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Research Article

**ANTIMICROBIAL AND ANTI ACNE ACTIVITY OF
BALANITES AEGYPTIACA**Mr. Vivek Thakur¹, Pragya Mishra¹, B. K. Dubey², Deepak Kumar Basediya²,
Prabhat Kumar Jain²¹TIT - College of Pharmacy, Bhopal (M.P.)²Technocrats Institute of Technology-pharmacy, Bhopal (M.P.)**Abstract:**

This study investigates the therapeutic potential of the hydroalcoholic extract of Balanites aegyptiaca in treating acne induced by Propionibacterium acnes in a rat model. The hydroalcoholic extract demonstrated a higher yield (6.20% w/w) compared to the pet ether extract (0.85%), indicating that this extraction method is more efficient in obtaining bioactive compounds. Phytochemical analysis revealed the presence of alkaloids, flavonoids, proteins, and saponins, which are known for their antimicrobial, anti-inflammatory, and immunomodulatory properties, suggesting their role in acne treatment. The extract showed moderate antimicrobial activity with a zone of inhibition of 14 mm against Propionibacterium acnes at 100 mg/ml, though less potent than Clindamycin, which exhibited a 21 mm zone at 30 µg/ml. In vivo, the hydroalcoholic extract significantly reduced skin thickness in rats treated with doses of 100 mg/kg and 200 mg/kg, with the higher dose demonstrating more pronounced effects. These results suggest that the extract's antimicrobial and anti-inflammatory properties contribute to its potential as an acne treatment. Although Clindamycin was more effective, the hydroalcoholic extract of Balanites aegyptiaca presents a promising natural alternative for acne treatment, particularly for individuals seeking plant-based options. This study underscores the therapeutic value of alkaloids and saponins in the extract and highlights its potential for further clinical exploration in human acne therapy.

Keywords: Balanites aegyptiaca, hydroalcoholic extract, acne, Propionibacterium acnes, antimicrobial activity, phytochemical screening, Clindamycin, skin thickness, anti-inflammatory properties, natural remedies, acne treatment, bioactive compounds.

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INTRODUCTION:

Acne vulgaris is a common dermatological condition, particularly affecting adolescents and young adults, characterized by inflammation of sebaceous glands and hair follicles. It is primarily caused by the overproduction of sebum and the proliferation of *Propionibacterium acnes* (*P. acnes*), a gram-positive bacterium that plays a central role in the pathogenesis of acne (Zaidi et al., 2018). Current treatments for acne predominantly include topical and oral antibiotics, such as Clindamycin and tetracyclines, as well as retinoids, corticosteroids, and hormonal therapies (Cunliffe et al., 2009). However, the growing concern over antibiotic resistance, adverse side effects, and the cost of conventional treatments has prompted the search for alternative therapies, particularly plant-based or natural remedies, with antimicrobial and anti-inflammatory properties (Marrelli et al., 2015). *Balanites aegyptiaca*, commonly known as the "desert date" or "sodom apple," is a medicinal plant widely distributed in Africa and parts of Asia. The plant has been traditionally used for its various therapeutic properties, including antimicrobial, anti-inflammatory, antioxidant, and antidiabetic activities (Sharma et al., 2014). The seeds, fruits, and bark of *Balanites aegyptiaca* are known to contain a rich array of bioactive compounds such as alkaloids, flavonoids, saponins, and tannins, which are believed to contribute to its pharmacological effects (Ahmed et al., 2016). Alkaloids and saponins, in particular, are known for their antimicrobial and anti-inflammatory properties, making them potentially useful in treating acne and other skin disorders (Ncube et al., 2013). Studies have reported the antimicrobial activity of *Balanites aegyptiaca* extracts against a range of pathogenic microorganisms, including *Staphylococcus aureus* and *P. acnes* (Oluwaseun et al., 2019). In addition to its antimicrobial activity, the anti-inflammatory effects of the plant may also play a crucial role in alleviating acne symptoms, which are often characterized by inflammation and skin irritation (Jiang et al., 2019). Recent research has focused on the efficacy of plant extracts as adjuncts or alternatives to conventional acne treatments, particularly in the context of rising antibiotic resistance and the increasing demand for natural products in skincare (Yasmin et al., 2021). Given the antimicrobial and anti-inflammatory properties of *Balanites aegyptiaca*, this study aims to investigate its potential as a therapeutic agent for acne treatment, specifically targeting *P. acnes* in an experimental rat model. The hydroalcoholic extract of *Balanites aegyptiaca* was chosen for its high yield and the presence of bioactive compounds, which could enhance its therapeutic potential. The antimicrobial efficacy of the extract will be evaluated, alongside its ability to reduce acne-

related lesions in vivo, in comparison to standard treatments like Clindamycin.

MATERIAL AND METHODS:

Material

The materials used in this study included a variety of chemicals sourced from reputable suppliers. These chemicals included reagents like potassium mercuric iodide, iodine, potassium iodide, and picric acid from Thomas Baker and Loba Chemie Pvt. Ltd. Additionally, compounds such as sodium hydroxide, pyridine, ferric chloride, and gelatin were obtained from S. D. Fine Chem. Ltd., while solvents like methanol, ethanol, and chloroform came from Qualigens Fine Chemicals. Other essential reagents included Fehling's solution, quercetin, gallic acid, and aluminum chloride from Hi Media, and Folin-Ciocalteu reagent sourced from Loba Chemie Pvt. Ltd. These materials were used to facilitate various biochemical and analytical processes in the

Methods

Extraction by soxhlet extraction method

Leaves of *Balanites aegyptiaca* were shade dried at room temperature. The shade dried plant material (60gram) were coarsely powdered and subjected to extraction with petroleum ether by soxhlet extraction. The extraction was continued till the defatting of the material had taken place. Dried powdered *Balanites aegyptiaca* has been extracted with hydroalcoholic solvent (ethanol: water; 70:30v/v) using soxhlet extraction process for 48 hrs, filtered and dried using vacuum evaporator at 40°C (Mukherjee, 2007; Kokate, 1994).

Determination of percentage yield

The percentage yield of an extract is a measure that quantifies the efficiency of the extraction process. It is calculated by comparing the amount of extract obtained to the initial weight of the raw material used. The percentage yield of each extract was calculated by using following formula:

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

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 (Parkhe and Bharti, 2019).

Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminium chloride method (Parkhe and Bharti, 2019). 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% AlCl₃ 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 alkaloids content

The plant extract (1mg) was dissolved in methanol, added 1ml of 2 N HCl and filtered (Shamsa *et al.*, 2008). This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. 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 antimicrobial activity of extract of *Balanites aegyptiaca*

The well diffusion method was used to determine the antimicrobial activity of the extract prepared from the *Balanites aegyptiaca* using standard procedure (Marasini *et al.*, 2015). 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 37°C 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 *Balanites aegyptiaca*

The *in vivo* antiacne activity of the extract of *Balanites aegyptiaca* 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 & 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 hydroalcoholic extract of *Balanites aegyptiaca* (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 *Balanites aegyptiaca* (100mg/kg, p.o.)

Group –III: Hydroalcoholic extract of *Balanites aegyptiaca* (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 *Balanites aegyptiaca* 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 10th 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:

This study aimed to investigate the antimicrobial and anti-acne activity of the hydroalcoholic extract of *Balanites aegyptiaca* using various in vitro and in vivo models. The results from the experiments demonstrate the potential therapeutic benefits of *Balanites aegyptiaca*, especially for acne treatment, through its antimicrobial, anti-inflammatory, and healing properties.

The hydroalcoholic extract of *Balanites aegyptiaca* exhibited a significantly higher yield (6.20% w/w) compared to the pet ether extract (0.85%) (Table 1). This higher yield indicates that the hydroalcoholic extraction method is more effective in isolating bioactive compounds, which are likely responsible for the extract's pharmacological effects. Phytochemical screening (Table 2) revealed the presence of several bioactive compounds in the hydroalcoholic extract, including alkaloids, flavonoids, proteins, and saponins. These compounds are known for their antimicrobial, anti-inflammatory, and antioxidant properties, which support their potential in treating acne. Notably, alkaloids and saponins are recognized for their antimicrobial and anti-inflammatory effects, which are essential in addressing the dual aspects of acne pathology microbial infection and skin inflammation.

The estimation of total flavonoids and alkaloids (Table 3) revealed that the hydroalcoholic extract contains 0.962 mg of flavonoids and 0.532 mg of alkaloids per 100 mg of dried extract. These bioactive compounds, particularly flavonoids and

alkaloids, are often linked to antimicrobial and anti-inflammatory actions, which may contribute to the therapeutic effects observed in acne treatment.

The antimicrobial activity of the hydroalcoholic extract was tested against *Staphylococcus aureus* and *Propionibacterium acnes*, which are both implicated in acne pathogenesis. As shown in Table 5, the hydroalcoholic extract demonstrated moderate antimicrobial activity, particularly against *P. acnes*. At a concentration of 100 mg/ml, the extract exhibited a zone of inhibition of 14 mm against *P. acnes*, suggesting its potential as a therapeutic agent for acne. While the antimicrobial activity of the extract was weaker compared to Clindamycin (Table 4), which showed a 21 mm zone of inhibition at 30 µg/ml, the results indicate that *Balanites aegyptiaca* could be a valuable natural alternative, especially for individuals seeking plant-based options.

The in vivo evaluation of the anti-acne potential of *Balanites aegyptiaca* was carried out by inducing acne-like lesions in rats using *P. acnes* and assessing the effect of the hydroalcoholic extract on skin thickness. Both doses (100 mg/kg and 200 mg/kg) of the extract significantly reduced skin thickness compared to the control group (Table 6). The higher dose (200 mg/kg) showed a more pronounced effect, indicating a dose-dependent reduction in skin inflammation, likely due to the combined antimicrobial and anti-inflammatory actions of the extract. The results were comparable, though less potent, to Clindamycin treatment, which almost completely resolved acne lesions by Day 10. These findings support the idea that *Balanites aegyptiaca* may serve as a potential adjunct or alternative treatment for acne, especially in individuals who prefer natural therapies.

Table 1: % Yield of hydroalcoholic extract of *Balanites aegyptiaca*

S. No.	Extracts	% Yield (W/W)
1.	Pet ether	0.85%
2.	Hydroalcoholic	6.20%

Table 2: Phytochemical screening of extract of *Balanites aegyptiaca*

S. No.	Constituents	Hydroalcoholic extract
1.	Alkaloids Dragendroff's test Hager's test	+ve +ve
2.	Glycosides Legal's test	-ve
3.	Flavonoids Lead acetate	+ve

	Alkaline test	-ve
4.	Phenol Ferric chloride test	-ve
5.	Proteins Xanthoproteic test	+ve
6.	Carbohydrates Fehling's test	-ve
7.	Saponins Foam test	+ve
8.	Diterpenes Copper acetate test	-ve
9.	Tannin Gelatin test	-ve

+ve= Positive; -ve= Negative

Table 3: Estimation of total flavonoids and alkaloid content of *Balanites aegyptiaca*

S. No.	Extract	Total flavonoids content (mg/ 100 mg of dried extract)	Total alkaloid content (mg/ 100 mg of dried extract)
1.	Hydroalcoholic	0.962	0.532

Table 4: Antimicrobial activity of standard drug against selected microbes

S.N.	Name of drug	Microbes	Zone of inhibition (mm)		
			10 µg/ml	20 µg/ml	30 µg/ml
1	Ciprofloxacin	<i>Staphylococcus aureus</i>	13±0.5	16±0.57	20±0.47
2	Clindamycin	<i>Propionibacterium acnes</i>	14±0.74	18±0.86	21±0.94

Table 5: Antimicrobial activity of hydroalcoholic extract of *Balanites aegyptiaca* against selected microbes

S. N.	Name of microbes	Zone of inhibition (mm)		
		Hydroalcoholic extract		
		25mg/ml	50 mg/ml	100mg/ml
1.	<i>Staphylococcus aureus</i>	10±0.57	12±0.47	15±0.5
2.	<i>Propionibacterium acnes</i>	9±0.94	11±0.86	14±0.5

Table 6: Effect of Clindamycin (Standard) and hydroalcoholic extract of *Balanites aegyptiaca* induced acne by *Propionibacterium acnes* in rats

Treatment	Dose	Mean thickness ±SEM				
		Day2	Day4	Day6	Day8	Day10
Control	140 µg	1.45± 0.15	1.38 ± 0.10	1.32± 0.18	1.30± 0.15	1.25± 0.10
<i>Balanites aegyptiaca</i>	100 mg/kg p.o.	1.39± 0.25*	0.42±0.05*	0.38±0.05*	0.35±0.03*	0.23±0.03*
<i>Balanites aegyptiaca</i>	200 mg/kg p.o.	1.28±0.15*	0.30±0.05**	0.25±0.05**	0.20±0.10**	0.18±0.05**
Clindamycin	200 mg/kg p.o.	1.15±0.30*	0.15±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 hydroalcoholic extract of *Balanites aegyptiaca* demonstrates significant antimicrobial and anti-inflammatory effects, supporting its therapeutic potential in acne treatment. While it was less effective than the standard antibiotic Clindamycin, the extract's bioactive compounds, particularly alkaloids and saponins, could play a critical role in reducing acne lesions. These promising results warrant further research, including clinical trials, to fully assess the extract's safety, efficacy, and potential as a natural acne remedy.

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