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(Research Article)

Formulation and Evaluation of Herbal Anti-Acne Gel Containing Argemone Mexicana Leaf Extract

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ABSTRACT

The Prickly Poppy (Argemone Mexicana L.) has been used for centuries due to its ethnomedicinal properties, being one of the most widespread plants in sub-tropical areas. It contains several bio-actives such as Isoquinolines Alkaloids (Sanguinarine, Berberine, Protopine and Chelerythrine), Flavonoids, Terpenes and Phenols, which are associated with Antimicrobial Activity, Anti-Oxidant Properties, Anti-Inflammatory Effects, Antimalarial Activities, and Anti-Cancerous Effects. The purpose of this research was to create and assess an Anti-Acne Gel that includes the Ethanolic Leaf Extract of A. Mexicana as the main Active Agent; Neem Oil and Aloe Vera Gel as Synergists, while utilizing Carbopol 940 as the Gelling Agent. Three different formulations (F1-F3) were developed at three different levels of concentration of the Plant Extract (0.1g, 0.2g and 0.3g respectively). Phyto-Chemical Screening confirmed the presence of alkaloids, tannins, flavonoids and glycosides in the extract. The evaluation parameters included Physical Appearance, pH Value, Viscosity, Spreadability, Extrudability, Homogenity, Drug Content, Washability, Skin Irritation and Antimicrobial Activity. F2 showed better than all the other formulations when it came to its Characteristics, these include a uniform appearance, pH value = 6.2 (compatible to skin), acceptable viscosity, good spreadability, high antimicrobial activity against Propionibacterium Acnes and Staphylococcus Aureus.

Keywords: *Argemone mexicana; anti-acne gel; Carbopol 940; isoquinoline alkaloids; Propionibacterium acnes; phytochemical screening; topical formulation***.**

I. INTRODUCTION

A large number of people all around the world suffer from Acne vulgaris, that's one of the most common dermatologic disorders. In fact, about 85 % of young adults suffer from some form of Acne vulgaris. This disease causes a lot of psychological distress. Most cases of this disease result from excessive Sebum production, Follicular Hyperkeratinization, Propionibacterium acnes (also called Cutibacterium acnes) infection of hair follicles, and resulting inflammation [6]. There are many treatments available to help deal with Acne vulgaris including Benzoyl peroxide, Retinoid creams or gels, Antibiotics (oral & topical); however, because there is increasing resistance to these drugs and side effects associated with them (such as Photosensitization, Skin Irritation, etc.), researchers have been looking for natural alternatives. One alternative source could be herbal remedies derived from plants. For example, Argemone mexicana L., (common name- Mexican Prickly Poppy), Papaveraceae family is a perennial or annual Herb native to Tropical and Subtropical regions. It grows in poor quality soil and Waste Lands. This makes it easy to find and harvest for commercial purposes [12]. Historically, A. mexicana has been used for generations in Traditional Medicine to treat various health problems like skin conditions, infections, Inflammations, Jaundice, Malaria and Rheumatism [4]. Many of these medicinal uses have been supported scientifically using both laboratory based "in-vitro" and animal model "in-vivo" testing [16]. Many of the pharmacological activities exhibited by A. mexicana are due to a wide variety of Phytochemicals present within the plant. The main types of Phytochemicals responsible for the pharmacological activities are Isoquinoline Alkaloids. Some examples of Isoquinoline Alkaloids found in A. mexicana are Berberine, Protopine, Chelerythrine and Sanguinarine [7]. Both Berberine and Sanguinarine have shown strong antibacterial activity against Gram-positive bacteria such as Staphylococcus aureus and P. acnes. These two are major contributors to Acne pathogenesis [4]. In addition to antibacterial activity exhibited by Isoquinoline Alkaloids present in A. mexicana, flavonoids and phenolic compounds also exhibit Antioxidant and Anti-Inflammatory Properties. As a result, the A. mexicana Extract may be suitable as a multi-faceted candidate for developing a product designed to prevent or treat Acne [12]. Gel formulations have become increasingly popular compared to cream or ointment formulations for treating Acne. Gels provide several advantages when applied topically for the treatment of Acne. They do not leave a greasy residue on the skin. They are easier to apply than creams or ointments. Patients tend

to be more compliant with using gels than they are with applying creams or ointments. And finally, since gels are liquid formulations, they allow the delivery of the active ingredients directly to the site where they are needed to produce therapeutic results [8]. Polyacrylate polymers such as Carbopol 940 are very effective as gelling agents due to their Bioadhesion properties, pH-dependent Swellability properties, and Compatibility with Botanical extracts [11]. Co-exipient additions of Aloe Vera Gel and Neem Oil may further enhance the Moisturizing and Anti-Inflammatory properties of the formulation [3]. Although the potential benefits of using A. mexicana appear promising, few studies have investigated its application in Standardized Topical Dosage Forms. Additionally, Toxicity studies have identified toxic components in Seed Extracts and oils from seeds of A. mexicana due to Sanguinarine and Dihydrosanguinarine. Therefore, the Safety Profile of Leaf Specific extracts of A. mexicana would need to be evaluated separately from those found in Seeds and Oils of Seeds [4]. Therefore, the objectives of this research project were to: (i) Prepare Ethanol-based extracts from Leaves of A. mexicana; (ii) Screen the prepared extracts for Phytochemical Constituents; (iii) Formulate Gel preparations incorporating the Prepared extracts at Three Different Concentration Levels; and (iv) Evaluate Physicochemical Parameters, Rheology, and Antimicrobial Activity of the Formulated Gels to Identify Optimal formulations for Further Development.

Figure 1. Argemone mexicana.

II. PLANT PROFILE

A. Botanical Classification

Argemone mexicana L. is placed in the Kingdom Plantae; Division Magnoliophyta (flowering angiosperm); Class Magnoliopsida (Dicotyledon); Order Ranunculales; Family Papaveraceae; Subfamily Papaveroideae; Genus Argemone; Species A. mexicana. [4] (Figure 1.)

B. Morphology

A. mexicana is a tall, upright, branching annual herb that grows from 30-100 cm in height. All parts of the plant produce a typical yellow latex which has significant characteristics for the identification and is a resource of many different bioactive alkaloids [4]. It is rooted on a developed taproot, that is provided with lateral roots that provide the anchoring function. The stems are green, have bluish tints (glaucous) are cylindrical, highly branched and have long spines. The leaves are alternate, sessile, pinnatifid at the base and very hairy; they have white veins and spiny borders. Flowers are solitary, located at the apex of the stem, actinomorphically symmetrical, bisexual and bright yellow with 6 petals and 3 sepals. Fruit is an ovoidal capsule covered with spines, it opens through apical pores releasing brownish-black rounded seeds.

III. METHODOLOGY

A. Collection of Plant Material

Fresh A. mexicana leaves were gathered in early January 2026 from a number of sites in Takalidokeshwar (Parner Taluka), Ahmednagar District, Maharashtra State, India. All samples were identified and verified as authentic by a trained botanist. The fresh leaves were removed from the rest of the plant material and then cleaned several times under a gentle flow of tap water; the last cleaning used deionized/sterile distilled water to ensure removal of all surface contaminants. Following cleaning the leaves were air dried at room temperature until completely dry over a period of approximately six to seven days. The dried leaves were ground into a coarse powder using an electrical grinding device, and then sieved to pass through a 40 mesh screen. Once sieved the fine powder was placed in an airtight container, protected from both light and moisture, until needed for analysis.

Figure 2. Extraction.

B. Preparation of Extract

The ethanol extract was prepared by applying the maceration method as described by [14]. 2g of the powdered leaves were precisely measured and mixed with 20mL of 95% (v/v) ethanol. This solution was then transferred into a sealed glass container and left at room temperature (25±2°C) for 24h; this interval included an intermittent shaking every four hours to facilitate maximum interaction between the solute and the solvent. After 24 h, the macerated solution was passed through a No.1 Whatman filter paper to produce a clear ethanol-based liquid. The resulting clear liquid extract was removed from the rotary evaporator at a low vacuum and heated to approximately 40°C. Once dried it was refrigerated at 4° C and stored until required [11].

C. Phytochemical Screening

Qualitative phytochemical screening of the ethanolic leaf extract was conducted according to standard procedures described by Lachman et al. [11] and the Indian Pharmacopoeia [9]. Tests were performed for the following secondary metabolite groups:

(1)Alkaloids — Dragendorff's test (orange-red precipitate), Mayer's test (white/cream precipitate), and Wagner's test (brown precipitate).

(2)Flavonoids — Shinoda test (pink/red coloration with magnesium and HCl) and alkaline reagent test (yellow color disappearing on acidification).

(3)Tannins — Ferric chloride test (blue-black or green coloration).

(4)Glycosides — Bornträger's test (pink/red coloration).

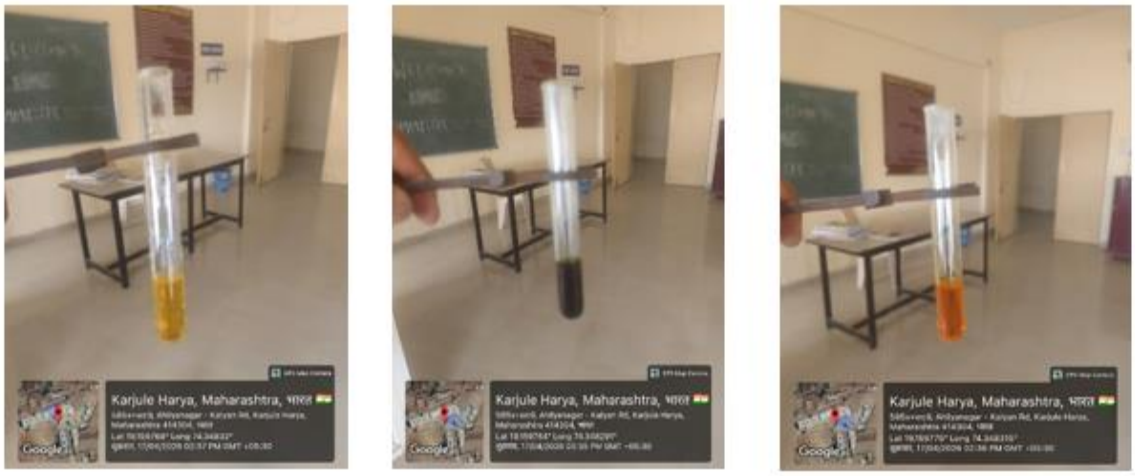

Figure 2. Phytochemical Screening.

D. Formulation of Herbal Anti-Acne Gel

Three different gel products (F1, F2, and F3) were made using three different amounts of A. mexicana. Other ingredients remained the same (see Table 1). A mixture of carbopol 940 (0.1g) and 2.2 ml of distilled H2O was mixed slowly until fully hydrated after thirty minutes. At that point, 7.0 grams of aloevera gel and 0.5 grams of glycerine were slowly mixed into the carbopol dispersion. To make sure no air bubbles formed during this process, it had to be done very slowly and continuously. The required amount of A. mexicana (0.1g, 0.2g, or 0.3g) along with the required amount of neem oil (0.1g), were then combined with the gel base as well. The final product was a smooth, clear, viscous gel which had been brought to a pH range of 6-6.8 by adding drop wise NaOH solution. Once it had achieved its desired consistency, the gel was stored in labelled amber glass jars at room temperature [8].

Table 1. Formulation Composition of Herbal Anti-Acne Gel.

Sr. No.	Ingredient	Role	F1	F2	F3
1	A. mexicana Extract	Active agent	0.1 g	0.2 g	0.3 g
2	Neem Oil	Antimicrobial, emollient	0.1 g	0.1 g	0.1 g
3	Aloe vera Gel	Humectant, anti-inflammatory	7.0 g	7.0 g	7.0 g
4	Carbopol 940	Gelling agent	0.1 g	0.1 g	0.1 g
5	Glycerin	Humectant, plasticizer	0.5 g	0.5 g	0.5 g
6	Sodium Hydroxide	pH adjuster	q.s.	q.s.	q.s.

7	Distilled Water	Vehicle	2.2 g	2.2 g	2.2 g

IV. EVALUATION PARAMETERS

All three formulations were evaluated for standard physicochemical and biological parameters as described below [1], [5], [6], [8].

A. Physical Appearance

A small quantity of each gel was visually inspected against white and black backgrounds for color, clarity, texture, uniformity, and the presence of any particulate matter or phase separation.

B. pH Determination

One gram of each formulation was dissolved in 25 mL of distilled water. The pH was measured using a calibrated digital pH meter (Systronics, Model 335) at room temperature (25 ± 0.5°C) in triplicate, and the mean value was reported.

C. Viscosity Measurement

Viscosity was determined using a Brookfield Viscometer (LV model, spindle No. 6) at 20 rpm and 25°C. Each measurement was performed in triplicate.

D. Spreadability

Spreadability was assessed by placing 0.5 g of gel between two glass slides (10 × 10 cm) and applying a standard weight of 500 g for 5 minutes. The diameter of spreading was measured, and spreadability (S) was calculated as: $S = m \times L / t$, where m = weight applied (g), L = length of glass slide (cm), and t = time taken for separation (s) [11].

E. Extrudability

Each formulation (10 g) was filled into collapsible aluminum tubes. A force of 100 g was applied to the closed end, and the amount of gel extruded per minute was recorded as a measure of extrudability [15].

F. Homogeneity, Drug Content, and Washability

Homogeneity was assessed by visual examination and touch. Drug content was determined by dissolving 1 g of gel in ethanol, filtering, and analyzing the filtrate by UV-Vis spectrophotometry (Shimadzu UV-1800) at the appropriate λmax of the extract. Washability was evaluated by applying gel to the dorsum of the hand and washing with tap water, observing ease and completeness of removal.

G. Skin Irritation Test

A patch test was performed on the inner forearm of healthy volunteers (n = 5, ethics approval obtained). The formulation was applied to a 2 × 2 cm demarcated area and evaluated at 24 and 48 hours for erythema, edema, itching, or other signs of irritation using the Draize scoring system [13].

H. Antimicrobial Activity

The antimicrobial effectiveness of the gels was evaluated using the agar well diffusion technique [1], [3]. The agar wells were tested against Propionibacterium acnes (ATCC #6919) and Staphylococcus aureus (ATCC #25923). Mueller-Hinton agar plates were inoculated with a standard microbial suspension (McFarland = 0.5), 6mm in diameter wells were created in each plate, 50µl of each gel formulation was then placed into each well. Plates were incubated for 24 hr at 37°C under anaerobic conditions for P.acnes and aerobic conditions for S.aureus. Zone diameters of inhibition were recorded in millimeters and compared to Clindamycin (1%), which served as the positive control; while the zone diameters of inhibition from the negative controls (plain gel base) were recorded as zero.

I. Stability Study

Stability was assessed following ICH Q1A(R2) guidelines [9]. Samples were stored at accelerated conditions (40 ± 2°C / 75 ± 5% RH) and room temperature (25 ± 2°C / 60% RH) for three months. pH, viscosity, appearance, and drug content were re-evaluated at monthly intervals.

V. RESULTS AND OBSERVATION

A. Phytochemical Screening

Phytochemical screening of the A. mexicana ethanolic leaf extract confirmed the presence of alkaloids (Dragendorff's, Mayer's, and Wagner's tests), tannins (ferric chloride test), flavonoids (Shinoda and alkaline reagent tests), and glycosides (Bornträger's test). These findings are consistent with previously reported chemical profiles of A. mexicana [4], [16].

Table 2. Phytochemical Screening Results of A. Mexicana Ethanolic Leaf Extract.

Phytochemical	Test Performed	Observation	Result
Alkaloids	Dragendorff's Test	Orange-red precipitate formed	+
Tannins	Ferric Chloride Test	Blue-black coloration	+
Flavonoids	Alkaline Reagent Test	Yellow color disappeared on acidification	+

Glycosides	Bornträger's Test	Pink/red coloration appeared	+

B. Evaluation of Gel Formulations

All three formulations were semi-transparent, light greenish gels with a characteristic herbal odor. No gritty particles were detected upon tactile examination. The results of physicochemical evaluation are summarized in Table 3.

Figure 3. Gel Formulation.

Table 3. Physicochemical Evaluation of Herbal Anti-Acne Gel Formulations.

Parameter	F1	F2	F3	Ideal Range
Appearance	Smooth, uniform	Smooth, uniform	Slightly viscous, uniform	Smooth, homogeneous
pH	6.4	6.2	6.0	6.0–6.8
Viscosity (cP)	4,200	5,800	7,400	Moderate (4,000–8,000)
Spreadability	Good	Very good	Moderate	Good to very good
Extrudability	Easy	Easy	Requires slight force	Easy extrusion
Homogeneity	Uniform	Uniform	Uniform	No phase separation
Drug Content (%)	95.4 ± 0.8	97.8 ± 0.6	96.2 ± 1.1	90–110%
Washability	Complete	Complete	Complete	Residue-free
Skin Irritation	None	None	None	No irritation

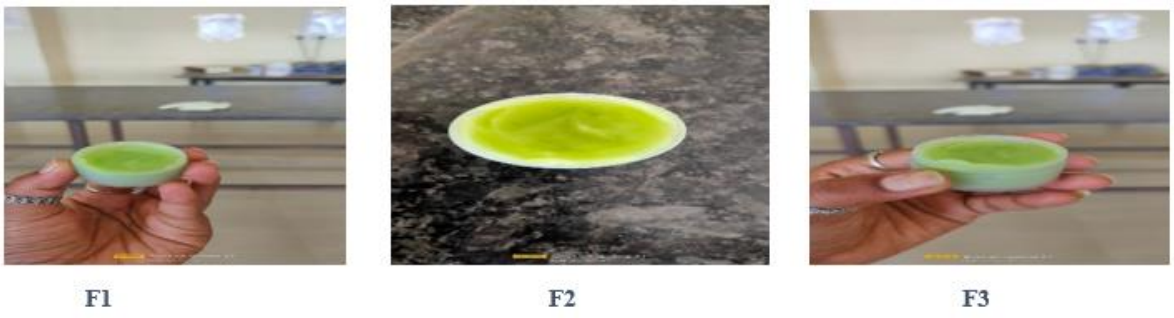

Figure 4. Formulation Comparisons.

C. Antimicrobial Activity

The agar well diffusion test confirmed that the antimicrobial efficacy of each formulation was dependent on their respective concentrations. In terms of size of zone of inhibition against P. acnes and S. aureus, F3 had the greatest zones of inhibition. However, due to a very high viscosity, the spreadability of F3 as well as acceptability by patients were negatively impacted. Zones of inhibition for F2 were similar in magnitude to those created by the positive control (clindamycin 1%), whereas F1 displayed minimal activity (see Table 4). As such, these results are consistent with prior research which have shown that sanguinarine and berberine exhibit broad spectrum antibacterial activities toward gram +ve acnegenic bacteria [4] [6].

Table 4. Antimicrobial Activity — Zones of Inhibition (mm).

Formulation	P. acnes (ATCC 6919)	S. aureus (ATCC 25923)
F1 (0.1 g extract)	12 ± 0.5	11 ± 0.4
F2 (0.2 g extract)	18 ± 0.6	17 ± 0.5
F3 (0.3 g extract)	22 ± 0.7	20 ± 0.6
Clindamycin 1% (Positive Control)	24 ± 0.4	23 ± 0.5
Plain Gel Base (Negative Control)	0	0

D. Stability Study

No significant changes were observed in the pH, viscosity, or appearance of F2 after three months of storage under both room temperature and accelerated conditions. Drug content remained above 95% at all-time points, confirming the chemical stability of the formulation. These results are consistent with Carbopol 940-based herbal gel systems reported in the literature [8], [15].

VI. DISCUSSION

The present study has shown that it is possible to develop a gel from an ethanolic leaf extract of A. mexicana that is stable, active and well tolerated topically. Phytochemically, this study supported previous findings [4] [12] on the presence of alkaloids, flavonoids, tannins and glycoside in A. mexicana ethanolic leaf extracts. The presence of these compounds explains the antimicrobial properties demonstrated by the ethanolic leaf extracts against two bacteria responsible for acne: Staphylococcus aureus and Propionibacterium acnes [7] due to their ability to inhibit DNA gyrase activity in the bacteria [4]. Amongst the three preparations tested, F2 (0.2g extract), proved to be the most promising. The pH value of F2 at 6.2 is within the acceptable range for human skin [13]. Therefore, there should be little chance of causing irritation to the skin or disrupting the natural acidic environment of the skin. In addition, the high viscosity of F2 (5800cP) provided sufficient adhesion to the skin to allow easy application and long enough contact time before removal – factors important for improving patient compliance when treating chronic conditions such as acne [11]. Further evidence supporting the suitability of F2 for topical use is derived from its high spreadability, measured using the standard glass-slide method [8]. Although the preparation F3 exhibited the greatest antimicrobial activity due to higher concentrations of extract; however, both its high viscosity and low spreadability would compromise patient adherence [10]. On the other hand, F1 contained lower concentrations of extract than required to produce satisfactory antimicrobial results. The dose dependent antimicrobial response reported for sanguinarine-based products used in treating acne [6]; supports this finding. Both neem oil and aloe vera added as additional excipients enhanced the formulation's anti-inflammatory and moisturising capabilities and acted synergistically with the antimicrobial properties of the extract [3][5]. Additionally, no signs of skin irritation were evident amongst all formulations tested (Draize score = 0). It should be noted that although some studies have identified potential toxicities with the ingestion of A. mexicana seed oil containing dihydrosanguinarine via oral routes, none have been reported when applied topically with ethanolic leaf extracts [4]. The results obtained from the stability assessment of F2 indicate an adequate shelf-life for commercialisation purposes; meeting ICH Q1A(R2) requirements for analogous herbal gel formulations described previously in literature [9],[15].

VII. CONCLUSION

This study effectively illustrated the development and assessment of a topical herbal gel for acne control using ethanolic extracts of the leaves of A. mexicana as the primary therapeutic agent along with complementary ingredients such as neem oil and aloevera gel to serve as synergy enhancing co-formulants. The use of Carbopol 940 as the gelling agent served as the base upon which these herbal agents were formulated. The phytochemically rich nature of the extracts of A. mexicana were confirmed via phytochemical analysis. Bioactive compounds present in this extract include bioactive alkaloids, flavonoids, tannins, and glycosides which are responsible for the medicinal effectiveness. Based on their physical characteristics among the three experimental formulations tested, F2 (which contained 0.2 g of herb extract) was found to be the most efficacious formulation due to its favorable chemical properties. These included skin compatibility pH (pH = 6.2), viscosity (5800 centipoise), ease of spreading, a high percentage of medicated compound content (97.8% + / - .6%) non-irritant status, and marked

inhibitory action against both P. acnes and S. aureus. Results from the three-month stability experiments indicated that F2 remained chemically stable under both standard laboratory temperatures (ambient) and accelerated testing conditions. Therefore, it can be concluded from this research that extracts of A. mexicana have considerable promise as an effective and relatively nontoxic source of the active pharmaceutical ingredient used in topical dermatologic products intended for the treatment of acne vulgaris. The results of this research will also serve as a basis for conducting additional advanced preclinical and clinical studies to assess efficacy and safety in humans. Future research is suggested in the form of assessing the in-vivo efficacy of topical application using in vitro and/or in vivo animal model systems designed to simulate acne vulgaris; toxicokinetics, i.e., identifying routes of absorption into the bloodstream and metabolizing enzymes; and randomized controlled clinical trials assessing efficacy and safety in subjects suffering from mild or moderate acne vulgaris.

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