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STUDY OF PHYTOCHEMICALS AND ANTIACNE ACTIVITY OF ETHANOLIC BARK EXTRACT OF *ZANTHOXYLUM ARMATUM*

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ABSTRACT

The ethanolic extract of *Zanthoxylum armatum*, a plant native to various regions of Asia, has emerged as a potential candidate for acne treatment due to its rich content of bioactive compounds. This study aimed to evaluate the phytochemical profile and anti-acne activity of the ethanolic extract of *Zanthoxylum armatum*. Phytochemical analysis revealed the presence of carbohydrates, flavonoids, phenols, saponins, proteins, and alkaloids, which are known for their antimicrobial and anti-inflammatory properties. The anti-acne activity of the extract was assessed against *Propionibacterium acnes* and, although less potent than Clindamycin, demonstrated significant antibacterial effects at higher concentrations. These findings suggest that the ethanolic extract of *Zanthoxylum armatum* could serve as a natural alternative or adjunct treatment for acne, leveraging its anti-inflammatory, antioxidant, and antimicrobial actions. Future studies should focus on enhancing the efficacy of the extract, understanding its specific mechanisms of action, and exploring its potential in clinical applications.

Keywords: *Zanthoxylum armatum,* ethanolic extract, phytochemicals, anti-acne, antibacterial activity, *Propionibacterium acnes*, natural treatment, bioactive compounds.

INTRODUCTION

Acne vulgaris, commonly referred to as acne, is a prevalent dermatological condition affecting individuals across all age groups, with a significant impact on adolescents and young adults. The condition is multifactorial in nature, involving the interplay of increased sebum production, abnormal keratinization of hair follicles, proliferation of bacteria such as *Cutibacterium acnes* (formerly *Propionibacterium acnes*), and an immune-mediated inflammatory response (Zaenglein *et al.,* 2016). While the pathogenesis of acne is well understood, its management remains challenging due to issues like antibiotic resistance, adverse drug reactions, and recurrence following treatment discontinuation. These limitations underscore the need for alternative, safer, and more effective therapeutic options.

In recent years, medicinal plants have gained attention as potential sources of bioactive compounds for acne management. Plant-based remedies are often associated with lower side effects and have shown promise in addressing the multifactorial nature of acne. Among these, *Zanthoxylum armatum* DC., a member of the Rutaceae family, stands out for its traditional and pharmacological significance. Commonly known as Timur, Prickly Ash, or Toothache Tree, *Z. armatum* is distributed across the Himalayan region, extending to parts of China, India, Nepal, and Bhutan (Singh *et al.,* 2014). Its bark, fruits, and seeds

have been utilized in traditional medicine for treating various ailments, including toothaches, gastrointestinal disorders, respiratory problems, and skin infections (Shrestha *et al.,* 2019).

Phytochemical investigations of *Z. armatum* have revealed the presence of diverse secondary metabolites, including alkaloids, flavonoids, tannins, terpenoids, and phenolic acids, which contribute to its wide-ranging pharmacological activities (Kumar *et al.,* 2020). These bioactive compounds exhibit antimicrobial, anti-inflammatory, antioxidant, and wound-healing properties, making the plant a promising candidate for dermatological applications. Specifically, flavonoids and phenolic compounds act as potent antioxidants that can neutralize free radicals, reduce oxidative stress, and inhibit inflammatory mediators involved in acne pathogenesis (Kumar *et al.,* 2018). Alkaloids and terpenoids, on the other hand, demonstrate strong antimicrobial effects against skin pathogens such as *C. acnes* and *Staphylococcus aureus* (Sharma *et al.,* 2021).

The bark extract of *Z. armatum* has been traditionally used to treat skin conditions; however, its scientific evaluation for anti-acne activity remains limited. Preliminary studies have indicated the plant's potential to inhibit bacterial growth and modulate inflammation, suggesting its relevance in acne treatment. The ethanolic extraction method, widely employed for isolating plant bioactives, enhances the yield of polyphenols and other bioactive compounds, ensuring a comprehensive analysis of the extract's therapeutic potential (Jain *et al.,* 2017).

This study aims to bridge the knowledge gap by systematically exploring the phytochemical composition of *Z. armatum* bark ethanolic extract and evaluating its anti-acne activity through in vitro and in silico methods. The in vitro studies focus on antimicrobial activity against acne-causing bacteria, while the in silico approaches involve molecular docking to identify bioactive compounds with potential inhibitory effects on acne-related enzymes and proteins. By integrating traditional knowledge with modern scientific techniques, this research seeks to establish *Z. armatum* as a sustainable, natural alternative for acne management and provide a foundation for further pharmacological studies.

MATERIALS AND METHODS

Collection of Plant materials

Bark of *Zanthoxylum armatum* were collected from local area of Bhopal in the month of February, 2024. Drying of fresh plant parts was carried out in under the shade. Dried plant parts were preserved in plastic bags, closed tightly and powdered as per the requirements.

Extraction by maceration method

Powdered material of plant parts was subjected to solvent extraction using maceration methods (Kokate, 1994; Mukherjee, 2007). Powdered plant materials were weighed (50 gram) and packed in air tight glass Bottle. The plant drug was defatted with petroleum ether for about 12 hrs. The defatted plant materials were subjected to extraction by ethanol solvents. The liquid extracts were collected in a tarred conical flask. The solvent removed from the extract by evaporation method using water bath.

Determination of percentage yield

Percentage yield measures the effectiveness of the entire extraction process. It shows how much product a researcher has obtained after running the procedures against how much is actually obtained. A higher % yield means the researcher obtained a greater amount of product after extraction. % yield is calculated using the formula below:

$$\textbf{Percentage Yield} = \frac{Weight\ of\ Extract}{Weight\ of\ Powder\ drug\ taken} x\ 100$$

Phytochemical Screening

Phytochemical examinations were carried out for all the extracts as per the standard methods (Parkhe and Bharti, 2019).

Detection of alkaloids: Extract were dissolved individually in dilute Hydrochloric acid and filtered.

Mayer's Test: Filtrates was treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Wagner's Test: Filtrates was treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Dragendroff's Test: Filtrates was treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Hager's Test: Filtrates was treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

Detection of carbohydrates: Extract was dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Fehling's Test: Filtrates was hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Benedicts Test: Filtrated was heated with few drops of Benedict's reagent solution. Formation of reddish brown precipitate indicates the presence of reducing sugar.

Detection of glycosides: Extract was hydrolysed with dil. HCl, and then subjected to test for glycosides.

Legal's Test: Extract was treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

Detection of saponins

Froth Test: Extract was diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

Detection of phenols

Ferric Chloride Test: Extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Detection of tannins

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate

indicates the presence of tannins.

Detection of flavonoids

Alkaline Reagent Test: Extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Lead acetate Test: Extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Detection of proteins

Xanthoproteic Test: The extract was treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

Detection of diterpenes

Copper acetate Test: Extract was dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

Detection of Glycoside

Conc. H_2SO_4 Test: Extract dissolved in distilled water and treated with few drops of conc. Sulphuric acid. Formation of red color indicates the presence of glycoside.

Detection of Sterols

Salkowski Test: 3-4 drops of Conc. Sulphuric acid were added to the extract in chloroform. Formation of red color appears at the lower layer indicates the presence of sterols.

In vivo* antiacne activity of ethanolic extract of *Zanthoxylum armatum

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 (Lim *et al.,* 2018).

Acute toxicity studies

Toxicity studies were carried out according to OECD guidelines, including an acute oral toxicity study of the ethanolic bark extract of *Zanthoxylum armatum*. 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 bark extract of *Zanthoxylum armatum* was given orally with a safe dose (Jonsson *et al.,* 2013).

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 bark extract of *Zanthoxylum armatum* (100mg/kg, p.o.)

Group –III: Ethanolic bark extract of *Zanthoxylum armatum* (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 bark extract of *Zanthoxylum armatum* 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 (Sahu ; 2011).

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 investigates the phytochemical profile and anti-acne potential of the ethanolic bark extract of *Zanthoxylum armatum*. Acne vulgaris, a common inflammatory skin condition primarily caused by *Propionibacterium acnes* (recently reclassified as *Cutibacterium acnes*), involves follicular hyperkeratinization, bacterial proliferation, and inflammation. The results of this study highlight the potential use of *Z. armatum* as a natural remedy for acne, offering an alternative to conventional treatments.

Phytochemical screening of the ethanolic extract of *Z. armatum* bark revealed the presence of carbohydrates, flavonoids, phenols, saponins, proteins, and alkaloids. These bioactive compounds are recognized for their antimicrobial, anti-inflammatory, and antioxidant properties, which are critical in combating acne. Flavonoids and phenols, for instance, act as potent antioxidants that neutralize free radicals and reduce oxidative stress, while also alleviating skin inflammation. Saponins and alkaloids contribute antimicrobial effects, likely inhibiting the growth of *C. acnes*. The presence of proteins indicates potential skin-repair properties, supporting tissue regeneration and healing. The absence of diterpenes, glycosides, lignins, sterols, and tannins further refines the specific bioactive profile of the bark extract.

The ethanolic extraction process yielded 8.5% (w/w) of the extract, emphasizing ethanol's effectiveness in isolating polar and semi-polar compounds. This yield aligns with previous findings that ethanol is a superior solvent for extracting a broad spectrum of phytochemicals, enhancing its utility in preparing plant-based therapeutics.

The anti-acne activity of the extract was evaluated using an acne model induced by *C. acnes* in rats. A dose-dependent reduction in acne severity was observed, with both low-dose (100 mg/kg) and high-dose (200 mg/kg) treatments showing significant improvements. By Day 10, the high-dose extract demonstrated a marked reduction in skin thickness, comparable to the standard treatment with clindamycin. These results suggest that the ethanolic extract exerts antimicrobial effects against *C. acnes*, reduces inflammation through its flavonoid and phenolic content, and promotes skin repair via its protein components.

The mechanism of action of the extract appears to involve a synergistic interaction of its bioactive compounds. The antimicrobial effects, likely driven by alkaloids and saponins, reduce bacterial load, while flavonoids and phenols alleviate inflammatory responses by modulating cytokine activity. The presence of proteins supports skin healing, making the extract effective in addressing both the microbial and inflammatory aspects of acne.

These findings underscore the therapeutic potential of *Z. armatum* bark extract as a natural alternative for acne management. Compared to conventional treatments like clindamycin, the extract offers benefits such as a reduced risk of antibiotic resistance and fewer side effects. Future research should aim to isolate and characterize the specific compounds responsible for the observed effects and explore their application in topical formulations.

Table 1: % Yield of *Zanthoxylum armatum*

Sr. No.	Extracts	% Yield (W/W)
1	Ethanolic	8.5%

Table 2: Phytochemical Test of *Zanthoxylum armatum* extract

Sr. No.	Test	Ethanolic extract
1.	**Carbohydrate** Fehlings Test Benedicts Test	 +ve - ve
2.	**Flavonoids** Lead acetate Test Alkaline Test	 + ve + ve
3.	**Phenols** Ferric chloride Test	 + ve
4.	**Saponins** Foam Test	 +ve
5.	**Proteins** Xanthoproteic Test	 + ve
6.	**Diterpenes** Copper Acetate Test	 - ve
7.	**Alkaloid** Wagner's Test	 +ve
8.	**Glycosides** Conc. Sulphuric acid Test	 - ve
9.	**Lignin** Labet Test	 - ve
10.	**Sterols** Salkowski Test	 - ve
11.	**Tannins** Gelatin Test	 - ve

[+ve= Positive; - ve= Negative]

Table 3: **Effect of Clindamycin (standard) and ethanolic bark extract of *Zanthoxylum armatum* induced acne by *Propionibacterium acnes* in rats**

Treatment	Mean thickness ±SEM				
	Day2	Day4	Day6	Day8	Day10
Control (0.14 mg)	1.65 ± 0.35	1.70 ± 0.40	1.82 ± 0.38	1.90 ± 0.42	1.85 ± 0.45
Ethanolic bark extract of *Zanthoxylum armatum* (100 mg/kg p.o.)	1.58 ± 0.32	1.05 ± 0.28*	0.82 ± 0.25*	0.78 ± 0.22*	0.70 ± 0.20*
Ethanolic bark extract of *Zanthoxylum armatum* (200 mg/kg p.o.)	1.52 ± 0.30	0.80 ± 0.20**	0.68 ± 0.18**	0.55 ± 0.15***	0.48 ± 0.12***
Clindamycin (200 mg/kg p.o.)	1.40 ± 0.28	0.65 ± 0.18**	0.52 ±0.15***	0.40 ± 0.10***	0.35 ± 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)

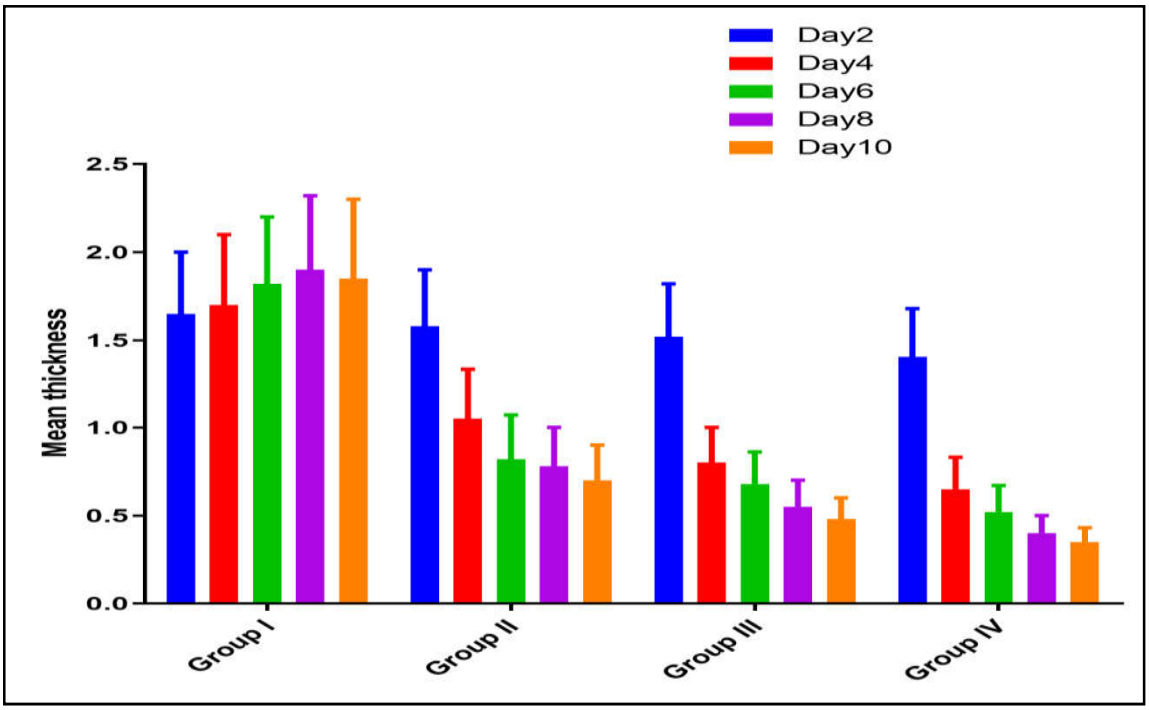

Figure 1: Effect of Clindamycin (Standard) and ethanolic extract of *Zanthoxylum armatum* induced acne by *Propionibacterium acnes* in rats

CONCLUSION

In conclusion, the ethanolic bark extract of *Zanthoxylum armatum* holds promise as a plant-based treatment for acne. Its phytochemical richness and notable anti-acne activity make it a compelling candidate for further development, aligning with the growing interest in natural and sustainable healthcare solutions.

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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