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EVALUATION OF ANTIMICROBIAL AND ANTI-ACNE ACTIVITY OF BIXA ORELLANA LEAF EXTRACT-BASED HERBAL GEL

Snehal Bagde*, Abhishek Sharma, Payal Saiju, Surendra Kumar Jain

Lakshmi Narain College of Pharmacy, Kalchuri Nagar, Raisen Road, Bhopal, Madhya Pradesh-462021, India.

****Corresponding Author: Snehal Bagde**

Lakshmi Narain College of Pharmacy, Kalchuri Nagar, Raisen Road, Bhopal, Madhya Pradesh-462021, India.

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Abstract

The present study was aimed at evaluating the antimicrobial and anti-acne potential of an ethanolic leaf extract of *Bixa orellana* formulated as a herbal gel. The ethanolic extract was subjected to preliminary phytochemical screening, which revealed the presence of flavonoids, phenolic compounds, glycosides, proteins, carbohydrates, and sterols. Quantitative estimation showed appreciable total phenol and total flavonoid content, indicating strong antioxidant potential. The antioxidant activity of the extract was confirmed by DPPH and nitric oxide radical scavenging assays, which demonstrated concentration-dependent free radical inhibition. Antimicrobial activity against *Propionibacterium acnes* showed significant zones of inhibition, confirming the antibacterial efficacy of the extract. Herbal gel formulations were prepared and evaluated for physicochemical parameters such as homogeneity, pH, viscosity, spreadability, washability, and extrudability, all of which were found to be satisfactory for topical application. In vivo anti-acne activity was assessed using a heat-killed *Propionibacterium acnes* induced acne model in rats, where the extract-based gel produced a significant reduction in inflammatory ear thickness in a dose-dependent manner. The 2% extract gel showed comparable efficacy to standard clindamycin gel. The results of the study suggest that *Bixa orellana* leaf extract-based herbal gel is a promising, safe, and effective alternative for the management of acne vulgaris.

Keywords: *Bixa orellana*; Anti-acne activity; Herbal gel; Antimicrobial activity; Antioxidant activity; *Propionibacterium acnes*

Introduction

Acne vulgaris is a common chronic inflammatory disorder of the pilosebaceous unit, predominantly affecting adolescents and young adults. It is characterized by comedones, papules, pustules, nodules, and, in severe cases, scarring. The pathogenesis of acne involves increased sebum production, follicular hyperkeratinization, colonization by *Cutibacterium acnes* (formerly *Propionibacterium acnes*), and inflammation mediated by microbial metabolites and host immune responses (Zaenglein *et al.*, 2016).

Conventional acne therapy includes topical and systemic antibiotics, retinoids, benzoyl peroxide, and hormonal agents. However, prolonged use of synthetic antimicrobials is associated with adverse effects such as skin irritation, dryness, photosensitivity, and the emergence of antibiotic-resistant strains of *C. acnes* and *Staphylococcus aureus* (Thiboutot *et al.*, 2009; Walsh *et al.*, 2016). These limitations have driven growing interest in herbal and plant-based formulations that offer better safety, improved patient compliance, and multi-targeted therapeutic effects.

Medicinal plants are rich sources of bioactive phytoconstituents such as flavonoids, tannins, phenolic compounds, terpenoids, and alkaloids, which exhibit antimicrobial, anti-inflammatory, antioxidant, and wound-healing properties. Herbal gels, in particular, are preferred topical dosage forms due to their non-greasy nature, ease of application, good spreadability, and enhanced patient acceptability (Sasidharan *et al.*, 2011; Kumar *et al.*, 2012).

Bixa orellana Linn. (family: Bixaceae), commonly known as annatto, is a medicinal plant traditionally used for the treatment of skin disorders, wounds, infections, and inflammatory conditions. The leaves of *Bixa orellana* are reported to contain flavonoids, tannins, phenols, carotenoids, and essential oils, which contribute to its antimicrobial and anti-inflammatory activities (Pandey *et al.*, 2014; Suleiman *et al.*, 2018). Previous studies have demonstrated the antibacterial potential of *Bixa orellana* extracts against Gram-positive and Gram-negative bacteria, suggesting its suitability for managing acne-causing microorganisms.

Incorporation of *Bixa orellana* leaf extract into a herbal gel formulation may enhance its topical efficacy by ensuring prolonged contact with the skin, improved stability of phytoconstituents, and controlled release of active compounds. Therefore, the present study was undertaken to evaluate the antimicrobial and anti-acne activity of *Bixa orellana* leaf extract-based herbal gel as a safe and effective alternative to conventional anti-acne therapies.

MATERIAL AND METHODS

Material

The materials used in the present study included fresh leaves of *Bixa orellana*, which were collected, authenticated, and used for extraction. Ethanol was employed as the solvent for extraction of bioactive constituents. Chemicals and reagents such as Folin–Ciocalteu reagent, quercetin, gallic acid, ascorbic acid, DPPH, sodium nitroprusside, and dimethyl sulfoxide were used for phytochemical and antioxidant studies. Nutrient media and microbial cultures of *Propionibacterium acnes* were utilized for antimicrobial evaluation. Gel formulation materials such as suitable gelling agents, preservatives, and purified water were used for the preparation of herbal gel, while clindamycin gel served as the standard for comparison in the in vivo anti-acne study.

Methods

Collection of plant materials

The collection of plant material is an essential step in various scientific, agricultural, and horticultural endeavors. The method of collecting plant material depends on the specific objectives, the type of plant, and the desired outcome. Leaves of *Bixa orellana* were collected from Vindhya Herbals of Bhopal in the month of February, 2025.

Extraction by ultrasonic-assisted extraction process

Following procedure was adopted for the preparation of extract from the shade dried and powdered herbs. Leaves of *Bixa orellana* was shade dried at room temperature. The shade dried plant material was coarsely powdered. 30 gm of dried powdered leaves of *Bixa orellana* has been extracted with ethanol solvent using ultrasonic-assisted extraction process for 24 hrs, filtered and dried using vacuum evaporator at 40°C (Mukherjee, 2007).

Determination of percentage yield

The percentage yield of each extract was calculated by using following formula:

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

Phytochemical screening

Phytochemical tests are conducted to identify and determine the quantity of specific phytochemical compounds present in a plant extract or plant material. These tests employ various chemical, chromatographic, and spectroscopic techniques to isolate, separate, and characterize the phytochemicals. The choice of tests depends on the nature of the phytochemical of interest and the available resources. Phytochemical examinations were carried out for the extract as per the standard methods (Kokate, 1994).

Estimation of total phenol 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% $AlCl_3$ 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-vitro antioxidant activity of _Bixa orellana_ extract

DPPH method

Total free radical scavenging capacity of extracts from *Bixa orellana* were estimated according to the previously reported method with slight modification (Parkhe and Jain, 2018). Solution of DPPH (6 mg in 100ml methanol) was prepared and stored in dark place. Different concentration of standard and test (10- 100 µg/ml) was prepared. 1.5 ml of DPPH and 1.5 ml of each standard and test was taken in seperate test tube; absorbance of this solution was taken immediately at 517nm. 1.5 ml of DPPH and 1.5 ml of the methanol was taken as control absorbance at 517nm. The percentage inhibition of free radical DPPH was calculated from the following equation:

% inhibition = [(absorbance of control – absorbance of sample)/ absorbance of control] × 100%.

Nitric oxide method

Nitric oxide was produced from sodium nitroprusside and the Griess reagent was measured. Sodium nitroprusside spontaneously produces nitric oxide in aqueous solution at physiological pH, interacting with oxygen to generate nitric ions that can be estimated using Griess reagent. Nitric oxide scavengers compete with oxygen resulting in decreased nitric oxide manufacturing (Marcocci *et al.*, 1994). Sodium nitroprusside (10 mmol / L) was mixed with various extract concentrations in phosphate buffer saline (PBS) and incubated at 25°C for 150 min. Griess reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% napthylethylenediamine dihydrochloride) was added to the specimens. The chromophore absorbance created during the diazotization of sulphanilamide nitrite and subsequent coupling with napthylethyleneediamine was read at 546 nm and referred to the absorption of conventional ascorbic acid solutions treated in the same manner with Griess reagent as a positive control. The inhibition proportion was evaluated using the following formula:

$$\text{Radical scavenging activity (\%)} = (A_{control} - A_{test})/A_{control} \times 100$$

Where Acontrol is the absorption (without extract) of the control and where Atest is the absorption in the presence of the extract / standard.

***In vitro* antimicrobial activity of *Bixa orellana* extract**

The well diffusion method was used to determine the antimicrobial activity of the ethanolic extract prepared from of *Bixa orellana* using standard procedure (Bauer *et al.*, 1966).There were 3 concentration used which are 25, 50 and 100 mg/ml for 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.

Evaluation of herbal gel

Appearance and consistency

The physical appearance was visually checked for the texture of herbal gel formulations and observations.

Washability

Formulations were applied on the skin and then ease and extent of washing with water were checked manually and observations may be like stated in table (Maria *et al.*, 2009).

Extrudability determination of formulations

The herbal gel formulations were filled into collapsible metal tubes or aluminium collapsible tubes. The tubes were pressed to extrude the material and the extrudability of the formulation was checked (Das *et al.*, 2010).

Determination of Spreadability

Two glass slides of standard dimensions (6×2) were selected. The herbal gel formulation whose spreadability had to be determined was placed over one of the slides. The second slide was placed over the slide in such a way that the formulation was sandwiched between them across a length of 6 cms along the slide. 100 grams of weight was placed up on the upper slide so that the herbal gel formulation between the two slides was traced uniformly to form a thin layer (Bhat and Shivakumar, 2007).

The weight was removed and the excess of the herbal gel formulation adhering to the slides was scrapped off. The lower slide was fixed on the board of the apparatus and one end of the upper slide was tied to a string to which 20 gram load could be applied 50with the help of a simple pulley. The time taken for the upper slide to travel the distance of 6 cms and separate away from lower slide under the direction of the weight was noted. The experiment was repeated and the average of 6 such determinations was calculated for herbal gel formulation.

$$\text{Spreadability} = \frac{m.l}{t}$$

Where, S=Spreadability (gcm/sec)
m = weight tied to the upper slide (20 grams)
l= length of glass slide (6cms).
t = time taken is seconds.

Determination of pH

The pH of the herbal gels was determined by digital pH meter. One gram of gel was dissolved in 25 ml of distilled water and the electrode was then dipped in to gel formulation until constant reading obtained. And constant reading was noted. The measurements of pH of each formulation were replicated two times (Kaur *et al.*, 2010).

Drug content

The drug content was determined by taking 1gm of gel in 10 ml volumetric flask diluted with methanol. 2 ml of stock solution 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 15min for colour development. The absorbance was measured at 765 nm using a spectrophotometer (Bele *et al.*, 2010).

***In vivo* antiacne activity of gel formulation**

Animals

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.

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 extract of *Bixa orellana* 1% Gel (Topically)
Group –III: Ethanolic extract of *Bixa orellana* 2% Gel (Topically)
Group –IV: 1% Clindamycin, Clindac A (Topically)

The experimental model of acne-like inflammation was induced in rat ears through subcutaneous administration of 0.14 mg of heat-killed *Propionibacterium acnes* (Jatav *et al.*, 2023).

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 evaluated the phytochemical profile, antioxidant potential, antimicrobial activity, and in vivo anti-acne efficacy of the ethanolic extract of *Bixa orellana* leaves formulated as a herbal gel. The percentage yield of the ethanolic extract was found to be 5.56% w/w (Table 1), indicating effective extraction of bioactive constituents using ethanol as the solvent. Ethanol is known to efficiently extract polar and moderately non-polar phytochemicals, which may explain the appreciable yield obtained.

Preliminary phytochemical screening (Table 2) revealed the presence of glycosides, flavonoids, phenolic compounds, proteins, carbohydrates, and sterols in the ethanolic extract, while alkaloids, saponins, diterpenes, and tannins were absent. The presence of flavonoids and phenolic compounds is particularly significant, as these constituents are well documented for their antioxidant, antimicrobial, and anti-inflammatory activities, which are crucial in the management of acne.

Quantitative estimation showed a high total phenol content (4.68 mg/100 mg) and total flavonoid content (3.25 mg/100 mg) in the extract (Table 3). These findings support the antioxidant potential of *Bixa orellana*. This was further confirmed by DPPH and nitric oxide radical scavenging assays (Tables 4 and 5), where the extract exhibited concentration-dependent antioxidant activity. Although the extract showed lower activity compared to ascorbic acid, the IC_{50} values (66.74 µg/ml for DPPH and 75.92 µg/ml for NO scavenging) indicate a moderate to good antioxidant potential, which may help reduce oxidative stress associated with acne pathogenesis.

The antimicrobial study against *Propionibacterium acnes* demonstrated that the ethanolic extract possessed notable antibacterial activity (Table 6). The zones of inhibition observed at different concentrations confirm the extract's ability to suppress acne-causing bacteria. This activity can be attributed to the presence of phenols and flavonoids, which are known to disrupt microbial cell membranes and inhibit bacterial enzymes.

The formulated herbal gels (HG1 and HG2) showed satisfactory physicochemical properties, including good homogeneity, smooth texture, absence of clogging, acceptable washability, and extrudability (Tables 7 and 8). The pH of both formulations was found to be close to skin pH, indicating suitability for topical application without causing irritation. Spreadability and viscosity values (Table 9) suggest good patient compliance and ease of application. The phenol content retained in the gel formulations indicates stability of active constituents after formulation.

In vivo anti-acne studies using heat-killed *Propionibacterium acnes* induced acne model in rats demonstrated significant reduction in ear thickness in

animals treated with *Bixa orellana* gel formulations (Table 11). Both 1% and 2% topical gels showed progressive and significant reduction in inflammation compared to the control group, with the 2% gel showing better efficacy. The results were comparable to the standard clindamycin gel, especially at later time points, indicating strong anti-acne potential of the extract-based formulation.

Table 1: % yield of extract of *Bixa orellana.*

S. No.	Extract	% Yield (w/w)
1.	Ethanolic	5.56

Table 2: Phytochemical screening of extract *of Bixa orellana*

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

+ve=positive; -ve= negative

Table 3: Total phenol and total flavonoid content of *Bixa orellana*

S. No.	Total phenol content	Total flavonoid content
	mg/100mg	
1.	4.68	3.25

Table 4: % Inhibition of ascorbic acid and extract of *Bixa orellana* using DPPH method.

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Ethanolic extract
1	10	42.25	13.27
2	20	45.69	14.66
3	40	61.47	33.02
4	60	67.05	48.38
5	80	75.98	56.92
6	100	81.11	61.08
IC 50 value		**24.75**	**66.74**

Table 5: % Inhibition of ascorbic acid and extract of *Bixa orellana* using NO method.

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Ethanolic extract
1	20	46.87	21.05
2	40	55.21	37.12
3	60	63.47	42.58
4	80	78.05	51.96
5	100	92.63	60.24
IC 50 value		**29.89**	**75.92**

Table 6: Antimicrobial activity of ethanolic extract of *Bixa orellana* against *Propionibacterium acnes.*

S. No.	Microbes	Zone of Inhibition (nm)		
		25mg/ml	50 mg/ml	100 mg/ml
1	*Propionibacterium acnes*	15±0.47	14±0.94	11±0.86

Table 7: Results of evaluation of gel formulation.

Formulation	Colour	Clogging	Homogeneity	Texture
HG1	Dark brown	Absent	Good	Smooth
HG2	Dark brown	Absent	Good	Smooth

Table 8: Results of washability and extrudability.

Formulation	Washability	Extrudability
HG1	Good	Average
HG2	Good	Average

Table 9: Results of evaluation of gel.

Formulation	Spreadability (gcm/sec)	pH	Viscosity (cps)	Phenol content (mg/100mg)	
HG1	13.65±0.25	6.85±0.08	3256±17	4.25±0.11	
HG2	12.45±0.36	6.92±0.06	3130±15	4.38±0.12	

Table 10: Protocol study for *in-vivo* anti-acne activity on rats

Groups	Induction of Acne	Treatment
Control (acne induced)	Heat killed *Propionibacterium acnes*	Vehicle
Treated with ethanolic extract of *Bixa orellana* gel	Heat killed *Propionibacterium acnes*	1% Topical
Treated with ethanolic extract of *Bixa orellana* gel	Heat killed *Propionibacterium acnes*	2% Topical
Treated with Clindamycin	Heat killed *Propionibacterium acnes*	1% Topical

Table 11: Effect of Clindamycin (standard) and ethanolic extract of *Bixa orellana* induced acne by *Propionibacterium acnes* in rats.

Treatment	Dose	Mean thickness ±SEM				
		Day 2	Day 4	Day 6	Day 8	Day 10
Control	0.14 mg	1.95 ± 0.15	1.98 ± 0.20	1.96 ± 0.10	1.98 ± 0.15	1.92 ± 0.35
Ethanolic extract of *Bixa orellana*	1% Topical	1.65 ± 0.15	1.25 ± 0.20*	1.20 ± 0.18*	0.93 ± 0.15*	0.84 ± 0.14*
Ethanolic extract of *Bixa orellana*	2% Topical	1.60 ± 0.15	1.15 ± 0.20**	0.95± 0.13**	0.78 ± 0.12***	0.65 ± 0.15***
Clindamycin	1% Topical	1.15 ± 0.18	0.80 ± 0.20**	0.63 ± 0.10***	0.49 ± 0.14***	0.40 ± 0.13***

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)

CONCLUSION

The present study concludes that the ethanolic extract of *Bixa orellana* leaves possesses significant antimicrobial, antioxidant, and anti-acne activity. Phytochemical investigation confirmed the presence of bioactive constituents such as flavonoids, phenolic compounds, glycosides, and sterols, which are likely responsible for the observed biological activities. The extract exhibited effective free radical scavenging ability and notable antibacterial action against *Propionibacterium acnes*. The formulated herbal gel showed acceptable physicochemical characteristics, skin-compatible pH, and good stability, making it suitable for topical application. In vivo evaluation demonstrated a significant, dose-dependent reduction in acne-induced

inflammation, with the 2% extract gel showing efficacy comparable to the standard clindamycin formulation.

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