Research Article

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DEVELOPMENT OF HERBAL ANTI ACNE GEL AND ITS EVALUATION AGAINST ACNE CAUSING BACTERIA *PROPIONIBACTERIUM ACNE* AND *STAPHYLOCOCCUS EPIDERMIDIS*

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ABSTRACT
Acne by definition is multifactorial chronic inflammatory disease of pilosebaceous units. *Propionibacterium acnes* and *Staphylococcus epidermidis* are considered as the major skin bacteria that cause the formation of acne. Although acne does not pose serious threat to general health, it is one of the most socially distressing conditions especially for adolescents. The objective of this study was to design a product to treat Acne with purely herbal actives as an effective and safe alternative to harmful antibiotics. For this purpose three essential oils and two herbal extracts having anti-microbial properties were selected. These were incorporated in a Gel Base in different concentrations and the *in vitro* antibacterial activity for the different formulations (F1, F2, F3) was studied against *Propionibacterium acnes* (*P. acnes*) and *Staphylococcus epidermidis* (*S.epidermidis*), a causative organism for Acne vulgaris using Agar Well Diffusion method. All the formulations showed satisfactory Anti-microbial activity with Formulation F3 showing highest activity. It was then subjected to stress testing for three months at various temperatures. The samples were found to be stable after three months of stability studies and showed satisfactory antibacterial activity against *Propionibacterium acnes* and *Staphylococcus epidermidis* at the end of the stress testing studies. Thus it was concluded that the formulated Herbal Anti Acne Gel with natural actives can be used effectively for treating acne on skin.
Keywords: Acne, Anti microbial, Herbal Gel, *Propionibacterium acne*, *Staphylococcus epidermidis*, Stress testing.

INTRODUCTION

Acne, from the Greek word "Akme" means peak or apex, is gemnetic or acquired affections of the pilosebaceous units.[1] The correct name for acne is Acne vulgaris. 70% - 80% of patients affected by this are aged 11-25 years old.[2] Acne vulgaris is characterized by the formation of inflammatory and non-inflammatory lesions of the hair follicles and/or sebaceous glands commonly referred to as the pilosebaceous unit.[3] A slight degree of acne is typical at puberty, but a serious case can cause unsightly appearance and leaves scarring in many cases even after treatment.[2] Non inflammatory lesions may be categorized as open comedons (blackheads) and closed comedons (white heads). Inflammatory lesions manifest themselves as papules, pustules, cysts, and nodules. In practical terms, acne may be grouped in terms of severity of the symptoms; i.e. mild, moderate, and severe.[4] *Propionibacterium acnes,* an anaerobic pathogen, play an important role in the pathogenesis of acne. It is implicated in the development of inflammatory acne by its capability to activate complements and by its ability to metabolize sebaceous triglycerides into fatty acids, which chemotactically attract neutrophils.[5] *Staphylococcus epidermidis*, an aerobic organism, and part of natural skin flora is usually involved in superficial infections within the sebaceous unit.[6]

Causes of Acne

- Hyperactive sebaceous glands (overactive lipid secretion).
- Hyperkeratosis (accelerated keratinization) at hair infundibulum.
- Activity of bacteria (*Propionibacterium acnes*) promoting comedogenesis.
- Cyclic hormonal levels in women.
- Occupational hazards such as chronic exposure to chemicals and air contaminants, high humidity.
- Other stimuli and events associated with acne include seasonal effects, excessive sexual activity, emotional or psychological stress, mechanical manipulation of the skin surface, and certain drugs such as corticosteroids.[7]

Sequence of events in Acne

Hormones, environmental factors as well as genetic susceptibility may be the cause for acne. Acne happens when hair follicles become clogged with dead skin cells and a sticky substance called sebum is produced by the sebaceous glands. This excess sebum causes skin cells to stick together inside the follicle, causing an obstruction. This leads to a comedone. Once bacteria nestle into the clogged pore or comedone, they release factors that cause inflammation. This causes comedones to turn into the

pimples and pustules. Some acne lesions become so inflamed that they rupture, which forms nodules. Due to confluence of affected glands nodules form cysts which may result into scar formation after healing[8].

Treatment of Acne

Treatment of acne depends on its condition and degree of severity which may vary from a mild non inflammatory comedons to an inflammatory papule or pustule. This usually signifies the presence of *Propionibacterium acnes*. Topical as well as systemic therapy is available for the treatment of acne. While traditional treatments in the inflammatory phase are topical and systemic antibiotics acting as both antimicrobial and anti-inflammatory agents, modern acne therapy has been designed to interrupt the pathogenic pathway at one or more points. Topical therapy includes comedolytic agents, antibiotics and various anti-inflammatory drugs. Systemic therapy includes antibiotics, zinc and hormones. The excessive use of antibiotics for long periods has lead to the increased resistance in acne causing bacteria i.e. *Propionibacterium acne* and *Staphylococcus epidermidis*, against a number of antibiotics used to treat acne.[9] The spread of drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases. Down the ages essential oils and other extracts of plants have evoked interest as sources of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases. World Health Organization (WHO) noted that majority of the world's population depends on traditional medicine for primary healthcare. Medicinal and aromatic plants which are widely used as medicine constitute a major source of natural organic compounds. Essential oils have been shown to possess antibacterial, antifungal, antiviral insecticidal and anti-oxidant properties. Also extracts from aromatic plants have been studied for their antimicrobial properties. Therefore, it is reasonable to expect a variety of plant compounds in these oils with specific as well as general antimicrobial activity.[10] In the present study, topical gel formulations were developed containing essential oils and extracts. The developed formulations were examined for antimicrobial activities against microorganism frequently involved in acne inflammation, *Propionibacterium acne* and *Staphylococcus epidermidis*.

Objectives

- To develop herbal anti acne gel formulation by incorporating essential oils such as Cinnamon oil, Rosemary oil, Tea tree oil and herbal extracts like *Berberis aristata* and *Azardirachta indica*.
- To evaluate anti microbial activity of herbal anti acne gel against *Propionibacterium acne* and *Staphylococcus epidermidis*.
- To Evaluate anti microbial activity of herbal anti acne gel after completion of three months of stress testing.

MATERIAL AND METHODS

Selection and Procurement of Herbs and Essential oils

On the basis of extensive literature study, pure essential oils of Cinnamon (*Cinnamomum zeylanicum*)[11], Rosemary (*Rosmarinus officinalis*)[12] and Tea tree (*Melaleuca alternifolia*)[13] and two botanical herbs of *Berberis aristata*[14] and *Azardirachta indica*[15] were selected for the study as they were indicated to possess anti microbial activity. The Cinnamon Oil, Rosemary Oil and Tea Tree oil were acquired in ready form from Katyani Exports, Blossom Kocher' Aroma Essential Oils and Ghaziabad Aromatics respectively. The two botanical herbs *Berberis aristata* and *Azardirachta indica* were purchased from local market of Nagpur, India and their extracts were prepared in house.

Preparation of Extracts

***Berberis aristata* extract**

Step 1: 20 g of *Berberis aristata* stems were taken and dried in hot air oven at 45ºC for 8 h. The dried stems were coarsely grinded in mixer.

Step 2: The coarse powder was refluxed in a reflux condenser for 3 h using Ethanol (99.9 %) and Water (80:20) as solvents.

Step 3: The extract was cooled at room temperature and filtered through vacuum filtration unit. This extract was then used for further study.

***Azardirachta indica* extract**

Step 1: 10 g of *Azardirachta indica* fresh leaves were taken and air dried in shade for 24 h. The dried leaves were grinded in mixer.

Step 2: The powder was refluxed in reflux condenser for 3 h using Ethanol (99.9 %) and Water (80:20) as a solvent.

Step 3: The extract was cooled at room temperature and filtered through vacuum filtration unit. This extract was then used for further study.

Procurement of Microorganism and Media

The media used for the analysis was Sheep blood agar plates for *Propionibacterium acnes* and Muller Hinton agar for *Staphylococcus epidermidis*. All media were purchased from Hi media laboratories Pvt. Limited. The test organism used in study was *Propionibacterium acnes* (MTCC No.1951) which was obtained from MTCC Chandigarh and *Staphylococcus epidermidis* (ATCC No.12228) which was obtained from NCL, Pune, India.

Methodology

Anti microbial activity of essential oils and herbal extracts was examined before incorporation in the product using 'Well Diffusion Method' and Zone of Inhibition was noted as in Table 1 and found to be satisfactory.

Development of herbal anti acne gel formulation

Four formulations of herbal anti acne gel were prepared. One formulation was kept as control which was without actives (F). Three formulations were prepared by varying concentrations of essential oils and herbal extracts (F1, F2 and F3) as in Table 2 and checked for anti-microbial activity against *Propionibacterium acnes* and *Staphylococcus epidermidis.*

Method of manufacture of Gels

- Out of total quantity of water, some water was kept aside.

- Remaining amount of water was taken in main mixing vessel. Phase A ingredients i.e. EDTA and Benzalkonium chloride were added and Carbopol was then sprinkled slowly to disperse it properly. After complete dispersion of Carbopol the dispersion was stirred for 15-20 minutes at slow speed.
- Sodium benzoate from Phase B was dissolved in 2 % water in a separate container/vessel, added to the main mixing vessel and stirred for 10 minutes.
- Phase C ingredients were added one by one to the above and stirred slowly for 30 minutes.
- Phase D (Triethanolamine dissolved in 5 % water) was added slowly to the above with constant stirring. The product was stirred for 30 minutes and the final yield was checked.
- The gel was then used for further study.

Anti-microbial assay of herbal anti acne gel

'Well Diffusion Method' was adopted for evaluation of antimicrobial activity:

- Lyophilized culture of *Propionibacterium acne* was revived in brain heart infusion broth at 37ºC for 48 to 72 h under anaerobic conditions.
- *Propionibacterium acne* inoculum was spread on the surface of sheep blood agar with the help of sterile swab stick.
- A well approximately 9 mm in diameter was bored on the surface of agar using a sterile cork borer.
- The samples (F, F1, F2, and F3) were introduced into the well on four separate plates. The plates were then incubated at 37ºC for 48 to 72 h under anaerobic conditions in an anaerobic jar (Hi-Media) with gas pack and indicator tablet. [Anaerobic gas pack - a disposable oxygen absorbing and carbon dioxide generating agent for use in anaerobic jar (Refer Figure 1) was used to maintain and check the anerobiosis. Anaerobic tablet having pink color was used, on introduction into the jar color remains pink indicating anaerobic conditions, original pink color if change to purplish-blue indicates aerobic condition because of the absorption of oxygen.][16]
- *Staphylococcus epidermidis* was inoculated in soybean casein digest medium (TSB) for 24 h at 37°C and adjusted to yield approximately 1.0×10^8 CFU/ml.
- *Staphylococcus epidermidis* inoculum was spread on the surface of Muller Hinton Agar with the help of sterile swab stick.
- The samples were then inoculated using same method performed above except that the plates were incubated at 37°C for 24 h under aerobic conditions.
- All the Well Diffusion tests were performed in duplicate and antibacterial activity was expressed as the mean of inhibition diameters (mm).

Measurement of Zone of Inhibition

The Zone of Inhibition for all gel formulations against Standard Clindamycin was measured using the known Standards for Bacterial Response[17] as shown in Table 3. The Activity Index (A.I.) and Percent Inhibition (P.I.) were then calculated using the following formula.[17] (Table 4)

A.I = Mean Zone of Inhibition of each sample / Zone of Inhibition obtained for Standard

P.I. = Activity Index x 100

No results were observed for formulation F (without actives). After calculation of Percent Inhibition, it was observed that formulation F3 possessed highest activity (Figure 2 and 3). The final product (F3) was then subjected to stress testing so as ensure prolonged sustainability of the anti-microbial effect.

Stress testing of herbal anti acne gel (Formulation F3)

As per the ICH (International Conference on Harmonization), Stress testing of the drug substance can help identify the likely degradation of products, which can in turn help establish the degradation pathways and the intrinsic stability of the molecule and validate the stability indicating power of the analytical procedures used. The nature of the stress testing will depend on the individual drug substance and the type of product involved. Stress testing is likely to be carried out on a single batch of the product. It includes the effect of temperatures (in 10°C increments (e.g., 50°C, 60°C etc.) above that for accelerated testing), humidity (e.g. 75 % RH or greater) where appropriate, oxidation, and photolysis on the product.[18]

Protocol

Herbal Anti Acne Gel (Formulation F3) was subjected to accelerated stress studies for a period of three months as per ICH Guidelines.[18] Two samples of gel were kept at RT while two in refrigerator and two in stability chamber.

Stress Parameters

Room Temperature (RT)
4°C (in refrigerator)
Stability Chambers - maintained at following temperature and humidity conditions.
40°C and 75 % relative humidity (RH) [Thermolab Stability Chamber, Equipment No.260/07/09-10]
30°C and 75 % relative humidity (RH) [Thermolab Stability Chamber, Equipment No.239/07/09-10]

They were then evaluated at the end of three months for the following parameters and the observations were noted in Table 5.

Parameters

- pH – Determined directly with pH meter equipped with Glass electrode (Equip-Tronic Digital pH meter Model no. EQ- 610.
- Water Content- Determined by Toulene Distillation Method using Dean and Stark apparatus (As per BIS: 9740-1981)
- Microbial Testing (Procedure followed as per Indian Pharmacopoeia- 2000)

Table 1: Zone of Inhibition of Essential oils and Herbal extracts against *Propionibacterium acnes* and *Staphylococcus epidermidis* after 24 h

S. No.	Name of Sample	*Propionibacterium acne*			*Staphylococcus epidermidis*		
		Zone of inhibitions in mm		Mean	Zone of inhibitions in mm		Mean
1	Cinnamon oil	32	33	32.5	31.5	35	33.2
2	Tea tree oil	18	17.5	17.75	19	18.5	18.75
3	Rosemary oil	12	13.5	12.75	14.75	15	13.5
4	*Berberis aristata*	18.5	19	18.75	19.5	20.2	19.85
5	*Azardirachta indica*	15	15.2	15.1	16	16.5	16.25

Table 2: Formulations of Herbal Anti Acne Gel

S. No.	Ingredients	Quantity (% w/w)			
	Phase A	**F (Control without actives)**	**F1**	**F2**	**F3**
1	EDTA	0.05	0.05	0.05	0.05
2	Benzalkonium chloride	0.02	0.02	0.02	0.02
3	Carbopol	0.8	0.8	0.8	0.8
4	DM water	Up to 10 0%	Up to 100 %	Up to 100 %	Up to 100 %
	Phase B				
5	Sodium benzoate	0.2	0.2	0.2	0.2
6	DM water	2	2	2	2
	Phase C				
7	Glycerin	3	3	3	3
8	Berberis aristata extract	-	5	7	10
9	Azardirachta indica extract	-	3	5	5
10	Cinnamon oil	-	0.2	0.3	0.5
11	Tea tree oil	-	0.3	0.3	0.3
12	Rosemary oil	-	0.1	0.3	0.3
	Phase D				
13	Triethanolamine	0.6	0.6	0.6	0.6
14	DM water	5	5	5	5

Table 3: Standard Values of Zone of Inhibition to Evaluate Bacterial Response

	Diameter of zone of inhibition (mm) [17]
Resistant	10 or less
Intermediate	11-15
Susceptible	16 or more

Table 4: Zone of Inhibition, Activity Index and Percent Inhibition of Finished Product against *Propionibacterium acne* and *Staphylococcus epidermidis*

Formulation No.	*Propionibacterium acne*					*Staphylococcus epidermidis*				
	Zone of inhibitions in mm		Mean	Activity Index	% Inhibition	Zone of inhibitions in mm		Mean	Activity index	% inhibition
F1	16.8	17.1	16.95	0.64	64.57	18.2	18.4	18.3	0.75	75
F2	18.9	18.5	18.7	0.71	71.23	19.4	19.7	19.5	0.79	79
F3	23.5	23.2	23.5	0.89	89.5	24	24.2	24.7	0.98	98
Standard (Clindamycin)	26.5	26	26.25	-	-	24.2	24.6	24.4	-	-

Table 5: Evaluation of Herbal Anti Acne Gel (F3)

S. No.	Parameters	Limits	Observation
1	Description	Yellowish brown colored gel with pleasant fragrance	Complies
2	pH	5.0 – 6.00	5.48
3	Water content	Not more than 90 %	73.60 %
4	**Microbiological analysis**		
	Total Viable Count	NMT 10^3 cfu/g	Complies
	Pathogens	Absent	Complies

Table 6: Zone of Inhibition of Herbal Anti Acne Gel after Three Months Stability Studies

Stability Parameters	*Propionibacterium acne*			*Staphylococcus epidermidis*		
	Zone of inhibitions in mm		Mean	Zone of inhibitions in mm		Mean
Room Temperature	21	21.5	21.25	23.2	23.1	23.15
4°C	22.6	22.2	22.4	23.3	23.7	23.5
40°C and 75 % RH	17.4	17.8	17.6	18.5	18.9	18.7
30°C and 75 % RH	19.2	19.7	19.45	19.7	19.4	19.55

Figure 1: Anaerobic Chamber

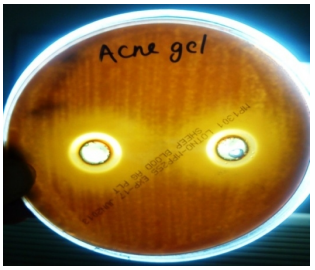

Figure 2: Zone of Inhibition of Herbal Anti Acne Gel (F3) against *Propionibacterium acnes*

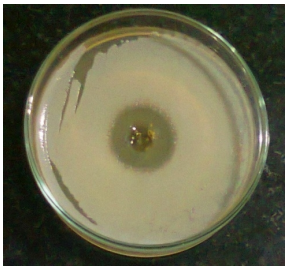

Figure 3: Zone of Inhibition of Herbal Anti Acne Gel (F3) against *Staphylococcus epidermidis*

RESULTS

The results of anti- microbial evaluation showed that all the selected herbal actives showed anti-microbial activity against acne organism. All the developed herbal anti acne gel formulations except Control (F) also showed inhibitory effect on *Propionibacterium acnes* and *Staphylococcus epidermidis*. Zone of inhibition, activity index and percent inhibition of formulation F3 was higher than all other formulations. It was also observed that this formulation showed sustained anti-microbial activity for both the organisms even after three months of stress testing studies (Table 6). The samples kept at room temperature and 4°C showed comparatively higher anti microbial activity than samples kept at 40°C and 30°C 75 % RH.

DISCUSSION AND CONCLUSION

Natural remedies are more acceptable in the belief that they are safer with fewer side effects than the synthetic ones. Herbs are safe, efficacious and multifunctional. Medicinal plant extracts are known to have enormous therapeutic potential. They serve as safer choice or in some cases is the only effective treatment. Essential oils are potential sources of novel antimicrobial compounds especially against bacterial pathogens.[19] From the entire study, it can be concluded that all three herbal anti acne gel formulations showed anti-acne activity against *P.acnes* and *S.epidermidis* however Formulation F3 showed highest activity and percent inhibition amongst all three formulated gels when compared with the standard Clindamycin, a known anti acne agent (Table 4). This could be because of the higher concentrations of herbal actives in Formulation F3 as against other formulations. This indicates that not just the right selection of actives but their concentrations in right quantities are also an essential controlling factor in achieving maximum activity. It is also important for the formulated product to be stable for the stipulated time period and maintain its efficacy. This was determined by accelerated stress studies as per ICH guidelines. The Formulation F3 was stable throughout the stress studies carried out for three months to various stresses. The pH as well as water content was found to be well within limits at the end of the study. The gel also maintained its anti-microbial activity at the end of the study time period as indicated in Table 5. Thus it can be concluded that the preparations using essential oils and herbal extracts can be effectively used as an alternative treatment for acne. However, further clinical trial evaluation should be done to check accurate efficacy of the product on acne which was beyond the scope of this study.

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