REPORT

Formulation and evaluation of herbal anti-acne moisturizer

Arun Rasheed*[1], Shaik Neelufar Shama[2], Jyothi Mulanjananiyil Joy[2], Bobbu Sravya Reddy[2] and Chirra Roja[2]

[1]Department of Pharmaceutical Chemistry, Sree Vidyanikethan College of Pharmacy, Andhra Pradesh, India
[2]Department of Pharmacognosy, Sree Vidyanikethan College of Pharmacy, Andhra Pradesh, India

Abstract: The moisture content present in human skin makes it look young and the use of moisturizer results in fastening the moisture with a surface film of oil. Acne vulgaris is one of the most commonly seen diseases among the youth. The present study is focused on the use of herbs as moisturizer for acne treatment. The anti-acne moisturizer was formulated from herbal crude extracts and investigated the physico-chemical parameters as well as antibacterial activity of the formulation. The study revealed that ethanol extract of *Andrographis paniculata, Glycyrrhiza glabra, Ocimum sanctum, Azadiracta indica* and Green tea possessed the potential for inhibiting acne. It was observed that the optimal formula of anti-acne moisturizer was satisfactorily effective to control acne inducing bacteria i.e., *Staphylococcus epidermis* and *Propionibacterium*. The physico-chemical parameters of the formulation were also optimal with no signs of irritation.

Keywords: *Andrographis paniculata, Glycyrrhiza glabra, Ocimum sanctum, Azadiracta indica,* Green tea, anti-microbial.

INTRODUCTION

The moisture content present in human skin makes it look young. The prime and foremost function of moisturizer is to hinder skin dehydration by preventing the loss of moisture apart from speeding up the process of cell renewal. This is achieved by fastening moisture in the stratum cornea, the outermost layer of the skin with a surface film of oil. The moisturizing treatment include various steps such as repairing the skin barrier, raising water content, restoring the lipid's water barrier function and decreasing transepidermal water loss. Occlusives, Humectants, Emollients, Proteins, preservatives are the moisturizing ingredients and based on the skin type the moisturizers are selected (Lieb *et al.,* 1988; Armold *et al.,* 1990). The moisture must contain the active ingredients that supply vitamins and minerals to the skin.

Acne vulgaris, a commonly seen disease among the youth, occurs at puberty and continues into adulthood. *Staphylococcus epidermis* and *Propionibacterium* are considered as the major skin bacteria that cause the formation of acne. Typical acne lesions are comedones, inflammatory papules, pustules as well as nodules and are affected on areas of face, upper part of the chest and the back (Udomlak Sukatta *et al.,* 2008). The aim of this research was to develop anti-acne moisturizer from herbal crude extracts and to investigate antibacterial activity of the anti-acne moisturizer. Ethanol extract of *Andrographis paniculata, Glycyrrhiza glabra, Ocimum sanctum, Azadirachta indica,* Green tea have been used in this study as they possess the potential to inhibit acne - causing bacteria.

MATERIALS AND METHODS

Plant material and extraction

The plant materials used in the formulation were procured from Herbal Crude Drugs, Mumbai, India and authenticated by the botanist Prof. Madhava Chetty, Department of Botany, S.V. University, Tirupati, India. The dried leaves of *A.paniculata, G.glabra, O.sanctum, A.indica* and Green tea were finely ground and individually passed through sieve no. 80. 500 g of each powder was macerated for 3 days with 95% ethanol and filtered. The filtrates were dried using a vacuum desiccator. 45 g of each extract was dissolved in 150 ml of ethanol (300 mg/ml). This was concentrated to a final volume of 135 ml. The obtained semisolid extracts were kept in a desiccator at 4°C until further used (Singha *et al.,* 2003).

Animals

Four groups of Wistar albino rats (150-200 g) each with three animals were selected for the study. The selected animals were housed in acrylic cages at standard environmental conditions at 25 ± 2ºC, relative humidity of 45-55%, in a well ventilated room maintained at 12: 12 h light: dark cycle, fed with standard rodent diet and water *ad libitum*. All the animals were acclimatized for a week

**Corresponding author:* e-mail: arunrasheed@rediffmail.com

before experiment. The animal experiments were carried out according to the guidelines of the Committee for the Purpose of Control of Experiments on Animals (Reg. No. 930/a/06/CPCSEA) and approval of the Institutional Animal Ethics Committee, Sree Vidyanikethan College of Pharmacy, Tirupati, India was obtained.

Method of formulation

Accurate quantities of cocoa butter, bees wax and carrier oils such as coconut oil and olive oil were melted in a glass container over low heat. 75 ml of distilled water was heated and then stirred with borax. Stirring was continued until the borax was completely dissolved. In another glass container, weighed extracts of *A. paniculata,* Liquorice, *A. indica, O. sanctum,* green tea and aloe gel were added along with HPMC, vitamin A, vitamin C and preservatives (table 1). Both the containers were removed and both the mixtures were combined together slowly and stirred until it becomes creamy. Finally flavoring agents like almond oil and rose oil were added.

Table 1: Formulation composition

Herbal Ingredients	Quantities
Andrographis paniculata	1.5 ml
Glycyrrhiza glabra	1 ml
Aloe barbadensis	5 gm
Ocimum sanctum	1.5 ml
Azadirachta indica	0.5 ml
Green tea	1 ml
Cocoa butter	1 gm
Bees wax	1 gm
Coconut oil	1.5 ml
Olive oil	1 ml
Rose oil	0.5 ml
Almond oil	0.5 ml

Chemical Ingredients	Quantities
Zinc oxide	1.2 g
Borax	2.0 g
Sulphur	0.5 g
HPMC	2 g
Methyl paraben	1 gm
Vitamin A	0.5 ml
Carbopol	0.5 g
Vitamin C	0.5 ml

Evaluation

i. Efficacy test

Efficacy analysis is an important step to verify the claim produced by finished products. In present study, efficacy of herbal acne moisturizer has been determined by anti-microbial test.

Disc diffusion method

The screening of antibacterial sensitivity of the extracts against pathogens was performed using disc diffusion method. Nutrient agar was prepared and sterilized. It was aseptically spread on three sets of Petri plates; each set containing three plates and was marked as test, control and standard. Test cultures used were *Staphylococcus epidermis* and *Propionibacterium*. The plates were inoculated with test cultures and incubated at 37°C for 24 h. Next day two filter paper discs were loaded with herbal anti-acne moisturizer and a commercial anti-acne moisturizer and each disc was placed in the respectively marked plate. It was taken care that the sterile discs completely absorb the formulation. Disc of SLS (Sodium Lauryl sulphate) was maintained as control. After 24 h, the test determines the efficacy of the product in terms of zone of inhibition of the organism. Higher the zone of inhibition, the more effective is the test product (Minakshi *et al.,* 2008).

ii. Stability Studies

All quality parameters evaluation were done according to the guidelines of Bureau of Indian Standard (BIS), World health Organization [WHO], European Cosmetic, Toiletry and Perfumery Association [COLIPA] and Scientific Committee of Cosmetics and Non-Food Products [SCCNFP].

Viscosity profile of the formulation was measured using a Brookfield viscometer at 10 to 100 rpm (Gaspar and Maia Campos 2003). Viscosity measurements were made under 25ºC, 8 ml samples and using LV-spindle. Spreadability and layer thickness were evaluated according to Multimer (Multimer 1956), Spreadability refers to the percentage area covered by a fixed amount of the sample after its uniform spreading and layer thickness refers to thickness of the layer in microns. Stability of the formulation was determined by centrifugation and freeze thaw method (Butler 2000). During centrifugation study the formulation was centrifuged at 3500-13500 rpm at the intervals of 500 rpm for 10 minutes, and further observed for phase separation. In freeze thaw study the formulation was kept alternatively at 20ºC and 40ºC, then observed for color change and phase separation. pH of the formulation was measured using digital pH meter (New Delhi, India). The skin irritation test was performed on albino rats of both sex weighing about 150-200 g. The animals were maintained on standard animal feed and free access to water. Hair was shaved from the back of the rats and an area of 2 cm^2 on both sides. One side served for control (5% SLS (Sodium lauryl sulphate) in distilled water) and the other side for test (formulation) with 2 animals (Hiremath *et al.,* 2008). The formulated moisturizer was applied twice a day for 3 days and the site was observed

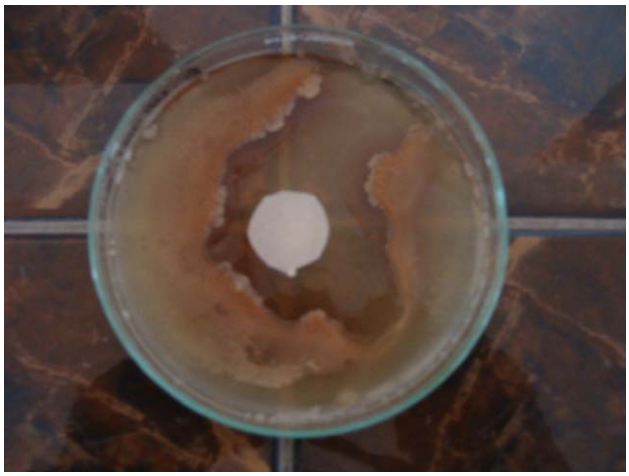

Fig. 1a: Zone of inhibition by herbal formulation

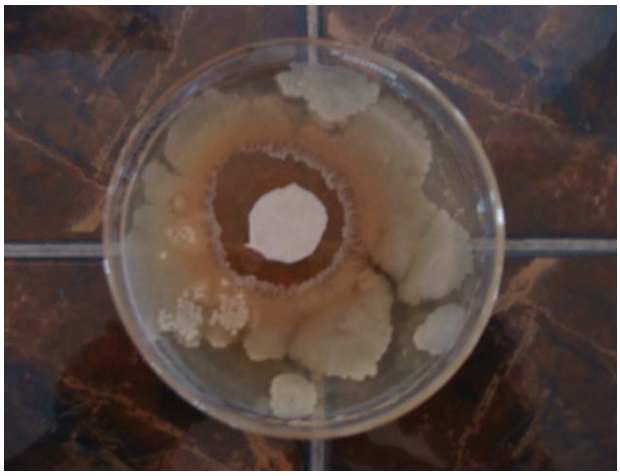

Fig. 1b: Zone of inhibition by marketed formulation

Table 2: Anti-microbial sensitivity result of the formulation

Organisms	Zone of inhibition (mm)		
	Herbal formulation	Commercial formulation	Control
Staphylococcus epidermis	29 ± 2	19 ± 2	6 ± 2
Propionibacterium acne	21 ± 2	17 ± 1	No inhibition

All the values are represented as Mean ± SD (n=3), $p < 0.001$.

for any sensitivity, edema and erythema. All evaluations were carried out in triplicate.

STATISTICAL ANALYSIS

Statistical analysis was carried out by using STATS [70] software and results were expressed as mean S.D. All the parameters were statistically analyzed at 95% confidence level in the column. Statistical result of psychometric evaluation was further tested by ANOVA [One way analysis].

RESULTS

The results of disc diffusion method (table 2) showed that the herbal anti-acne moisturizer prepared from ethanol extract of the combined plant materials had greater activity than the activity of the commercially available product (figs. 1a & 1b). Color of the formulated moisturizer was found to be pale green, pH of the formulation was found to be in range of 6.98 ± 0.03.

Spreadability and layer thickness were found to be 96 ± 0.8% and 28.99 ± 1.55 µm for the formulation (table 3). As the speed of rotation increased, viscosity of the tested sample decreased (table 4), revealing the pseudo plastic behavior of the products. Stability results, tested by centrifugation and freeze thaw method are shown (table 3). No phase separation at 13500 rpm was observed in the formulation; this shows the stability of the formulation at high stress conditions. Water was not separated from the formulation during freeze thaw study. The skin irritation test performed showed no signs of sensitivity, erythema and edema (table 3). So the prepared formulation was considered to be non-irritant.

Table 3: Physicochemical evaluation parameters

Parameter	Value
Color	Pale green
pH	6.98 ± 0.03
Spreadability (%)	96 ± 0.8%
LT (µm)	28.99 ± 1.55
Centrifugation (13500 rpm)	+
Freeze thaw	+
Erythema score	0

All of the values are represented as Mean ± SD (n=3), $p < 0.001$.
(LT) Layer thickness; (rpm) rotation per minute;
(+) indicates stable, (-) indicates unstable; 0 indicates no irritation

Table 4: Viscosity profile of herbal anti-acne moisturizer

Rotation per minute	Viscosity (cps)
10	178.0
20	89.2
30	58.4
50	28.7
60	25.4
100	16.8

(cps) Centipoise

DISCUSSION

The leaves of *Azadiracta indica*, *Aloe*, *Andrographis paniculata*, Liquorice are widely used for medicinal purposes. In the present context the plants under study are rich in these varied compounds and hence are more effective against skin pathogens. The ethanol extract is efficient extracting the phytochemicals and acids which act on pathogens. The main ideology behind combining the plant materials is to observe the additive affect of the active constituents from different plants. The combination proves to be beneficial and hence it is used in preparation of an herbal anti-acne moisturizer. The herbal anti-acne moisturizer prepared was checked for its efficacy using disc diffusion method. Hence a new way can be found to combat antibiotic resistance of pathogenic organism and provide safe and healthy living through germ free skin although the removal is not 100% but a major number can be reduced. From this study, it can be concluded that the formulated herbal anti-acne moisturizer was associated with significant reduction in microbial growth which causes acne and also was found to produce moisturizing effect with no erythema.

REFERENCES

Armold HL, Odom RB and James WD (1990). Diseases of the Skin, Clinical Dermatology, Philadelphia, USA, p.702.

Hiremath SSP, Dasankoppa FS, Nadaf A, Jamakandi VG, Mulla JS, Sreenivas SA, Sholapur HN, Ahmed A and Nanjunda Swamy NG (2008). Formulation and evaluation of a novel *in situ* gum based ophthalmic drug delivery system of linezolid. *Sci. Pharm*., **76**: 515-532.

Lieb LM, Nash RA, Matias JR and Orentreich N (1988). A new *in vitro* method for transepidermal water loss: A Possible method for moisturizer evaluation. *J. Soc. Cosmet. Chem*., **39**: 107-119.

Minakshi Joshi G, Kamat DV and Kamat SD (2008). Evaluation of herbal handwash formulation. *Natural Product Radiance*, **7**(5): 413-415.

Singha PK, Roy S and Dey S (2003). Antimicrobial activity of Andrographis paniculata. *Fitoterapia*, **74(**7-8): 692-694

Sukatta U, Rugthaworn P, Pitpiangchan P and Dilokkunanant U (2008). Development of Mangosteen Anti-Acne Gel. *Kasetsart J. (Nat. Sci.),* **42**: 163-168.