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Research Article

FORMULATION AND EVALUATION OF HERBAL ANTIACNE GEL OF *MYRICA ESCULENTA*

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ABSTRACT

Objective: The present study was conducted to formulate and evaluate the topical anti-acne formulation of *Myrica esculenta* extract and its isolated essential oil.

Methods: The antibacterial activity of crude extract and essential oil of *M. esculenta* against *Propionibacterium acne, Staphylococcus epidermidis, and Staphyloccocus aureus* was investigated using disc diffusion method. After finding active, the topical gel formulations were developed and tested for physical parameters, drug content uniformity, and *in-vitro* diffusion.

Results and Discussions: The results revealed that *M. esculenta* crude extract and essential oil showed the minimum inhibitory concentration values of 2.1 mg/ml and 1.7 mg/ml respectively against *P. acne*. It was revealed from the results that formulation F5 showed the maximum drug content (94.6%), *in-vitro* diffusion (93%), maximum stability and the zone of inhibition among all the formulations.

Conclusions: From the present study, it can be concluded that the *M. esculenta* extract and essential oil have potential against *Acne vulgaris*. Among the formulations developed, F5 was considered as the optimized formulation with desired profile.

Keywords: *Myrica esculenta*, *Acne vulgaris*, Hydrogel, *In-vitro* evaluation, *Propionibacterium acne.*

INTRODUCTION

Acne vulgaris commonly known as pimples involves many factors for its manifestation and it mainly affects the pilosebaceous follicle (Fig. 1). It mainly indicates open and closed comedones and inflammation causing various conditions such as papules, pustules, and nodules [1]. The main micro-organisms responsible to cause the condition include *Propionibacterium acnes, Staphylococcus aureus,* and *Staphylococcus epidermidis* [2]. These micro-organisms proliferate rapidly which ultimately result in development of acne.

To treat the problem of *A. vulgaris*, a large number of medicinal products including antibiotics and chemotherapeutic agents from synthetic as well as natural origin are available in the market which can be used externally as well as internally to treat the ailment [3]. For milder condition of *A. vulgaris*, only topical therapy can be effective but for the moderate and severe type of acne, systemic therapy along with topical application is of choice. Herbal therapies are getting worldwide acceptance over the currently available ones as majority of the present formulations give harsh side effects such as skin dryness, rashes, wrinkling, erythema, pruritus, skin eruption, and development of resistance [4].

In previous study, we have found that *Myrica esculenta* stem bark crude extract, as well as isolated essential oil, have antimicrobial potential [5]. Hence, the study has been extended to evaluate anti-acne potential of the crude extract as well as essential oil against *P. acne, S. aureus,* and *S. epidermidis*. Furthermore, hydrogel formulations were prepared, and evaluation was carried out for the most effective one. Despite having potential, the formulation development aspect for the selected plant drug has not been explored so far. Hence, this is an attempt to take the study one step ahead for its commercial use.

METHODS

The stem bark of *M. esculenta* was collected from the provinces around Sunder Nagar, Mandi, Himachal Pradesh. The herbarium was submitted, and the plant material was authenticated by senior botanist at National Bureau of Plant and Genomic Research, Pusa Campus, New Delhi (Voucher number: EP 533). The test organisms, *P. acne* (MTCC 1951), *S. aureous* (MTCC 3160), and *S. epidermidis* (MTCC 931), were obtained from microbial culture collection and Genebank, IMTECH, Chandigarh, India. All media were purchased from Hi-Media. All reagents used were of analytical grade.

Extraction and isolation

The stem bark was coarsely powdered and extracted in a Soxhlet apparatus with methanol for 72 hrs. The methanolic extract was concentrated on a steam bath and dried under reduced pressure to get brown colored powder. To obtain essential oil, the air-dried plant material was hydrodistilled in an all-glass apparatus according to the method recommended by the British Pharmacopoeia, 2007. The pale yellow oil was dried over anhydrous sodium sulfate and stored at 4°C in the dark.

Determination of antimicrobial activity

Antibacterial activity

The antibacterial activity was determined by disc diffusion method. *P. acne* was incubated in ASLA agar medium for 48 hrs under anaerobic conditions. The agar plates were swabbed with inoculums. The sterile filter paper disc of diameter 6 mm was aseptically placed on the inoculated plates and was impregnated with the test material (100 mg/ml of extracts). The plates were left at ambient temperature for 30 minutes to allow exceed pre-diffusion before incubation at 37°C for 72 hrs under anaerobic conditions in an anaerobic bag (Hi-Media) with the gas pack, and indicator tablets and the bag was kept in an incubator for 72 hrs at 37±1°C. Gas packs containing citric acid, sodium carbonate, and sodium borohydride were used to maintain and check the anaerobiosis. The indicator tablet of methylene blue changed from dark pink-blue-light pink finally, which indicated the achievement of anaerobic condition. The cultures of *S. aureus and S. epidermidis* were prepared in nutrient agar medium at 24 hrs under aerobic conditions. Test samples of these aerobic bacteria were incubated at 37°C for 24 hrs

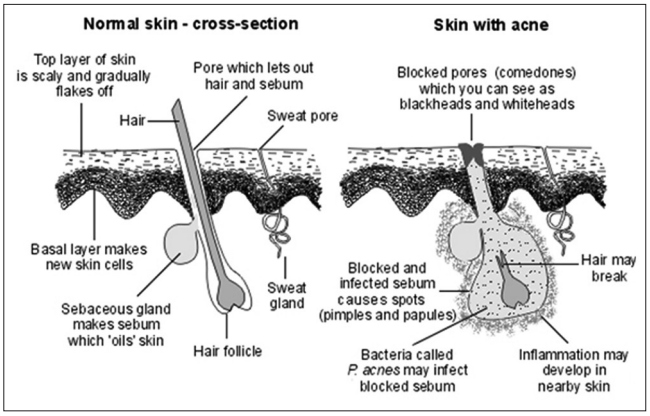

Fig. 1: Changes in skin physiology due to acne. Available from: http://www.patient.info/health/acne-leaflet

under aerobic conditions. The antibacterial activity was estimated by measuring the diameter of the zone of inhibition. All disc diffusion tests were performed in three separate experiments and antibacterial activity was expressed as the mean±standard deviation. Ciprofloxacin was used as a standard, and control was used to determine the growth capacity of medium [6].

Minimum inhibitory concentration (MIC)

The MIC values were determined by agar dilution method. The test material was added aseptically to 20 mL aliquots of sterile molten agar at an appropriate range of test material (0.05 mg/ml-5 mg/ml). The resulting agar solutions were vortexed at high speed for 15 seconds or until completely dispersed, immediately poured into sterile petri plates then allowed to set for 30 minutes. The plates were then inoculated with the *P. acne*. The inoculated plates were left until the inoculums had set and then incubated under anaerobic conditions at 37°C for 72 hrs in gas bag (Hi-Media) with gas pack and indicator tablets, and the bag was kept in an incubator for specified duration at specified temperature.

The test samples of *S. aureus* and *S. epidermidis* were prepared in nutrient agar medium and incubated for 24 hrs at 37°C under aerobic conditions. Following the incubation period, the plates were observed and recorded for the presence or absence of growth. From the results, the MIC was recorded as the lowest concentration of test substance where the absence of growth was observed [7-9].

Preparation of gel

The weighted amount of Methyl Paraben was dissolved in 5 mL of hot water, and propylparaben was added on slight cooling of water. To this beaker carbopol 934 was dispersed with continuous stirring for 20 minutes after addition of 50 mL of distilled water. This dispersion was kept overnight for soaking. In another beaker, the required quantity of propylene glycol and polyethylene glycol 400 were added. This mixture along with the concentration of aqueous extract corresponding to its MIC was incorporated to carbopol beaker with stirring. The volume was made up with distilled water, and stirring was done vigorously. Triethanolamine was added from the gel by adjusting pH to 6.8 [10,11].

Physical parameters

Physical appearance- The physical appearance of the formulation was checked visually which comprised Color - The color of the formulations was checked out against white background. Consistency - The consistency was checked by applying on skin. Greasiness - The greasiness was assessed by the application onto the skin. Odor- The odor of the gels was checked by mixing the gel in water and taking the smell.

pH

An amount of 20 mg of the formulation was taken in a beaker and was subjected to the pH measurement using a digital pH meter within 24 hrs of manufacture [12].

Viscosity

Viscosities of formulated gels were determined using Brookfield viscometer spindle # 7 at 50 rmps and 25°C. The corresponding dial reading on the viscometer was noted. Then the spindle was lowered successively. The dial reading was multiplied by the factor mentioned in catalog [12].

Extrudability

Extrudability is defined as the weight in grams required for extruding 0.5 cm long ribbon of formulation in 10 seconds. The gel formulation was filled in a standard capped collapsible aluminum tubes and sealed by crimping to the end. The tubes were placed between two slides and were clamped. 500 g weight was placed over the slides, and then the cap was removed. The length of the ribbon of the formulation that came out in 10 seconds was recorded [13].

Spreadability

Spreadability denotes the extent of area to which a gel readily spreads on the application to the skin or affected part. The bioavailability efficiency of the gel also depends on Spreadability value. Spreadability is defined in terms of time in seconds required taken by the upper slide to slip off the gel placed between the two slides, under certain load. The lesser the time taken for the separation of two slides, the better the spreadability. An amount of 500 mg of the formulation was sandwiched between the two slides, each with dimensions of 6 cm × 2 cm. A weight of 100 g was placed upon the upper slide so that the formulation between the two slides get pressured uniformly to form a thin layer. The weight was removed, and the excess of the formulation adhering to the slides was scrapped off. The lower slide was fixed on the board of apparatus, and the upper slide was held to the non-flexible string to which 20g load was applied with the help of a simple pulley which was in horizontal level with the fixed slide. The time taken by the upper slide to slip off the lower slide was noted [11].

$$\text{Spreadability} = m \times l/t$$

Where m=Weight tied to upper slide, l=Length of the glass slide (6 cm), t=Time in seconds.

Antimicrobial studies of the formulation

The solutions of the gels were prepared using 100 mg of gel in 10 ml of dimethyl sulfoxide. The antibacterial activity was tested by well diffusion method. *P. acne* was incubated in ASLA agar medium for 48 hrs under anaerobic conditions. The solidified agar plates were swabbed with inoculums on the surface. The equidistance wells were cut in the plates with the help of 8 mm borer. In each of these wells the gel solutions in dimethyl sulfoxide were placed and the plates were left at ambient temperature for 30 minutes to allow pre-diffusion prior to incubation at 37°C for 72 hrs under anaerobic conditions in an anaerobic bag (Hi-Media) with gas pack and indicator tablets and the bag was kept in an incubator for 72 hrs at 37±1°C. Gas packs containing citric acid, sodium carbonate and sodium borohydride were used to maintain and check the anaerobiosis. The indicator tablet of methylene blue changed from dark pink-blue-light pink finally, which indicated the achievement of anaerobic condition. The culture of *S. aureus* and *S. epidermidis* was prepared in nutrient agar medium at 24 hrs under aerobic conditions. Test samples of this aerobic bacterium were incubated at 37°C for 24 hrs under aerobic conditions. The antibacterial activity was estimated by measuring the diameter of the zone of inhibition. All well diffusion tests were performed in three separate experiments and antibacterial activity was expressed as the mean±standard deviation [14].

Stability studies

The stability of the formulations was assessed according to the guidelines issued by International Conference on Harmonisation on October 27, 1993 [15,16].

Drug content

The drug content of the formulations was determined by dissolving an accurately weighed quantity of gel 1 g in 100 ml of solvent (phosphate buffer pH 6.8 + ethanol in ratio 40:60). The solutions were kept for shaking for 4 hrs and then kept for 6 hrs for complete dissolution of the formulations. Then the solutions were filtered through 0.45 mm membrane filters and proper dilutions were made, and the solution was subjected to the spectrophotometric analysis. The drug content was calculated from the linear regression equation obtained from the calibration data.

***In-vitro* diffusion studies**

The *in-vitro* diffusion studies for all formulations (F1-F8) were carried out using the Franz-diffusion cell. The diffusion cell apparatus was fabricated as an open ended cylindrical tube. A weighed quantity of formulation equivalent to 1gm of the drug was placed onto the dialysis membrane-70 (Hi- Media) and was immersed slightly in 100ml of receptor medium (phosphate buffer pH 6.8+ethanol in ratio 40:60) which was continuously stirred and the temperature was maintained at 37±1°C. Aliquots of 1ml were withdrawn from each of the system at time intervals of 5, 10, 15, 30, 60, 120, 240, and 360 minutes were analyzed for drug content using ultraviolet spectrophotometer [13].

RESULTS AND DISCUSSION

In total eight formulations, four each of *M. esculenta* extract and *M. esculenta* essential oil were prepared. The gelling agent Carbopol 934 was used in varying quantities to optimize the best out of all.

The methanolic extract of *M. esculenta* as well as its isolated essential oil respectively showed the zone of inhibition of 21±1.1 and 24±1.2 mm as well as the MIC values of 2.1 mg/ml and 1.7 mg/ml against *P. acne*. This activity of the essential oil was more than that of the crude extract (Table 1).

The formulations were developed with methanolic extract of *M. esculenta* as well as its isolated essential oil using carbopol 934 as gelling agent in the concentration of 0.5% (F1 and F5), 1% (F2 and F6), 1.5% (F3 and F7), and 2% (F4 and F8) w/w (Table 2). Initially, the formulations were observed for their organoleptic properties.

The skin care formulations specifically, single-phase gels are of choice due to their user compliance [17]. In such formulations, the active ingredient may be the organic macromolecules are uniformly distributed throughout a liquid leaving no apparent boundaries between the dispersed macromolecules and the liquid [18]. Specifically, for acne vulgaris, the primary requisite is the formulation should spread easily and leave minimal residue or oiliness as it has been seen that oily skin type is more prone to acne problems. Carbopol 934, gelling agent with good plastic flow properties is one of the excellent viscosity builders effective at low concentration and does not support microbial growth. Propylene glycol is a water miscible cosolvent for carbopol®940 and acts as a preservative, humectant, plasticizer, or stabilizer in a variety of pharmaceutical formulations [19]. Its penetration enhancement capability has attributed to increased transdermal flux of many drugs [20].

All the formulations were brown in color and had characteristic odor of *M. esculenta*. The aromatic odor was more prominent in the case of isolated essential oil. All formulations were glossy and translucent. Formulation F1 and F5 were found to have excellent consistency. As indicated in Table 3, the pH of the formulation ranged from 6.7 to 7.0, which may be suitable for topical application without discomfort. The apparent viscosity of the formulations ranged from 35.2±0.4 to 38.6±0.5 cps. The viscosity of formulation increased with increase in the concentration of carbopol content. The spreadability and extrudability of the formulations were found to range from 42.5±0.3 to 48±0.4 g/sec and from 542.1±0.1 to 548.2±0.3 g, respectively. The viscosity was observed to increase with a decrease in the spreadability and vice versa. The drug content release was found to range from 89.6 to 94.6% (Table 4). This proved that the method was suitable for the preparation of topical dosage forms.

As indicated by the value of the drug content, there was no degradation of the drug during the preparation process. Cumulative drug diffusion after 6 hrs was found to be 93% for F5, which is the highest among all the formulations. This is ascribed to the increased drug diffusion from the gel due to its low viscosity, soft nature and the nature of gelling agent used. Thus, the formulation with the 0.5%w/w carbopol content (F5) was found to have the most optimized results.

The results from the stability studies showed that formulations showed no changes in the pH, viscosity, spreadability, consistency, extrudability, and drug content and hence are found to be stable.

When formulations were subjected to antimicrobial investigation, then it was found that all had inhibitory activity against *P. acne S. aureus*

Table 1: Antibacterial activity of *M. esculenta* crude extract and essential oil

Test sample	Zone of inhibition (mm) (±SD)			MIC (mg/ml)		
	P. acne	*S. aureus*	*S. epidermis*	*P. acne*	*S. aureus*	*S. epidermis*
M. esculenta extract	21±1.1	15±1.2	20±1.3	2.1	2.8	3
M. esculenta essential oil	24±1.2	18±1.4	22±1.2	1.7	2.4	2.0

M. esculenta: *Myrica esculenta*, *P. acne*: *Propionibacterium acne, S. aureus*: *Staphyloccocus aureus*, *S. epidermis*: *Staphyloccocus epidermis*, SD: Standard deviation, MIC: Minimum inhibitory concentration

Table 2: Composition of topical formulations

Ingredients	Weight (mg)							
	M. esculenta extract				*M. esculenta* essential oil			
	F1	F2	F3	F4	F5	F6	F7	F8
Carbopol 934	0.5	1	1.5	2	0.5	1	1.5	2
PEG 400	5	5	5	5	5	5	5	5
Propylene glycol	15	15	15	15	15	15	15	15
Methyl paraben	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Propyl paraben	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Triethanolamine	qs	qs	qs	qs	qs	qs	qs	qs
Distilled water qs	100	100	100	100	100	100	100	100

M. esculenta: *Myrica esculenta*, PEG: Polyethylene glycol

Table 3: Physicochemical properties of semisolid formulations of coriander aqueous extract

Formulation	Results (±SD)				
	pH	Consistency	Spreadability (g/second)	Extrudability (g)	Viscosity (cps)
F1	6.8±0.03	***	43±0.3	548.2±0.2	35.6±0.5
F2	6.7±0.01	**	46±0.2	546.4±0.6	36.1±0.5
F3	7.0±0.02	**	48±0.4	545.5±0.5	36.4±0.5
F4	6.7±0.02	**	45.5±0.2	542.2±0.4	38.4±0.2
F5	7.0±0.03	***	42.5±0.3	548.2±0.3	35.2±0.4
F6	6.8±0.02	**	44±0.3	544.3±0.3	37.4±0.4
F7	6.8±0.04	**	46.5±0.4	543.1±0.1	38.2±0.2
F8	6.7±0.04	**	44.5±0.3	542.2±0.4	38.6±0.5

***: Very good, **: Good. All experiments were performed in triplicate

Table 4: Drug content and *in-vitro* release

Formulation	Drug content (% m/m)	% Cumulative release
F1	92.4	91
F2	90.3	90
F3	89.6	88
F4	90.2	89
F5	94.6	93
F6	93.1	92
F7	92.4	91
F8	92.7	91

Table 5: Antibacterial activity of formulations

Formulation	Zone of inhibition (mm) mean±SD		
	P. acne	*S. aureus*	*S. epidermidis*
F1	22.8±1.1	17.4±1.2	22±1.12
F2	20.4±1.11	16.8±1.2	19.6±0.8
F3	20±1.11	16.5±1.12	19.2±0.8
F4	20.2±1.2	16±0.12	18.6±1.3
F5	23.5±0.8	18±1.1	22.4±1.12
F6	20.6±0.8	16.2±1.3	18.8±1.1
F7	20.4±1.12	17.2±1.2	18.6±1.3
F8	20.8±1.1	17.5±1.2	18.8±1.1
Marketed formulation	24.2±1.11	19.2±0.8	23.2±1.11
Ciprofloxacin	24±1.1	20±1.2	23.6±0.9

P. acne: *Propionibacterium acne, S. aureus*: *Staphyloccocus aureus*, SD: Standard deviation

and *S. epidermidis*. Among all the formulations, the formulation F5 had shown almost the same zone of inhibition as that shown by the aqueous extract (Table 5). Ciprofloxacin was found to have slightly greater activity than the developed formulations.

Thus, from the whole study carried out, it can be said that formulation F5 having 0.5 mg concentration of gelling agent, Carbopol 934, with good consistency, better spreadability, and viscosity and higher extrudability was found to be the most optimized formulation.

CONCLUSION

The *M. esculenta* extract and essential oil were found to be active against bacterial responsible to cause *A. vulgaris*. Among the formulations developed, F5 was considered as the optimized formulation with desired profile. Hence, it can be further developed and used commercially for treatment of acne.

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