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Research

Antimicrobial and *In- Vivo* Antiacne Activity of Ethanolic Extract of *Notonia Grandiflora* Leaves

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Abstract:
The present study investigated the antimicrobial and in vivo antiacne activities of the ethanolic extract of *Notonia grandiflora* leaves. Phytochemical analysis revealed the presence of several bioactive compounds including alkaloids, flavonoids, phenols, tannins, saponins, and glycosides, which are known for their pharmacological properties. The antimicrobial potential was assessed against *Propionibacterium acnes* and *Klebsiella pneumoniae* using the agar well diffusion method, demonstrating dose-dependent inhibition, with the highest zone of inhibition observed at 100 mg/ml. In vivo antiacne activity was evaluated in rats using a *P. acnes*-induced acne model. The extract showed a significant reduction in lesion thickness in a dose-dependent manner, comparable to the standard drug Clindamycin. The findings suggest that the ethanolic extract of *Notonia grandiflora* possesses promising antiacne potential, likely due to its antimicrobial and anti-inflammatory properties. This supports its potential application as a natural alternative for acne treatment.

Keywords: *Notonia grandiflora*, antiacne activity, antimicrobial activity, phytochemicals, ethanolic extract, *Propionibacterium acnes*, flavonoids, inflammation, in vivo model, herbal medicine.

Introduction

Acne vulgaris is one of the most common dermatological conditions, affecting nearly 85% of adolescents and young adults globally. It is a multifactorial inflammatory skin disorder primarily involving *Propionibacterium acnes* (recently renamed *Cutibacterium acnes*), which contributes to follicular hyperkeratinization, sebum overproduction, bacterial colonization, and inflammation (Kong et al., 2012). Standard treatments include topical and systemic antibiotics such as clindamycin and tetracyclines; however, their long-term use is often associated with adverse effects and the development of antibiotic resistance (Walsh et al., 2016). This has intensified the search for alternative therapies, particularly from plant-based sources that exhibit fewer side effects and broad-spectrum antimicrobial activity.

Notonia grandiflora, a traditionally used medicinal plant, has shown promise due to its diverse phytochemical profile. Preliminary reports suggest the presence of flavonoids, tannins, phenols, and glycosides, all of which are known for their anti-inflammatory, antimicrobial, and antioxidant properties (Harborne, 1998; Kumar & Pandey, 2013). These phytoconstituents are known to play a vital role in modulating inflammatory pathways and controlling microbial proliferation, thereby supporting the potential application of *Notonia grandiflora* in acne management.

The antimicrobial activity of plant extracts can be attributed to their ability to disrupt microbial membranes, inhibit protein synthesis, or interfere with bacterial DNA (Cowan, 1999). The ethanolic extract of *Notonia grandiflora* has previously shown activity against various pathogens, including *Klebsiella pneumoniae* and *Staphylococcus aureus*, suggesting a broad-spectrum antimicrobial potential.

In addition to in vitro antimicrobial screening, in vivo animal models provide a crucial understanding of the antiacne efficacy of plant extracts. Animal studies simulating acne-like inflammation through *P. acnes* inoculation allow for the evaluation of anti-inflammatory and lesion-reducing effects, thus confirming therapeutic viability in real biological systems (Kong et al., 2012).

Therefore, the present study was designed to evaluate the antimicrobial activity of ethanolic extract of *Notonia grandiflora* against acne-causing bacteria and assess its in vivo antiacne potential in a rat model. This research could contribute to the development of a novel herbal alternative for acne treatment.

Material and Methods

Defatting of plant material

50 gram shade dried leaves were 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.

Extraction by soxhlet extraction process

Defatted powdered of *Notonia grandiflora* has been extracted with ethanolic solvent using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40°C (Mukherjee, 2007).

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 analyses were performed according to the normal protocols for extract. Phytochemical examinations were carried out for all the extracts as per the standard methods (Kokate, 1994).

In vitro* antimicrobial activity extract of *Notonia grandiflora

At first, all instruments which were used in laboratory were made sterile, all glassware's like Erlenmeyer flask, graduated cylinders, stirring rods, beakers, test tubes, petri dishes, inoculating loops, that were used in the assay were placed in an autoclave at 121°C under 15 psi pressure for 25 min by using Autoclave and followed aseptic technique method. Nutrient agar media (NAM) was prepared for growing of bacteria inside the laboratory. The standard size (100mm× 15mm) petri dishes as required for whole experiment. For preparation of NAM, 13 gram powder was mixed with 1000 ml of distilled water and stirred to obtain homogenized mixture. After which, NAM mixture were placed in Autoclave under 15 psi pressure, at 121°C for 25 min for sterilization of media. After that poured the culture media into petri dishes at ratio of 20 ml/dish and was left half covered on the table to let the agar cool down and solidify at room temperature.

Agar well diffusion method

Agar well-diffusion method was followed to determine the antimicrobial activity (Bauer *et al.,* 1966). Nutrient agar (NA) plates were swabbed (sterile cotton swabs) with fresh broth culture of bacteria. Wells (6mm diameter) were made in each of these plates using sterile cork borer. 100mg/ml, 50mg/ml and 25mg/ml solution was prepared in different extracts. About 100 µl of different concentrations of leaves extract were added sterile micropipette into the wells and allowed to diffuse at room temperature for 2hrs. Control experiments comprising inoculums distilled water were set up. The plates were incubated at 37°C for 24 h for bacterial pathogens. The diameter of the inhibition zone (mm) was measured and the activity index was also calculated. Triplicates were maintained and the experiment was repeated thrice, for each replicates the readings were taken in three different fixed directions and the average values were recorded.

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 water ethanolic 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: Ethanolic extract of *Notonia grandiflora* (100mg/kg, p.o.)

Group –III: Ethanolic 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 ethanolic 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.

Results and Discussion

The present study evaluated the antimicrobial and in vivo antiacne activities of the ethanolic extract of *Notonia grandiflora* leaves, with supporting evidence from phytochemical screening. The results demonstrate that the extract exhibits promising biological activity, which could be attributed to its phytochemical constituents.

Phytochemical screening (Table 1) confirmed the presence of several bioactive compounds, including alkaloids, flavonoids, phenols, tannins, proteins, saponins, and glycosides. Notably, alkaloids and flavonoids are known to possess antimicrobial and anti-inflammatory properties, supporting the rationale for their role in treating acne. The presence of phenolic compounds further suggests antioxidant activity, which is crucial in reducing oxidative stress associated with acne pathogenesis.

The in vitro antimicrobial activity (Tables 2 and 3) revealed that the ethanolic extract exhibited a dose-dependent inhibitory effect against *Propionibacterium acnes* and *Klebsiella pneumoniae*. At the highest concentration tested (100 mg/ml), the zone of inhibition was 15 ± 0.5 mm for *P. acnes* and 12 ± 0.57 mm for *K. pneumoniae*. Although the extract was less potent than the standard antibiotics (Clindamycin and Ciprofloxacin), it demonstrated a significant antibacterial effect, indicating the potential for therapeutic use as a natural antimicrobial agent.

The in vivo antiacne study using a *Propionibacterium acnes*-induced rat acne model (Table 4) demonstrated a significant reduction in inflammatory lesion thickness in animals treated with *Notonia grandiflora* extract. The effect was dose-dependent, with 200 mg/kg showing a marked decrease in lesion thickness from Day 2 (1.28 ± 0.05 mm) to Day 10 (0.21 ± 0.07 mm). This performance was comparable to the standard drug Clindamycin, which reduced the lesion thickness to 0.12 ± 0.06 mm. The extract's efficacy may be attributed to its anti-inflammatory phytoconstituents such as flavonoids, tannins, and saponins, which are known to modulate inflammatory pathways and reduce bacterial proliferation.

These results are consistent with previously published literature highlighting the role of plant-based phytochemicals in the management of acne and microbial infections. The significant in vivo activity observed further reinforces the extract's potential for development into a topical or systemic antiacne formulation.

Table 1: Phytochemical screening of extract of *Notonia grandiflora*

S. No.	Constituents	Ethanolic extract
1.	**Alkaloids**	
	Mayer's Test	-ve
	Wagner's Test	-ve
	Dragendroff'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**	
	Molisch's Test	-ve
	Benedict's Test	-ve
	Fehling's Test	+ve
7.	**Saponins**	
	Froth Test	+ve
8.	**Diterpenes**	
	Copper acetate test	-ve
9.	**Tannins**	
	Gelatin Test	+ve

Table 2: *In vitro* Antimicrobial 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.	Clindamycin	*Propionibacterium acnes*	15±0.57	17±0	20±0.6
2.	Ciprofloxacin	*Klebsiella pneumoniae*	16±0.47	18±0.87	22±0.74

Table 3: Antimicrobial activity of extract of *Notonia grandiflora*

S. No.	Name of microbes	Zone of inhibition (mm)		
		25mg/ml	**50 mg/ml**	**100mg/ml**
1.	*Propionibacterium acnes*	10±0.94	12±0.86	15±0.5
2.	*Klebsiella pneumoniae*	9±0.47	10±0.74	12±0.57

Table 4: Effect of Clindamycin (standard) and ethanolic extract of *Notonia grandiflora* induced acne by *Propionibacterium acnes* in rats

Treatment	Dose	Mean thickness ±SEM				
		Day 2	Day 4	Day 6	Day 8	Day 10
Control	140 µg	1.42±0.05	1.35±0.06	1.32±0.08	1.32±0.03	1.32±0.03
Notonia grandiflora	100 mg/kg p.o.	1.35±0.09	0.45±0.03	0.42±0.06	0.38±0.02	0.37±0.06
Notonia grandiflora	200 mg/kg p.o.	1.28±0.05	0.35±0.08	0.28±0.06	0.25±0.08	0.21±0.07
Clindamycin	200 mg/kg p.o.	1.12±0.03	0.19±0.04	0.15±0.03	0.14±0.06	0.12±0.06

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

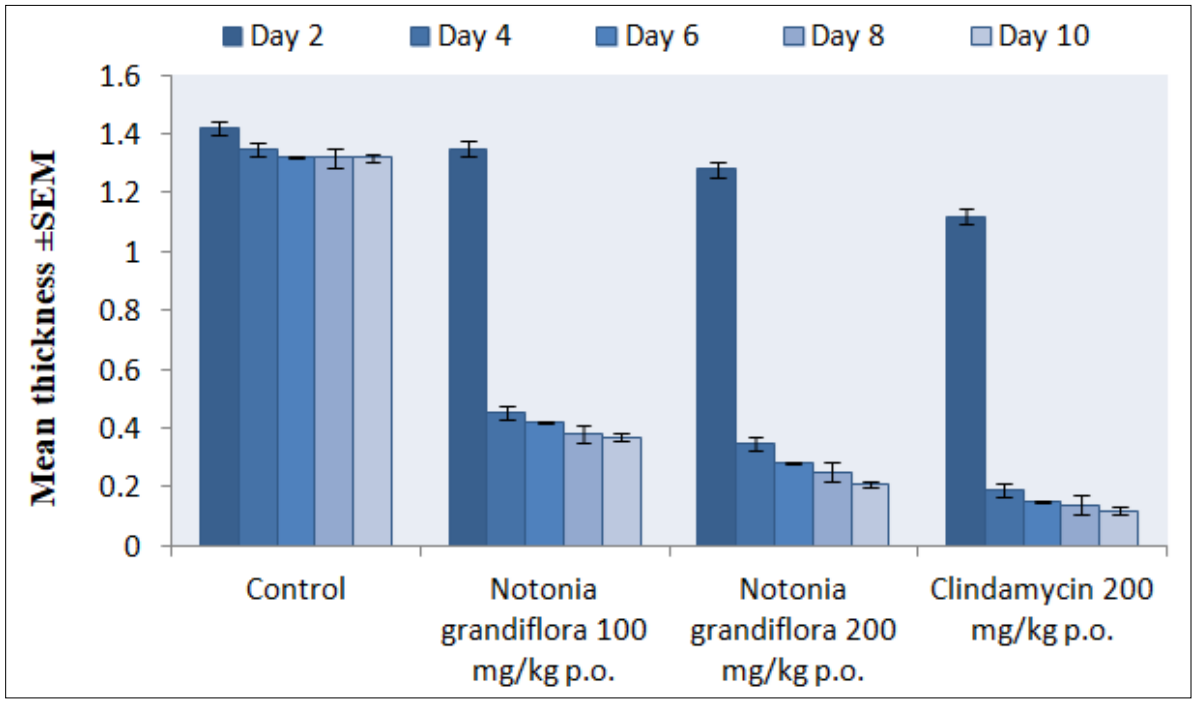

Figure 1: Effect of Clindamycin (standard) and ethanolic extract of *Notonia grandiflora* induced acne by *Propionibacterium acnes* in rats

Conclusion

The ethanolic extract of *Notonia grandiflora* exhibits notable antimicrobial activity against acne-associated pathogens and demonstrates significant anti-inflammatory effects in an in vivo acne model. These findings validate its traditional use and suggest its potential as a natural antiacne therapeutic agent. Further studies on formulation development and clinical evaluation are warranted to establish its utility in dermatological applications.

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