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Original Research Article

EVALUATION OF ANTI-ACNE ACTIVITY OF CRATAEVA NURVALA STEM BARK

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ABSTRACT

Acne is a common skin condition that affects a significant portion of the population, causing physical and psychological distress. Current treatments for acne often come with side effects and limitations. In the search for safer and more effective alternatives, traditional medicinal plants have garnered interest due to their potential anti-acne properties. *Crataeva nurvala*, a plant with a rich history in traditional medicine, has been reported to possess various therapeutic properties. This study aimed to evaluate the anti-acne activity of the hydroalcoholic extract of *Crataeva nurvala* stem bark. The extraction of *Crataeva nurvala* stem bark was performed using the maceration method, and the crude extract was concentrated on a water bath to obtain the actual yield. Phytochemical screening was conducted using standard methods to identify the presence of alkaloids, glycosides, saponins, flavonoids, phenols, proteins, carbohydrates, diterpenes, and tannins. The total phenolic content (TPC) and total flavonoid content (TFC) of the extract were determined using calibration curves based on the absorbance values obtained from the reaction with gallic acid and quercetin standards, respectively. The anti-acne activity was evaluated in a rat model induced by Propionibacterium acnes. The rats were treated with different doses of the hydroalcoholic extract of *Crataeva nurvala* stem bark, and ear thickness was measured as an index of inflammation. The results of the phytochemical screening revealed the presence of alkaloids, flavonoids, phenolics, proteins, reducing sugars, saponins, diterpenes, and tannins in the extract. The TPC and TFC values indicated that *Crataeva nurvala* stem bark is a rich source of phenolic and flavonoid compounds. In the anti-acne evaluation, the extract demonstrated significant anti-inflammatory effects, as evidenced by the reduction in ear thickness in the rat model. The anti-acne activity of the extract was comparable to that of the standard treatment, Clindamycin**.**

Keywords: *Crataeva nurvala*, Phytochemical screening, Total phenolic content, *Propionibacterium acnes*.

INTRODUCTION

Acne vulgaris is a chronic inflammatory disease mainly affecting the teenagers. As it is chronic and affects face mainly, it always causes negative psychological impact on human life. In a study conducted in 2015 acne affected 650 million people globally making it the most common skin disease worldwide. In a study of "Global burden of disease" it is revealed that acne was the 8th common prevalent disease worldwide (Leung *et al.*, 2021).

Acne vulgaris is a disfiguring prolonged inflammatory disorder of the pilosebaceous units. The psychological impacts of acne include loss of self-confidence, depression, anxiety, and interpersonal and work-related difficulties. The clinical presentation of acne comprises black and whiteheads (comedones), pinheads (papules), pustules, nodules, and pitted or hypertrophic scars. The face, shoulders, upper chest, and back may be affected. Acne vulgaris is mainly attributed to the increased production of androgens present in males and females during puberty. Accordingly, the pilosebaceous units produce more sebum, followed by follicular hyperkeratinization and plugging of the hair follicles. Thus, sebum cannot reach the skin surface, which encourages anaerobic bacteria, including Cutibacterium acnes (formerly Propionibacterium acnes), to grow in the plugged follicle. These bacteria trigger an inflammatory response in the skin, manifested as heat, swelling, redness, and pus (Kapoor *et al.*, 2011).

Grounded on the type and severity of acne lesions, rational use of existing treatment choices is currently an essential component of successful acne therapy. The current armamentarium available consists mainly of topical and oral retinoids, topical antimicrobials, systemic antibiotics, keratolytics, and hormonal therapy that consisted of oral contraceptives as well as androgen blocking agents, in addition to combination therapy of all the aforementioned agents (Gollnick *et al.*, 1998).

Topical retinoids (vitamin A derivatives) are comedolytic agents which reduce abnormal mitosis of keratinocytes, hyperkeratinization, and inflammation. Modified slow release formulations and a third generation retinoid adapalene are reported to be less irritating. Azelaic acid is a naturally occurring dicarboxylic acid with modest antibacterial and comedolytic effects. Erythromycin and clindamycin are the most commonly used topical antibiotics in acne.

They are useful in inflamed lesions with associated antibiotic resistant as a major problem. Oral antibiotics, namely, tetracyclines and macrolides, are prescribed in moderate to severe inflammatory acne, thereby precluding the practicality of applying topical therapies (Ramanathan & Hebert, 2011).

Conventional acne treatment involves topical alone or in combination with systemic therapies in severe cases. Topically used agents include comedolytic agents, antibiotics, and various anti-inflammatory drugs. Systemically used agents include retinoids, antibiotics, zinc, and hormones. Topical treatment is the standard for most patients with comedo-papular acne; however, local and systemic treatments are needed for pustulocystic scarring acne. Drawbacks of conventional therapy include increased antibiotic resistance in acne-causing bacteria (C. acnes and S. epidermidis). Moreover, the increased incidence of pregnant women exposed to oral tretinoin, a known teratogen, and the poor safety profile of systemic retinoid therapy needs attention to search for safer alternative of acne treatment.

Herbal therapy of acne has been encouraged due to the advantages of better patient tolerance, long history of use, fewer side effects, and being relatively more cost-effective. Considering this fact this study aims

to evaluate anti acne prospective of plant *Crataeva nurvala*.

MATERIALS AND METHODS

Extraction by maceration process

56 gm of dried powdered stem bark of *Crataeva nurvala* has been extracted with hydroalcoholic solvent (ethanol : water; 80:20 v/v) using maceration process for 24 hrs, filtered and dried using vacuum evaporator at 40ºC.

Phytochemical Screening

Phytochemical examinations were carried out for all the extracts as per the standard methods.

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

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

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

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

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

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

Molisch's Test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

Benedict's Test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Fehling's Test: Filtrates were 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.

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

Legal's Test: Extracts were 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: Extracts were 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: Extracts were 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: Extracts were 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: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Detection of proteins

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

9. **Detection of diterpenes**

Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes (Audu *et al.*, 2007).

Estimation of total phenolic content

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method (Parkhe and Bharti, 2019).

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.

Estimation of total flavonoids content

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% AlCl3 methanolic 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 vivo* anti acne activity**

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) (OECD, 2001). Animals were kept fasting providing only water, leaves hydroalcoholic extract of *Crataeva nurvala* (250, 500, 1000, 2000mg/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: Leaves hydroalcoholic extract of *Crataeva nurvala* (100mg/kg, p.o.)

Group –III: Leaves hydroalcoholic extract of *Crataeva nurvala* (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 140μg of heat-killed bacteria. The groups II, III and IV received 100 mg/kg and 200 mg/kg of leaves hydroalcoholic extract of *Crataeva nurvala* 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 two day until the 10th day.

Table 1: Phytochemical screening of extract of *Crataeva nurvala*

S. No.	Constituents	Hydroalcoholic 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	+ve
	Alkaline Reagent Test:	+ve
4.	**Phenolic**s	
	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: Foam Test:	 +ve -ve
8.	**Diterpins** Copper acetate test	 +ve
9.	**Tannins** Gelatin Test:	 +ve

Table 2: Total phenolic and total flavonoid content of *Crataeva nurvala*

S. No.	Total phenol content	Total flavonoid content
1.	0.455 mg/100mg	0.847 mg/100mg

Table 3: Effect of Clindamycin (standard) and hydroalcoholic extract of *Crataeva nurvala* on acneinduced by *Propionibacterium acnes* in rats

Treatment	Dose	Mean thickness ±SEM				
		Day2	Day4	Day6	Day8	Day10
Control	140 µg	1.48± 0.25	1.35 ± 0.15	1.28± 0.20	1.25± 0.15	1.24± 0.12
Crataeva nurvala extract	100mg/kg p.o.	1.45±0.20*	0.35±0.25*	0.22±0.32*	0.22±0.32*	0.22±0.30*
Crataeva nurvala extract	200mg/kg p.o.	1.07±0.25***	0.24±0.30**	0.20±0.35**	0.18±0.25**	0.18±0.35**
Clindamycin	200 mg/kg p.o.	0.93±0.30***	0.17±0.15***	0.10±0.25***	0.09±0.18***	0.09±0.25***

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

RESULTS AND DISCUSSION

Table showed the percentage yield of hydroalcoholic extract of *Crataeva nurvala* exhibited comparable yield 7.8% respectively. Preliminary phytochemical analysis generally helps identify and classify the plant extracts' bioactive constituents. For extracts of all samples, a small portion of the dried extract of plant underwent phytochemical screening using Kokate (1994) methods for chemical testing of alkaloids, glycosides, flavonoids, saponins, phenolics, proteins and amino acids, tannins separately.

The content of total phenolic compounds (TPC) content was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: Y = 0.042X-0.002, R2= 0.999, where X is the gallic acid equivalent (GAE) and Y is the absorbance. The content of total flavonoid compounds (TFC) content was expressed as mg/100mg of quercetin equivalent of dry extract sample using the equation obtained from the calibration curve: Y = 0.06X+0.019, R2= 0.999, where X is the quercetin equivalent (QE) and Y is the

absorbance. The total phenolic content in hydroalcoholic extract was found 0.455 mg/100mg and total flavonoid content was found 0.847 mg/100mg respectively in hydroalcoholic extract of *Crataeva nurvala*.

Acne vulgaris is a chronic inflammatory disease results in the formation of inflamed and/or noninflamed eruptions *Propionibacterium acnes* are the anaerobes, in the skin which grow in the sebaceous region. Various antibiotics like tetracycline, Clindamycin, and erythromycin etc and other drugs like benzoyl peroxide are used for acne treatment.

The evaluation of the anti-acne activity of *Crataeva nurvala* stem bark demonstrated promising results. The hydroalcoholic extract of *Crataeva nurvala* exhibited significant anti-acne effects in a rat model induced by *Propionibacterium acnes*. The reduction in inflammation observed in the rats' ears indicates the potential of the extract as a natural remedy for acne treatment.

CONCLUSION

The findings of this study support the traditional use of *Crataeva nurvala* in herbal medicine for treating skin conditions like acne. The anti-acne activity of the hydroalcoholic extract, combined with its phytochemical profile rich in bioactive compounds, provides a strong rationale for further exploration of this natural remedy. However, to validate these findings and establish its safety and efficacy in human subjects, additional studies, including clinical trials, are necessary. The potential of *Crataeva nurvala* stem bark extract as an anti-acne agent opens up opportunities for the development of novel natural skincare products or topical formulations for acne management.

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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