



Elucidating Anti-Acne Properties of a Novel Herbal Moisturizer

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Authors' contributions

This work was carried out in collaboration among all authors. Author AH designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SA and AAT managed the analyses of the study. Author AAT managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Acne vulgaris is most common dermatological condition that affects more than 70% of teenagers and more than 10% of adults. The moisture content of human skin makes it appear young and primary function of moisturizer is to avoid the dehydration by preventing the moisture loss. Natural therapies are more suitable and they are more reliable with less or no side effects. The current research focused on the use of herbs for treating acne vulgaris as a moisturizer. This research focused on the use of *Azadirachta indica*, *Curcuma longa*, and *Ocimum sanctum* to prepare the formulation, *Azadirachta indica*, *Ocimum sanctum* and *Curcuma longa* possessed the significant capability for inhibiting acne. The antimicrobial activity was evaluated against the *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Propionibacterium* by disc diffusion method. Prepared formulations were tested for physical parameters such as pH, spreadability, viscosity and color. Formulation studies was found to optimum for all parameters. The study showed the anti-acne moisturizer was significantly effective in controlling the bacteria which cause acne, i.e. *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Propionibacterium*. From this research, can be inferred that formulated herbal anti-acne moisturizer was related with a substantial decrease in the growth of microbes that causes acne.

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1. INTRODUCTION

The moisture content of human skin makes it appear young. The primary function of the moisturizer is to avoid skin dehydration by inhibiting the moisture loss, apart from speeding up the cell regeneration cycle. This is attained by attaching moisture in the stratum corneum, the skin's outermost layer with a film of oil on the surface. Treatment with moisturizer includes several steps like raising water content, repairing skin barriers, decreasing trans-epidermal loss of water and restoring lipid's water barrier functions [1]. The effective moisture must contain active ingredients that provide minerals and vitamins to the skin. Acne vulgaris is a common skin disorder that affects almost all individuals once during life. The occurrence of acne peaks at teenage but a large number of individuals between the age of 20-30 years are also affected by this disorder. *Propionibacterium* and *Staphylococcus epidermidis* are the major acne causing bacteria [2].

Acne can be categorized as papular, pustular, comedonal, nodular and cystic. Comedonal acne is the non-inflammatory and subdivided into the 2 types: Whiteheads and blackheads consist of follicular cells, melanin and sebum, present as an open pores comprising dark colored skin roughage [2]. Pustules are small bumps on skin which contains pus. Papules seem as red and elevated lesions frequently less than 5mm in the diameter. Nodules and cysts are solid and elevated lesions involving deeper and subcutaneous tissue. Nodules are more than 5 mm in the diameter and cysts are less than 5 mm in size.

Treatment of acne includes topical applications of retinoids, benzoyl peroxide and antibiotics. Regarding this, natural elements have been used in the treatment of acne vulgaris. The purpose of the present study was to formulate herbal anti-acne moisturizer and to evaluate antibacterial activity of the synthesized moisturizer. Ethanol extract of *Ocimum sanctum* (Tulsi), *Curcuma longa* (Turmeric), *Azadirachta indica* (Neem) have been used in this present research as they show the potential to inhibit acne causing bacteria (Figs. 1-3).

The literature study reveals the method of formation and evaluation of anti-acne moisturizer using herbal extracts.



Fig. 1. *Ocimum sanctum* [3]



Fig. 2. *Curcuma longa* [4]



Fig. 3. *Azadirachta indica* [5]

Dipanwita Chowdhury et al. [5], study showed the formulation of face mist using aqueous extract of apple cider, rosa vinegar also contains Ananuscomosus, Allium cepa, and Aloe Vera. Rose oil is used for fragrance. Antibacterial properties of formulation were evaluated against the *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* by disc plate and streak plate method. Formulation proved to have the antibacterial and anti-allergic properties [6].

Charde YM et al. [7], study showed the development and evaluation of the herbal formulation for the treatment of acne along with their antimicrobial activity against acne causing microorganisms. In this study ethanolic extracts of the Nutmeg (fruit), Neem (leaves) and Black pepper (fruit) were used to prepared and formulated into the gel. Antibacterial activity of the formulation was evaluated using agar well diffusion method against the *S. aureus*, *P. acnes* and *S. epidermidis*. Prepared gels were evaluated in vitro for pH, spreadability, stability, and acute skin irritancy. Results concluded that the prepared gel showed significant antimicrobial properties [7].

Arun Rasheed et al. [1], the research study showed the development and evaluation of anti-acne moisturizer using herbal extracts along with their antibacterial properties against *Staphylococcus epidermidis* and *Propionibacterium* [1].

Dhanshri Sanjay Koli et al. [2], the present study showed that development and evaluation of anti-acne face wash using herbal extracts containing extracts of turmeric, neem leaves, orange peel, shahi jeera, liquorice root. Prepared formulations were evaluated for the physical parameters such as color, consistency, spreadability and pH. Synthesized formulation showed good antimicrobial, anti-inflammatory and antioxidant properties [2].

In the prior research conducted, although the moisturizer and face mist prepared using herbal extract were found to be effective in treatment of the acne vulgaris but moisturizer showed minor effects than face wash and other commercial products. Therefore, there is need to develop herbal moisturizer for the treatment of acne vulgaris and prevent chances of chances of contamination. In the present research moisturizer was prepared by using *Azadirachta indica*, *Curcuma longa* and *Ocimum sanctum*. Plants extract showed significant antimicrobial properties against the acne causing bacteria.

2. METHODOLOGY

2.1 Design

Prepare the herbal anti-acne moisturizer, using *Azadirachta indica*, *Curcuma longa*, *Ocimum sanctum* and the antimicrobial activity of the synthesized formulation were evaluated against *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Propionibacterium* by disc diffusion method.

2.2 Plant Materials and Extraction

Plants used in the formulations were collected. Dried leaves of *Azadirachta indica*, *Curcuma longa*, *Ocimum sanctum* were grounded magnificently and passed individually through sieve number 70. 400 g of each powder was soaked with 95% ethanol for 3 days and vacuum filtered using 0.25 micron filter to obtain a sterilized extract. Using a vacuum desiccator, filterates were dried. 40g of extract was dissolved in ethanol in 150ml. Final volume of 130ml was concentrated and obtained semi solid extracts were placed in desiccators at 4°C for future use [8].

2.3 Method of Formation

Precise quantities of beeswax, cocoa butter, carrier oil such as olive and almond oil were liquefied over low heat in a glass container. 70ml of distilled water was stirred & boiled and with borax. Stirring was continued until the borax completely dissolved. Weighed extracts of *Azadirachta indica*, *Curcuma longa*, *Ocimum sanctum* were added in a glass container along with the vitamin A, vitamin C, HPC (Hydroxypropyl cellulose) and preservatives. Further, mentioned amounts of carbopol was added as formulation agent for providing viscosity. Sulphur has been used as it has been used in providing inflammatory relief to the skin and zinc oxide has been used a preservative. Both mixtures were slowly mixed together and stirred until it became creamy. Flavoring agents such as rose and vitamin oil were added. Table 1 presents the ingredients along with their compositions used in preparation of final formulation.

2.4 Efficacy Test

In the present research, efficacy of the anti-acne moisturizer had been evaluated by antimicrobial test.

2.5 Disc Diffusion Method

Analysis of the antimicrobial activity of the extracts to microorganisms was carried out using disc diffusion process. Agar was sterilized & packed. It was distributed aseptically on the 3 petri-plates, each of the set had 3 plates and was classified as check, control & normal. *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Propionibacterium* were used as a test cultures. Petri-plates were inoculated with the cultures and incubated at 37°C for 24 hours. 2 discs of filter paper were loaded with the herbal anti-acne moisturizer & marketed anti-acne moisturizer the next day, each disc was put in designated plate. Care was taken to ensure the sterile discs absorb formulation completely. Sodium lauryl sulphate (SLS) was continued as a control. Test regulates the efficacy of product in the terms of inhibition zone after 24 hr. Larger the zone of inhibition, more efficacy of test product [9].

Table 1. Composition of formulation

Herbal ingredients	Quantities
<i>Azadirachtaindica</i>	0.8 ml
<i>Curcuma longa</i>	1 ml
<i>Ocimum sanctum</i>	1.5 ml
Cocoa butter	1.5 g
Beeswax	1 g
Almond oil	1 ml
Olive oil	1.5 ml
Rose oil	0.8 ml
Chemicals	Quantities
Borax	2.5 g
Carbopol	0.5 g
Hydroxypropyl cellulose (HPC)	1.5 g
Propyl paraben	0.8 g
Sulphur	0.5 g
Vitamin A	0.5 ml
Vitamin C	0.5 ml
Zinc oxide	1.5 g

2.6 Stability Studies

Viscosity of formulations was determined using Brookfield viscometer at 10 to 100 rpm. Viscosity

tests were performed in samples below 25°C, 8 ml and using the LV-spindle. According to Multimer, layer thickness and spreadability were evaluated, spreadability refers to the percentage area enclosed by the fixed sum of sample after it is evenly spread and layer thickness states to layer thickness in microns. Formulations stability was measured by freeze thaw method & centrifugation. Formulations were centrifuged at 4000-14000 rpm and later observed for the phase separation. Formulation was reserved alternatively at 20°C and 40°C in freeze thaw study, and then detected for a change in color and phase separation. The formulation pH was calculated with the help of the digital pH meter.

3. RESULTS AND DISCUSSION

The result of the disc diffusion method showed (Table 2) anti-acne moisturizer synthesized by using the ethanol extract of the plants had larger activity than commercial product (clindamycin). It was observed that, MIC of *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Propionibacterium acne* 27 ± 3 , 25 ± 2 and 22 ± 2 respectively which clearly indicates that the present formulation is very effective in eradicating the bacteria (Fig. 4).

The color and pH of synthesized moisturizer was found to be green and 6.79 ± 0.02 . pH of the formulation had somewhat alkaline which was well-suited with the physiology of skin. Spreadability and the thickness of layer for formulation were found to be 94.8% and $27.02 \pm 1.23 \mu\text{m}$ (Table 3). As the rotation speed of centrifuge increased, the viscosity of sample was decreased (Table 4), revealing the products pseudo-plastic behavior. Stability results were shown, which were tested by using the freeze thaw method and centrifugation (Table 3). No separation of phase in the formulation was observed at 14000 rpm, this shows the formulation's stability under high pressure conditions. During the freeze thaw study, water was not isolated from the formulation.

Table 2. Anti-microbial activity result of the formulation. Herbal formulation showed larger zone of inhibition in comparison to commercial formulation. All the values are calculated as Mean \pm SD (n=3), p < 0.001

Organisms	Herbal formulation	Zone of inhibition commercial formulation	Control
<i>Staphylococcus epidermidis</i>	27 ± 3	20 ± 2	5 ± 2
<i>Staphylococcus aureus</i>	25 ± 2	18 ± 2	4 ± 1
<i>Propionibacterium acne</i>	22 ± 2	16 ± 2	No inhibition

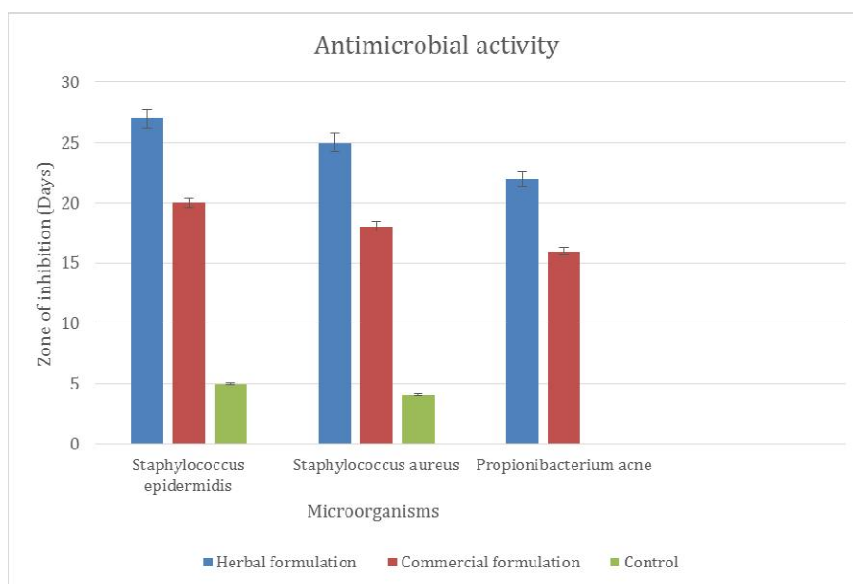


Fig. 4. Antimicrobial activity of formulation. Herbal cream showed larger zone of inhibition than commercial products. All the values are calculated as Mean ± SD (n=3), p < 0.001

Table 3. Physicochemical evaluation parameters. Formulation were found to be green in color and the pH and spreadability showed significant result. All of values are calculated as Mean ± SD (n=3), p < 0.001

Parameter	Value
Color	Green
Centrifugation (14000)	Stable
Freeze thaw	Stable
Layer thickness (µm)	27.02 ± 1.23
pH	6.79 ± 0.02
Spreadability (%)	94.8%

Table 4. Viscosity of herbal anti-acne moisturizer. Viscosity of the sample decreased when the rotation per minute increased

Rotation per minute	Viscosity (N.sm ⁻²)
10	168.0
30	65.6
50	32.4
100	15.6

4. CONCLUSION

The natural materials are suitable in the belief, they are safe and have less side effects than synthetic one. In the world market, herbal solutions have increasing demand. The leaves of *Azadirachta indica*, *Curcuma longa* and *Ocimum sanctum* are widely used for medicinal purposes.

The extract of ethanol effectively extracts phytochemicals & the acids that act upon pathogens. The thought behind the combination of plants is the observation of preservative effect of active ingredients from various plants. The combination of plant materials proved advantageous and is therefore used to prepare herbal anti-acne moisturizer. Prepared herbal anti-acne moisturizer was tested for its antimicrobial activity using the method of disc diffusion. A new way of combating antibiotic resistance of pathogenic species and ensuring healthy living by germ-free skin can thus be identified, while elimination is not the 100%, but a large number of microorganisms can be reduced. With this research, can be inferred that formulated herbal anti-acne moisturizer was related with a substantial decrease in the growth of microbes that caused acne.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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