RESEARCH PAPER

In Vitro and In Vivo Evaluation of Anti-Acne Potential of Hydroalcoholic Extract of Achyranthes aspera and Its Topical Herbal Gel Formulation

Vaishali Yadav*[1], Abhishek Sharma[2], Surendra Kumar Jain[3], Reetesh Malvi[4]

1. *Research Scholar, Lakshmi Narain College of Pharmacy, Bhopal, India, vyadav13042001@gmail.com*
2. *Associate Professor, Lakshmi Narain College of Pharmacy, Bhopal, India, prof.abhisheksharma30@gmail.com*
3. *Professor, Lakshmi Narain College of Pharmacy, Bhopal, India, prof.surendrajain@gmail.com*
4. *Associate Professor, Lakshmi Narain College of Pharmacy, Bhopal, India, reeteshmalvi@gmail.com*

ABSTRACT

Acne vulgaris is an inflammatory skin disease, which is predominantly associated with Propionibacterium acnes and Staphylococcus aureus. The present study was aimed to investigate the anti-acne activity of hydroalcoholic extract of Achyranthes aspera leaves and to develop and test a topical gel for the treatment of acne. The plant extract was prepared by maceration using hydroalcoholic solvent system (ethanol: water 65:35) and the amount of extract obtained was 5.70% w/w. Preliminary phytochemical screening showed presence of alkaloids, flavonoids, phenolics, tannins, saponins, carbohydrates, proteins and diterpenes, but not glycosides or sterols. The total phenolics and flavonoids were determined to be 0.85mg/100mg and 0.72mg/100mg respectively. The extract was found to have concentration-dependent in vitro antimicrobial activity against P. acnes (ZI 10±0.5 to 14±0.66 mm) and S. aureus (8.3±0.57 to 10±0.94 mm) at above 25 to 100 mg/ml. Two herbal gel formulations (1% Carbopol 940 gel (F1) and 2% Carbopol 940 gel (F2)) were developed and their physicochemical parameters such as spreadability, washability, extrudability, viscosity, pH and phenolic content were evaluated; both formulations had acceptable properties, F2 being better. The anti-acne effect of the gel was also validated using in vitro (zone of inhibition up to 16±0.57 mm at 100 mg/ml) and in vivo studies. Topical application of 1% and 2% Achyranthes aspera gel resulted in a significant decrease in ear thickness in a dose dependent manner (1.72±0.14 mm and 1.63±0.13 mm on Day 2; 0.82±0.12 mm and 0.58±0.10 mm on Day 10, respectively) compared to the standard 1% Clindamycin gel (0.25±0.07 mm). Based on the above findings, Achyranthes aspera proved to have promising anti-acne activity and could be considered as a herbal remedy for acne treatment without causing any side effects.

Keywords: Achyranthes aspera; Acne vulgaris; Hydroalcoholic extract; Anti-acne activity; Propionibacterium acnes; Herbal gel; Phenolic content; Flavonoids; Topical formulation.

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1. INTRODUCTION

Acne vulgaris is a chronic inflammatory disorder of the pilosebaceous unit that predominantly affects adolescents and young adults, with a global prevalence estimated at over 650 million people, making it one of the most common skin diseases worldwide. The condition arises from a complex interplay of increased sebum production, follicular hyperkeratinization, colonization by Propionibacterium acnes (Cutibacterium acnes), and a subsequent local inflammatory response. Clinically, acne manifests as comedones, papules, pustules, nodules, and, in severe cases, scarring, often resulting in considerable psychological distress including loss of self-confidence, anxiety, and depression [1].

Conventional therapeutic options for acne, including topical and oral retinoids, benzoyl peroxide, and antibiotics such as clindamycin and erythromycin, are frequently associated with limitations such as skin irritation, antibiotic resistance, and long-term side effects [2]. These concerns have intensified interest in plant-derived alternatives that combine antimicrobial, anti-inflammatory, and antioxidant properties with a favourable safety profile. Medicinal plants represent a vast and largely underexploited reservoir of bioactive phytoconstituents capable of modulating the multifactorial pathogenesis of acne [3].

Achyranthes aspera Linn. (*Family Amaranthaceae*), commonly known as Latjeera or Chirchita, is a perennial herb widely distributed throughout India and used extensively in Ayurvedic, Unani, Siddha, and folk medicine for the treatment of a broad spectrum of ailments, including inflammatory, dermatological, and infectious conditions. Phytochemical investigations have revealed that the plant is rich in alkaloids, flavonoids, saponins, tannins, triterpenoids, and phenolic compounds, many of which possess documented antimicrobial, antioxidant, and anti-inflammatory activities relevant to acne management. Despite considerable literature on the general pharmacological profile of A. aspera, comprehensive data correlating its phytochemical composition with in vitro and in vivo anti-acne efficacy, alongside the development of a stable topical formulation, remain limited [4,5].

The present study was therefore designed to prepare a hydroalcoholic extract of A. aspera leaves, characterize its phytochemical composition and total phenolic/flavonoid content, evaluate its antimicrobial activity against acne-associated pathogens, formulate topical herbal gels incorporating the extract, and assess their anti-acne efficacy through in vitro antimicrobial assays and an in vivo Propionibacterium acnes-induced rat model [6,7,8].

2. MATERIALS AND METHODS

2.1 Selection, Collection, and Extraction of Plant Material

Fresh leaves of Achyranthes aspera were collected from the local area of Bhopal, Madhya Pradesh, India. The plant material was shade-dried, powdered, and stored in airtight containers until further use. The whole plant of Achyranthes aspera was selected for the present study owing to its reported antimicrobial, anti-inflammatory, antioxidant, and wound-healing properties, which make it a promising candidate for the management of acne vulgaris. Fresh plant material was collected from the local region during the flowering season and authenticated by a qualified taxonomist. The collected material was thoroughly washed to remove adhering soil and foreign matter, shade dried at room temperature, and coarsely powdered for extraction [8,9].

The dried powdered plant material was subjected to maceration using a hydroalcoholic solvent system (ethanol:water) for 72 hours with occasional stirring to facilitate the extraction of phytoconstituents. After completion of extraction, the mixture was filtered and the filtrate was concentrated under reduced pressure to obtain a semisolid hydroalcoholic extract [10,11]. The obtained extract was stored in airtight containers until further use [12]. The hydroalcoholic solvent system was selected because of its ability to efficiently extract both polar and moderately non-polar bioactive constituents present in the plant material [13].

2.2 Preparation of Hydroalcoholic Extract

Fifty grams of the dried, powdered leaf material was extracted by the maceration method using a hydroalcoholic solvent system (ethanol:water, 65:35). The powdered drug was soaked in the solvent for 24 hours with intermittent stirring, filtered, and the filtrate concentrated under reduced pressure at 40°C using a rotary vacuum evaporator to obtain the dried extract. The percentage yield was calculated using the standard formula: % yield = (weight of extract / weight of powdered drug) × 100 [14,15].

2.3 Preliminary Phytochemical Screening

The hydroalcoholic extract was subjected to qualitative phytochemical analysis for the detection of alkaloids (Hager's test), carbohydrates (Benedict's and Fehling's tests), glycosides (concentrated H_2SO_4 test), saponins (froth test), phenols (ferric chloride test), tannins (gelatin test), flavonoids (lead acetate and alkaline reagent tests), proteins (xanthoproteic test), diterpenes (copper acetate test), and sterols (Salkowski test), following standard procedures [16,17].

2.4 Estimation of Total Phenolic Content (TPC)

The total phenolic content of the hydroalcoholic extract of Achyranthes aspera was determined using the Folin–Ciocalteu colorimetric method, employing gallic acid as the reference standard. Briefly, an aliquot of the extract was mixed with Folin–Ciocalteu reagent followed by the addition of sodium carbonate solution and incubated for color development. The absorbance of the resulting blue-colored complex was measured spectrophotometrically at 765 nm. The total phenolic content was calculated from the gallic acid calibration curve and expressed as milligrams of gallic acid equivalent (GAE) per 100 mg of extract. This assay provides an estimate of the phenolic constituents contributing to the antioxidant potential of the extract [18,19].

2.5 Estimation of Total Flavonoid Content (TFC)

The total flavonoid content of the hydroalcoholic extract was estimated by the aluminium chloride colorimetric method using quercetin as the standard reference compound. The extract was reacted with

aluminium chloride reagent to form a stable flavonoid–aluminium complex, and the absorbance was measured at 420 nm using a UV-visible spectrophotometer. The flavonoid content was calculated from the quercetin calibration curve and expressed as milligrams of quercetin equivalent (QE) per 100 mg of extract. The determination of flavonoid content provides an indication of the antioxidant and anti-inflammatory constituents present in the extract [19].

2.6 In Vitro Antimicrobial Activity

The in vitro antimicrobial activity of the hydroalcoholic extract of Achyranthes aspera was evaluated by the agar well diffusion method against acne-associated microorganisms, namely Propionibacterium acnes and Staphylococcus aureus. Sterile nutrient agar plates were inoculated with standardized microbial suspensions, and wells were prepared aseptically in the agar medium. Different concentrations of the extract (25, 50, and 100 mg/mL) were introduced into the respective wells, while appropriate controls were maintained for comparison. The plates were incubated at 37 ± 1°C for 24 hours, after which the antimicrobial activity was assessed by measuring the diameter of the zones of inhibition surrounding each well. The results demonstrated concentration-dependent antimicrobial activity of the extract against the tested microorganisms [20].

2.7 Formulation of Herbal Gel

Herbal gel formulations containing the hydroalcoholic extract of Achyranthes aspera were prepared using Carbopol 940 as the gelling agent at different concentrations. Two formulations were developed, namely F1 containing 1% w/w Carbopol 940 and F2 containing 2% w/w Carbopol 940, each incorporating 1% w/w plant extract. The formulations also contained methyl paraben as a preservative, polyethylene glycol as a humectant and co-solvent, and triethanolamine as a neutralizing agent to adjust the pH and facilitate gel formation, while distilled water served as the vehicle.

The required quantity of Carbopol 940 was dispersed in distilled water and allowed to hydrate completely. Subsequently, the extract and other excipients were incorporated with continuous stirring until a homogeneous gel was obtained. The pH was adjusted using triethanolamine to obtain clear and stable gel formulations suitable for topical application. The prepared gels were evaluated for various physicochemical and pharmaceutical parameters, including physical appearance, homogeneity, washability, extrudability, spreadability, pH, viscosity, total phenolic content, and in vitro anti-acne activity to determine their suitability for topical anti-acne therapy.

Table 1: Composition of Herbal Gel Formulations

Ingredient	**F1**	**F2**
Achyranthes aspera extract	1%	1%
Carbopol 940	1%	2%
Methyl Paraben	0.08%	0.08%
PEG	0.2 mL	0.2 mL
Triethanolamine	q.s.	q.s.
Distilled Water	q.s. to 100%	q.s. to 100%

2.8 In Vivo Anti-Acne Study

The in vivo anti-acne activity of the formulated herbal gels was evaluated using a Wistar rat acne model induced by Propionibacterium acnes. Acne-like inflammation was produced by subcutaneous injection of 0.14 mg of heat-killed *P. acnes* into the ear tissue of the animals. The rats were randomly divided into four groups (n = 6): normal control, negative control (acne-induced and vehicle-treated), 1% *Achyranthes aspera* gel-treated group, 2% *Achyranthes aspera* gel-treated group, and a standard-treated group receiving 1% Clindamycin gel. Topical treatments were applied once daily for a period of 14 days. The anti-inflammatory effect of the formulations was assessed by measuring ear thickness using a vernier caliper on Days 2, 4, 6, 8, and 10 following treatments. The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC), under the registration no 2149/po/Re/S/22/CPCSEA and all procedures were conducted in accordance with established ethical guidelines for animal experimentation in Pharmacology Lab-Lakshmi Narain College of Pharmacy, Bhopal.

2.9 Statistical Analysis

All experimental data were expressed as mean ± standard error of the mean (SEM) and analyzed using appropriate statistical methods. Statistical comparisons among experimental groups were performed using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison post hoc test to determine the significance of differences between treatment groups and the control group. Statistical analysis was carried out using suitable statistical software, and a value of $p < 0.05$ was considered statistically significant.

3. RESULTS

3.1 Percentage Yield and Phytochemical Screening

Figure 1: Phytochemical Screening of Hydroalcohplic Extract of Achyranthes Aspera

The hydroalcoholic extraction of A. aspera leaves yielded 5.70% w/w of a black, sticky extract with a characteristic odour. Phytochemical screening revealed the presence of alkaloids, flavonoids, phenolic compounds, proteins, carbohydrates, saponins, diterpenes, and tannins, while glycosides and sterols were not detected (Table 1).

Table 2: Phytochemical screening of hydroalcoholic extract of Achyranthes aspera

S. No.	Phytoconstituent	Test Performed	Result
1	Alkaloids	Hager's Test	+ve
2	Glycosides	Conc. H_2SO_4 Test	−ve
3	Flavonoids	Lead Acetate / Alkaline Reagent	+ve
4	Phenols	Ferric Chloride Test	+ve
5	Proteins	Xanthoproteic Test	+ve
6	Carbohydrates	Benedict's Test	+ve
7	Saponins	Froth Test	+ve
8	Diterpenes	Copper Acetate Test	+ve
9	Tannins	Gelatin Test	+ve
10	Sterols	Salkowski Test	−ve

3.2 Total Phenolic and Flavonoid Content

The total phenolic content (TPC) and total flavonoid content (TFC) of the extract were found to be 0.85 mg/100 mg GAE/100 mg extract and 0.72 mg QE/100 mg extract, respectively, indicating substantial antioxidant potential consistent with the phytochemical screening results.

3.3 In Vitro Antimicrobial Activity

Figure 2: Photo plate of Antimicrobial Activity of Hydroalcoholic Extract of Achyranthes Aspera Against Microbes

The hydroalcoholic extract of Achyranthes aspera exhibited appreciable antimicrobial activity against the acne-associated microorganisms tested. At a concentration of 25 mg/mL, the extract produced a zone of inhibition of 9.0 ± 0.6 mm against *Propionibacterium acnes* and 6.5 ± 0.5 mm against *Staphylococcus aureus***.** The extract demonstrated greater inhibitory activity against *P. acnes* compared to *S. aureus*, suggesting a higher susceptibility of the primary acne-causing organism to the phytoconstituents present in the extract. The observed antimicrobial activity may be attributed to the presence of bioactive compounds such as flavonoids, phenolics, tannins, alkaloids, and saponins, which are known to possess antimicrobial and anti-inflammatory properties. These findings support the potential application of *Achyranthes aspera* extract as a natural therapeutic agent for the management of acne vulgaris.

Table 3: Zone of inhibition (mm) of hydroalcoholic extract of Achyranthes aspera against selected microbes

S. No.	Microorganism	25 mg/ml	50 mg/ml	100 mg/ml
1	Propionibacterium acnes	9±0.6	10±0	15±0.47
2	Staphylococcus aureus	6.5±0.5	8±0.33	9±0.6

3.4 Physicochemical Evaluation of Gel Formulations

Both gel formulations (F1 and F2) exhibited satisfactory physicochemical properties suitable for topical application (Table 3). Formulation F2 (2% Carbopol) demonstrated superior homogeneity,

washability, extrudability, spreadability, and viscosity compared to F1, while both formulations retained comparable phenolic content after processing.

Table 4: Physicochemical evaluation of herbal gel formulations F1 and F2

Parameter	F1 (1% Carbopol)	F2 (2% Carbopol)
Colour	Dark Brown	Brownish Dark Green
Homogeneity	Good	Excellent
Washability	Good	Excellent
Extrudability	Good	Very Good
Spreadability (g·cm/sec)	12.98 ± 0.27	14.25 ± 0.34
pH	6.79 ± 0.06	6.95 ± 0.04
Viscosity (cps)	3156 ± 17	3168 ± 15
Phenolic Content (mg/100 mg)	0.80 ± 0.11	0.82 ± 0.12

3.5 In Vitro Anti-Acne Activity of Gel Formulation

The optimized gel formulation exhibited a concentration-dependent zone of inhibition against P. acnes of 11±0.94 mm, 12±0.86 mm, and 16±0.57 mm at 25, 50, and 100 mg/ml, respectively, exceeding the activity of the crude extract alone, suggesting improved diffusion and contact time of the active constituents within the gel matrix.

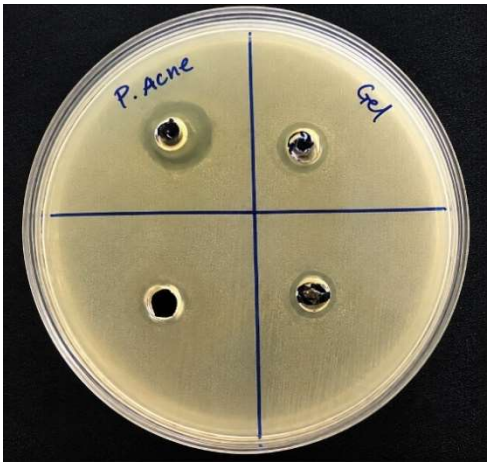

Figure 3: Photo plate of Antimicrobial Activity of Gel of Achyranthes Aspera Against Microbes

3.6 In Vivo Anti-Acne Activity

Table 5: Effect of Achyranthes aspera gel and standard clindamycin gel on ear thickness (mm, mean ± SEM) in P. acnes-induced rats

Group	Day 2	Day 4	Day 6	Day 8	Day 10
Negative Control (0.14 mg)	1.98 ±0.16	2.01± 0.18	1.97± 0.12	1.95± 0.14	1.93± 0.20
A. aspera Gel (1%)	1.72 ±0.14	1.38± 0.18*	1.15± 0.16*	0.98± 0.14**	0.82± 0.12**
A. aspera Gel (2%)	1.63 ±0.13	1.20± 0.16**	0.98± 0.14**	0.75± 0.11***	0.58± 0.10***
Standard (1% Clindamycin)	1.12 ±0.15	0.78± 0.13***	0.55± 0.10***	0.38± 0.08***	0.25± 0.07***

*p<0.05, **p<0.001, ***p<0.0001 vs. negative control (one-way ANOVA followed by Dunnett's test).

In the *Propionibacterium acnes*-induced rat ear inflammation model, the negative control group maintained persistently elevated ear thickness throughout the 10-day study (1.98 ± 0.16 mm on Day 2 to 1.93 ± 0.20 mm on Day 10), confirming sustained inflammation. In contrast, treatment with 1% and 2% *Achyranthes aspera* gel produced a progressive, dose-dependent reduction in ear thickness. The mean ear thickness decreased from 1.72 ± 0.14 mm to 0.82 ± 0.12 mm in the 1% gel-treated group and from 1.63 ± 0.13 mm to 0.58 ± 0.10 mm in the 2% gel-treated group between Day 2 and Day 10, as shown in Table 4. The standard 1% clindamycin gel exhibited the greatest therapeutic effect, reducing ear thickness from 1.12 ± 0.15 mm on Day 2 to 0.25 ± 0.07 mm on Day 10. Statistically significant reductions in ear thickness compared with the negative control were observed from Day 4 onward in all treatment groups, with significance levels ranging from $p < 0.05$ to $p < 0.0001$. These findings demonstrate the anti-inflammatory and anti-acne potential of *A. aspera* gel, with the 2% formulation exhibiting greater efficacy than the 1% formulation, although both remained less effective than the standard clindamycin gel.

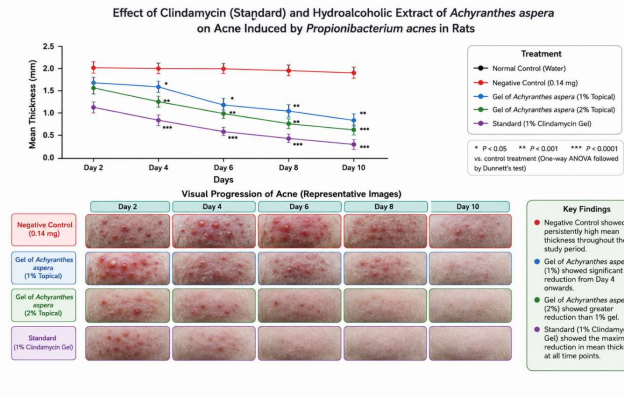

Figure 4: Effect of Clindamycin and Hydroalcoholic Extract of Achyranthes Aspera Gel on Acne induced by Propionibacterium in Rat

Table 6: Effect of Treatment on Ear Thickness in Acne-Induced Rats

Group	Day 2	Day 4	Day 6	Day 8	Day 10
Negative Control	Highest inflammation	Persistent inflammation	Persistent inflammation	Persistent inflammation	Persistent inflammation
1% Extract Gel	Moderate reduction	Significant reduction	Improved	Improved	Reduced inflammation
2% Extract Gel	Better reduction	Marked reduction	Significant reduction	Significant reduction	Near normal
Standard Clindamycin	Maximum reduction	Maximum reduction	Near normal	Near normal	Normal

4. DISCUSSION

The findings of this study demonstrate that the hydroalcoholic extract of Achyranthes aspera possesses notable anti-acne potential, attributable to its rich phytochemical profile. The presence of flavonoids, phenolics, tannins, saponins, alkaloids, and diterpenes is consistent with reports on related medicinal plants in which such constituents are associated with antimicrobial, antioxidant, and anti-inflammatory activities relevant to acne pathogenesis.

The concentration-dependent antimicrobial activity against P. acnes and S. aureus supports the traditional use of A. aspera in dermatological conditions and aligns with its reported broad-spectrum antibacterial properties. The enhanced antimicrobial efficacy of the gel formulation relative to the crude extract may be explained by improved retention and diffusion of active constituents from the semisolid gel matrix at the site of application, prolonging contact time with the causative organism.

The in vivo results, showing a significant and dose-dependent reduction in P. acnes-induced ear inflammation with both 1% and 2% gel formulations, corroborate the in vitro antimicrobial and anti-inflammatory findings. Although the standard clindamycin gel exhibited superior efficacy, the 2% A. aspera gel produced a comparable trend of inflammation reduction, suggesting its potential as an effective, plant-based alternative for managing mild to moderate acne, particularly in light of growing concerns regarding antibiotic resistance associated with conventional anti-acne agents. The acceptable physicochemical characteristics of the formulated gels, including suitable pH close to that of normal skin, satisfactory viscosity, spreadability, and good washability and extrudability, further support the suitability of the developed formulation for topical dermatological application.

5. CONCLUSION

The present study demonstrates that the hydroalcoholic extract of Achyranthes aspera contains a diverse array of bioactive phytoconstituents, including alkaloids, flavonoids, phenolics, tannins, saponins, and diterpenes, which collectively contribute to its significant antimicrobial activity against acne-associated pathogens. The developed herbal gel formulation exhibited satisfactory physicochemical properties and enhanced anti-acne activity compared with the crude extract, both in vitro and in an in vivo P. acnes-induced rat model. These findings support the potential of Achyranthes aspera as a safe, effective, and accessible herbal alternative for the topical management of acne vulgaris, warranting further clinical investigation.

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Conflict of Interest

The authors declare no conflict of interest.

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