mediated through antibacterial and anti-inflammatory mechanisms. The study supports further investigation and development of *N. grandiflora*-based topical formulations as a potential alternative therapy for acne vulgaris.

Keywords: *Notonia grandiflora*, hydroalcoholic extract, phytochemical screening, flavonoids, alkaloids, antibacterial activity, *Propionibacterium acnes*, anti-acne, *in vivo* study.

INTRODUCTION

Acne vulgaris is a common, chronic inflammatory disorder of the pilosebaceous unit characterized by comedones, papules, pustules and, in severe cases, nodules and scarring (Eichenfield *et al.*, 2021). Its pathogenesis is multifactorial and driven by four interrelated processes: increased sebum production, abnormal follicular keratinization, colonization and activity of Cutibacterium acnes, and a downstream inflammatory response that amplifies lesion formation and

tissue damage (Mawardi *et al.,* 2021). Acne causes substantial psychosocial burden, which motivates ongoing search for safer, effective topical and systemic therapies (Dreno *et al.,* 2021).

Limitations of current treatments including local or systemic antibiotic resistance, side effects from retinoids and benzoyl peroxide, and incomplete responses have renewed interest in plant-derived agents with antimicrobial, anti-inflammatory and sebum-modulating properties (AlSheikh *et al.,* 2020). Phytochemicals such as flavonoids, triterpenoids (e.g., lupeol), sterols (e.g., β-sitosterol), phenolic acids and essential oil constituents have documented antibacterial, antioxidant and immunomodulatory activities that can target multiple pathogenic steps in acne (direct inhibition of C. acnes, reduction of oxidative stress, and suppression of pro-inflammatory mediators) (Berganayeva *et al.,* 2023). These multimodal mechanisms make botanicals attractive candidates for developing topical anti-acne formulations (Abozeid *et al.,* 2025).

Notonia grandiflora (Asteraceae; synonym: *Kleinia grandiflora*) is a traditionally used medicinal plant found in parts of India and neighboring regions. Phytochemical investigations of the leaves have reported isolation of flavonoid glycosides (for example, kaempferol derivatives), lupeol, β-sitosterol and organic acids, while several recent and older studies describe antibacterial and antioxidant activity of leaf and whole-plant extracts. Given (a) the known roles of flavonoids and triterpenoids in antimicrobial and anti-inflammatory effects relevant to acne and (b) published reports of *Notonia* extracts inhibiting bacterial pathogens and scavenging free radicals, *Notonia grandiflora* leaves merit systematic evaluation as a source of anti-acne agents (Tegegne *et al.,* 2025).

The present study therefore aims to (1) perform phytochemical screening and quantify key groups (total phenolics, total flavonoids) in successive extracts of *Notonia grandiflora* leaves; (2) isolate or profile major constituents where feasible (guided by previous reports of kaempferol glycosides, lupeol and β-sitosterol); and (3) evaluate in vitro anti-acne relevant activities antibacterial activity against *Cutibacterium acnes* (and representative staphylococci), antioxidant potential, and anti-inflammatory effects in simple cell-based assays to determine whether *N. grandiflora* leaves are a promising source for topical anti-acne development.

MATERIALS AND METHODS

Materials

Fresh leaves of *Notonia grandiflora* were collected, shade-dried, and coarsely powdered for extraction. Hydroalcoholic solvent (ethanol:water, 70:30 v/v) was used for maceration. Analytical grade reagents and chemicals such as Wagner's, Hager's, Legal's, lead acetate, Benedict's, Fehling's, and ferric chloride reagents were procured from standard suppliers for phytochemical tests. Standard drugs ciprofloxacin and clindamycin were used as positive controls for antibacterial and in vivo studies. Culture strains of *Staphylococcus aureus* and *Propionibacterium acnes* were obtained from a recognized microbiology laboratory. Healthy Wistar albino rats (150–200 g) were used for in vivo anti-acne activity following CPCSEA guidelines, with institutional animal ethical committee approval.

Methods

Collection of plant material

The plants have been selected on the basis of its availability and folk use of the plant. The leaves of *Notonia grandiflora* were collected from local area of Bhopal in the month of February, 2025. Drying of fresh plant parts was carried out in sun but under the shade. Dried leaves of *Notonia grandiflora* were preserved in plastic bags, closed tightly and powdered as per the requirements.

Extraction by Maceration process

50 gram powdered of *Notonia grandiflora* has been extracted with hydroalcoholic solvent (ethanol: water; 70:30v/v) using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40°C (Mukherjee, 2007).

Determination of percentage yield

The extraction yield is an assessment of the efficiency of the solvent in extracting bioactive components from the selected natural plant samples and was defined as the quantity of plant extracts recovered after solvent extraction compared to the original quantity of plant samples. The yield of the collected plant extracts was measured in grams after extraction, and then converted into percentage. By using the following formula the percentage yield of extract was calculated:

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

Phytochemical screening

Medicinal plants are traditional pharmaceutical commodities and many of the current medicinal drugs are derived indirectly from plants. Phytochemical materials consist of two main bioactive components (chlorophyll, vitamins, amino acids, sugar etc.) and secondary bioactive components; (Alkaloids, terpenoids, phenols, flavonoids etc.). Phytochemical examinations were carried out for all the extracts as per the standard methods (Kokate, 1994).

Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminium chloride method (Mishra *et al.,* 2017). 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5-25µg/ml were prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filtered. 3 ml (1mg/ml) of this solution was used for the estimation of flavonoid. 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.

Estimation of total alkaloid content

The plant extract (20mg) was dissolved in 1ml of 2 N HCl and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added (Shamsa *et al.*, 2008). The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (40, 60, 80, 100 and 120 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/100mg of extract.

***In vitro* antibacterial activity against acne causing bacteria**

The well diffusion method was used to determine the antibacterial activity of the extract prepared from *Notonia grandiflora* using standard procedure (Bauer, 1966). There were 3 concentration used which are 25, 50 and 100 mg/ml for each extracted phytochemicals in studies. It's essential feature is the placing of wells with the antibiotics on the surfaces of agar immediately after inoculation with the organism tested. Undiluted over night broth cultures should never be used as an inoculums. The plates were incubated at 37ºC for 24 hr. and then examined for clear zones of inhibition around the wells impregnated with particular concentration of drug.

In vivo* antiacne activity of extract of *Notonia grandiflora

The *in vivo* antiacne activity of the extract of *Notonia grandiflora* was assessed through a well-structured experimental method. The study aimed to evaluate the potential therapeutic effects of the plant extract on acne, a common skin condition characterized by inflammation and the formation of comedones.

Animals

Wistar rats (180-220g) 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. 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.

Drugs and Chemicals

All chemicals and other biochemical used in the experiments were of analytical grade from different firms.

Acute toxicity studies

Acute oral toxicity was conducted according to the method of Organisation for Economic Co-operation and Development (OECD, 2001). Animals were kept fasting providing only hydroalcoholic extract of *Notonia grandiflora* (50,100,150,200,300 mg/kg/day) was administered orally for 4 days of five groups of rats (n=6) and the animals were kept under observation for mortality as well as any behavioral changes for evaluation of a possible anti-acne activity.

Induction of acne by *Propionibacterium acnes*

The acne like inflammatory model was produced in the ears of rats by subcutaneous injection of 140 µg of heat-killed bacteria (65°C for 30 min) (Pandey *et al.*, 2012).

Experimental designs

Group –I: control (acne induced)

Group -II: Hydroalcoholic extract of *Notonia grandiflora* (100mg/kg, p.o.)

Group –III: Hydroalcoholic extract of *Notonia grandiflora* (200mg/kg, p.o.)

Group –IV: Clindamycin (200mg/kg, p.o.)

Animals were divided into four groups of 6 animals each. The group I received subcutaneous injection of 140 µg of heat-killed bacteria. The groups II, III and IV received 100 mg/kg and 200 mg/kg of

hydroalcoholic extract of *Notonia grandiflora* and Clindamycin (200 mg/kg p.o.), respectively.

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. Data on the relevant parameters were systematically collected and subjected to statistical analysis. Statistical methods such as analysis of variance (ANOVA) or Student's t-test were likely employed to determine the significance of differences between the control and treatment groups. The results were presented as mean values with standard deviations to provide a measure of data variability.

RESULTS AND DISCUSSION

The present investigation was aimed at evaluating the phytochemical profile and anti-acne activity of the hydroalcoholic extract of *Notonia grandiflora* leaves. The percentage yield of the hydroalcoholic extract was found to be 10.4% w/w, with a dark black appearance (Table 1), indicating efficient extraction of phytoconstituents using hydroalcoholic solvent.

Phytochemical screening (Table 2) confirmed the presence of alkaloids, flavonoids, saponins, and diterpenes, which are bioactive secondary metabolites with known antimicrobial, antioxidant, and anti-inflammatory potential. The presence of alkaloids and flavonoids was further substantiated by quantitative estimation, which revealed total flavonoid and alkaloid contents of 0.87 mg/100 mg and 0.63 mg/100 mg of dried extract, respectively (Table 3). These results suggest that the observed pharmacological effects may be attributed to these classes of compounds, as flavonoids are reported to inhibit lipase activity of *C. acnes* and reduce inflammation, while alkaloids possess antibacterial activity.

The hydroalcoholic extract exhibited promising antibacterial activity against *Staphylococcus aureus* and *Propionibacterium acnes* in a concentration-dependent manner (Table 5). At 100 mg/ml, the extract produced a zone of inhibition of 14 ± 0.57 mm and 16 ± 0.47 mm, respectively, indicating significant activity compared to lower concentrations. Although the standard drugs ciprofloxacin and clindamycin showed higher potency (Table 4), the extract's activity is noteworthy given its crude nature and potential synergistic effects of multiple phytoconstituents.

In the *in vivo* anti-acne model, oral administration of the extract demonstrated a significant, dose-dependent reduction in the mean thickness of acne lesions induced by *P. acnes* in rats (Table 6). The 200 mg/kg dose exhibited a marked reduction from Day 4 onwards (0.75 ± 0.05 mm) and reached near-normal thickness by Day 10 (0.20 ± 0.05 mm), which was statistically significant ($P < 0.001$) compared to control. This effect was comparable, though slightly less potent, than clindamycin (200 mg/kg), which showed almost complete suppression of lesion

thickness (0.10 ± 0.03 mm). These findings indicate that *N. grandiflora* extract not only inhibits bacterial growth but also exerts an anti-inflammatory effect that contributes to resolution of acne lesions.

Table 1: % yield of hydroalcoholic extract of *Notonia grandiflora*

S. No.	Colour	Weight of Extract	% Yield (w/w)
1.	Dark black	5.2	10.4

Table 2: Phytochemical screening of extract of *Notonia grandiflora*

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids**	
	Wagner's Test	+ve
	Hager's Test	+ve
2.	**Glycosides**	
	Legal's Test	-ve
3.	**Flavonoids**	
	Lead acetate test	+ve
	Alkaline test	+ve
4.	**Phenol**	
	Ferric chloride test	-ve
5.	**Proteins**	
	Xanthoproteic test	-ve
6.	**Carbohydrates**	
	Fehling's Test	-ve
	Benedict's Test	-ve
7.	**Saponins**	
	Froth Test	+ve
8.	**Diterpenes**	
	Copper acetate test	+ve
9.	**Tannins**	
	Gelatin Test	-ve

Table 3: Estimation of total flavonoids and alkaloid content of extract of *Notonia grandiflora*

S. No.	Extract	Total flavonoids content	Total alkaloid content
		(**mg/ 100 mg of dried extract)**	
1.	Hydroalcoholic	0.87	0.63

Table 4: Antibacterial activity of standard drug against selected microbes

S. No.	Name of drug	Microbes	Zone of Inhibition (mm)		
			10 µg/ml	20 µg/ml	30 µg/ml
1.	Ciprofloxacin	*Staphylococcus aureus*	13±0.5	16±0.47	20±0.57
2.	Clindamycin	*Propionibacterium acnes*	15±0.94	19±0.86	22±0.5

Table 5: Antibacterial activity of hydroalcoholic extract of *Notonia grandiflora* against selected microbes

S. No.	Name of microbes	Zone of inhibition (mm)		
		25mg/ml	50 mg/ml	100mg/ml
1.	*Staphylococcus aureus*	9±0.74	12±0.5	14±0.57
2.	*Propionibacterium acnes*	10±0.5	13±0.86	16±0.47

Table 6: Effect of Clindamycin (standard) and hydroalcoholic extract of *Notonia grandiflora* induced acne by *Propionibacterium acnes* in rats

Treatment	Dose	Mean thickness ±SEM				
		Day2	Day4	Day6	Day8	Day10
Control		1.45± 0.15	1.36 ± 0.10	1.32± 0.18	1.30± 0.15	1.29± 0.10
Notonia grandiflora	100 mg/kg p.o.	1.36± 0.25*	0.85±0.05*	0.45±0.05*	0.36±0.03*	0.30±0.03*
Notonia grandiflora	200 mg/kg p.o.	1.30±0.15**	0.75±0.05**	0.42±0.05**	0.29±0.10**	0.20±0.05**
Clindamycin	200 mg/kg p.o.	1.10±0.30**	0.19±0.02***	0.15±0.03***	0.13±0.02***	0.10±0.03***

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

CONCLUSION

The present study demonstrated that the hydroalcoholic extract of *Notonia grandiflora* leaves exhibits significant anti-acne potential, supported by both in vitro and *in vivo* findings. The extract was found to be rich in secondary metabolites such as alkaloids, flavonoids, saponins, and diterpenes, which may contribute to its observed biological activities. Quantitative analysis confirmed appreciable levels of flavonoids and alkaloids, while antibacterial studies revealed a concentration-dependent inhibitory effect, particularly against *Propionibacterium acnes*. Furthermore, in vivo evaluation showed that treatment with the extract markedly reduced acne lesion thickness in a dose-dependent manner, with efficacy comparable to the standard drug clindamycin. These results suggest that *N. grandiflora* leaves possess promising antimicrobial and anti-inflammatory properties, making them a potential natural alternative for the management of acne vulgaris. Future research focusing on isolation of active constituents, formulation development, and clinical

validation is warranted to establish its therapeutic utility.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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