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Formulation and Evaluation of Herbal Anti-Acne Gel Containing Neem and Garlic Extract

	
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ABSTRACT

Acne vulgaris is a long term inflammatory disorder of the pilosebaceous unit that leads to the formation of inflammatory lesions, seborrhea, comedones, etc. *Propionibacterium acnes* and *Staphylococcus epidermidis* have been recognized as pus-forming bacteria triggering inflammation in acne. *Staphylococcus aureus* support to cause inflammation in acne. Natural remedies are more acceptable in the belief that they are suffering from fewer side effects than the synthetic ones. Herbal formulations have a growing demand in the global market. This present research work aims to formulate and evaluate herbal antiacne gel containing ethanolic extract of Neem (*Azadirachta indica*) and Garlic (*Allium sativum*). The herbal antiacne gel was optimized by preparing 3 formulations (F1, F2, F3) using an extract of Neem, Garlic, and a combination of these two extracts. The formulation was evaluated for various parameters like Physical appearance, pH, Drug content, Spreadability, Extrudability, Anti-acne activity assay against *S. aureus* was successfully studied. Amongst all the formulation studied, batch F3 was found optimum for all the parameters. Both extracts i.e. ethanolic extract of *Azadirachta indica* and *Allium sativum* on combination show potential effect against *Acne vulgaris* and also exert a synergistic effect on the bacteria.

INTRODUCTION

Acne, from the Greek word "Akme", means peak or apex, is genetic or acquired affections of the pilosebaceous units. The correct name for acne is *Acne vulgaris*. Acne is the most common disorder found among youngsters usually 18-25 years of age. *Acne vulgaris*, which is a skin disorder of the pilosebaceous gland which is characterized by formation of seborrhea, comedones, inflammatory lesions and presence of bacteria *Propionibacterium acnes*, *Staphylococcus epidermidis* and *Staphylococcus aureus* in the follicular canal and sebum production. It is almost a universal disease occurring in all races and affecting 95% of boys and 83% of girls.

P. acnes have been described as an obligate anaerobic microorganism. 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.

On the contrary, *S. epidermidis*, anaerobic organism, usually involves in superficial infections within the sebaceous unit. When the chemicals produced by *P. acnes* destroy the cellular structure of skin cells, *Staphylococcus aureus*, grows causing acne lesions. These factors provide a potential target for treatment. *P. acnes*, *S. epidermidis* and *S. aureus* are the target sites of anti-acne drugs.

The excessive use of antibiotics for long periods has led to the increased resistance in acne-causing bacteria i.e. *P. acnes*, *S. epidermidis*, and *S. aureus*. The development of antibiotic resistance is multifactorial, including the specific nature of the relationship of bacteria to antibiotics, how the antibacterial is used, host characteristics, and environmental factors. To overcome the problem of antibiotic resistance, medicinal plants have been extensively studied as alternative treatments for diseases. ^[1, 2]

Medicinal plants such as *Azadirachta indica* (Neem) & *Allium sativum* (Garlic) which have been traditionally used as antimicrobial and anti-inflammatory agents were examined for antimicrobial activity against microorganism frequently involved in acne inflammation, such as *Staphylococcus aureus*. Applications of Garlic and Neem juice directly on the acne are troublesome. Hence, it is necessary to develop topical dosage forms, such as a gel. The gel gives more cold sensations on the skin, easily absorbed and forms a film that is easy to wash.

The main objective of this present research work is to develop an effective herbal Anti-acne gel containing Neem and Garlic and to evaluate the same by preliminary methods.^[3,4]

MATERIALS AND METHODS

MATERIALS

Neem, Garlic, Carbapol, PEG 400, Methylparaben, Glycerin, Triethanolamine is used in present work.

DETAIL PROFILE OF MATERIAL USED

1) Neem^[5,6]

Synonyms: Nira, Nimb, Vespa, Limba, Nimba

Biological source: Neem consists of the fresh or dried leaves and seed oil of *Azadirachta indica* belonging to family Meliaceae.



Figure No. 1: *Azadirachta Indica*

Chemical Constituents:

Nimbin, 6-desacetylnimbinene, Nimbiene, Nimbandiol, nimbolide, Quercetin, Ascorbic acid, n-hexacosanol, amino acid, Nimbin & Nimbidinin

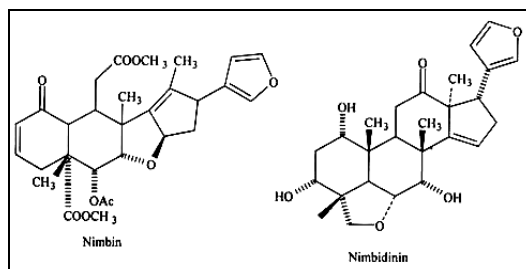


Figure No. 2: Structure of Nimbin & Nimbidinin

Geographical source:

It is found in India, Pakistan, Sri Lanka, Malaya, Indonesia, Japan, Tropical region of Australia and Africa. In India, it is found in Uttar Pradesh, Maharashtra, Tamil Nadu, Rajasthan, and M.P.

Uses:

- Poultice, applied to boils.
- In worm, jaundice
- Ulceration of cowpox
- Antiviral and anti-fungal



Pharmacological uses:

Anti-ulcer, antifertility, antifungal, antiviral, anti-pyretic.

2) Garlic

Synonym: Garlic, Allium, lasun

Biological source: It consists of the bulb part of the plant known as *Allium sativum* Linn. belonging to family Liliaceae.



Figure No. 3: *Allium sativum*

Chemical Constituents: Allicin, Allin, 29% Carbohydrates, Volatile oil, S-allyl mercapto cysteine, S-allyl cysteine.

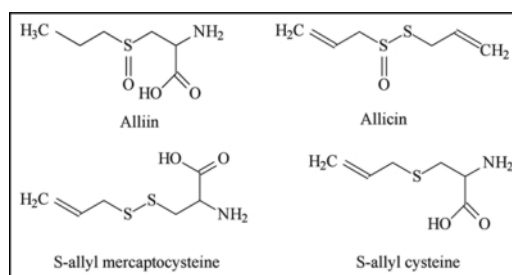


Figure No. 4: Structure of S-allyl mercapto cysteine & S-allyl cysteine

Geographical Source:

Garlic is cultivated in India, Russia, USA, Italy, and southern Europe

Uses:

- Carminative
- Expectorant
- Stimulant
- Disinfectant.

Pharmacological uses:

Anti-bacterial, Anthelmintic, Rubefacient.

METHODS

Procurement of plant material:

The fresh leaves of Neem (*Azadirachta indica*) were collected from the Medicinal Plant garden of P.S.G.V.P.Mandal's College Of Pharmacy, Shahada, District-Nandurbar and the bulb part of Garlic (*Allium Sativum*) were purchased from the local market of Shahada. The plant specimens were authenticated by Dr. Santosh K Tayade, HOD of Botany, Art's Science and Commerce College, Lonkheda, Shahada, Dist-Nandurbar (MS).

Extraction Procedure: ^[7]

- Weigh accurately the quantity of Neem and Garlic powders.
- Place each powder in the separate chamber of the soxhlet apparatus.
- This soxhlet extractor placed into RBF containing the extraction solvent i.e. Alcohol and water in a ratio of 1:1.
- Take the extraction solvent i.e. water + Alcohol in the ratio of 1:1 and pass at least the three cycles from thimble containing the drug.
- Place the reflux condenser on top of the soxhlet apparatus which closed with cotton plug from the top and allow to pass water from top to the bottom of the condenser.
- Then switch ON the assembly and pass the 5-6 cycles into the apparatus.
- After complete, the extraction removes the soxhlet apparatus and collect the extract from RBF.
- After collecting the extracts it allows to evaporate on the water bath to get the concentrated extract.

Formulation of Gel: ^[2]

The gel was prepared by using a 1% concentration of the extracts. In a separate beaker, Carbopol 940 was dispersed uniformly in distilled water with continuous stirring, avoiding air entrapment and allowed to soak overnight. In another beaker, methylparaben was dissolved in the remaining amount of distilled water by gently heating. To this solution, the

herbal extracts were added and triturated well. The above mixture was then added to the carbopol mixture and stirred well. Finally, propylene glycol and triethanolamine were added and the pH was adjusted to 6.8-7. The prepared formulation was filled in a suitable container and labeled. Various formulation batches were prepared; the composition of formulations was shown in Table No. 1.

Table No. 1: Composition of Developed Formulation

Sr. No.	Ingredient Name	Formulation Code			Role of Ingredients
		F1	F2	F3	
1.	Ethanollic extract of Neem	1%	-	0.5%	Heals Acne Scars, Coolant, Soothing, Moistening
2.	Ethanollic extract of Garlic	-	1%	0.5%	Anti-inflammatory, Anti-fungal
3.	Carbazole 940	2%	2%	2%	Gelling agent
4.	PEG 400	2%	2%	2%	Humectant, Solvent
5.	Methyl paraben	0.1%	0.1%	0.1%	Preservative
6.	Triethanolamine	2%	2%	2%	Stabilizer or Neutralizer
7.	Distilled Water	q.s	q.s	q.s	Vehicle



Figure No. 5: Formulation F1, F2 &F3

Evaluation of Gel

1) Physical appearance:

Physical parameters such as Colour, odor, and consistency were checked visually.

2) Washability:

Formulations were applied on the skin and then easy and the extent of washing with water was checked manually.

3) pH:

the pH of 1% aqueous solution of the formulation was measured by using a calibrated digital pH meter at a constant temperature.

4) Spreadability:

It indicates the extent of the area to which gel readily spreads on application to the skin or affected part. The therapeutic potency also depends upon spreading value. The time in sec taken by two slides to slip off from gel which is placed in between the slides under the direction of a certain load is expressed as spreadability. Lesser the time is taken for the separation of two slides, better the spreadability. The following formula is used to calculate the Spreadability:

$$\text{Spreadability (S)} = M \times L / T \text{ Where, } M = \text{weight tied to upper slide}$$

L = length of glass slides, T = time taken to separate the slides.

5) Extrudability:

It is a usual empirical test to measure the force required to extrude the material from the tube. The method applied for the determination of applied shear in the region of the rheogram corresponding to a shear rate exceeding the yield value and exhibiting consequent plug flow. In the present study, the method adopted for evaluating gel formulation for extrudability was based upon the quantity of gel extruded from a lacquered aluminum collapsible tube on the application of weight in grams required to extrude ribbon of study gel in 10 seconds. The measurement of the extrudability of each formulation was in triplicate and the average values were presented.

6) Percentage of Drug content:

Each formulation (1g) was accurately weighed and transferred to a 100 ml volumetric flask to which about 70 ml of methanol was added. After shaking, the volume was made up to 100 ml

with methanol. The content was filtered through a suitable filter paper. 1ml filtrate was taken and suitable diluted and the drug content (extract) was estimated by using UV/Visible spectrophotometer at 250nm, 216nm, 252nm respectively. [8]

7) *In-vitro* diffusion study:

Permeation of different Gel formulations was studied using a Franz glass diffusion cell. The effective permeation area of the diffusion cell and receptor cell volume was 1 cm² and 10 ml, respectively. The receptor compartment contained PBS (pH 7.4) and maintained at 37°C ± 1°C by a magnetic stirrer. The egg membrane was mounted between the donor and receptor compartment.

The donor compartment was filled with 1 gm. gel formulation. A 10 ml aliquot pH 7.4 phosphate buffer was used as a receptor medium to maintain a sink condition. At appropriate intervals, 1 ml aliquots of the receptor medium were withdrawn and immediately replaced by an equal volume of fresh receptor solution and analyzed by UV-Visible Spectrophotometer. [1, 5]

8) *Antiacne* activity: [6,9,10]

The antiacne and antibacterial activities of different formulations were determined by a modified agar well diffusion method. In this method, nutrient agar plates were seeded with 0.2 ml of 24 h broth culture of *S. aureus*. The agar plates were allowed to solidify. A sterile 8 mm borer was used to cut wells of equidistance in each of the plates. 0.5 ml of formulations, the herbal gel was introduced into the wells at randomly. The plates were incubated at 37°C for 24 hours. The antiacne activities were evaluated by measuring the zones of inhibition (in mm).

RESULTS AND DISCUSSION

The results of the evaluation are shown in Table no.2. The gels were Slight yellowish to brownish with a specific odor. All formulations were found homogenous easily washable. All the formulation has slightly alkaline pH. Amongst all the formulation F3 showed very optimum Spreadability. The Formulations F1, F2 shows good Extrudability whereas formulation F3 shows excellent Extrudability. Formulation F3 shows better drug content than F1&F2. The results showed that all developed gel formulation had an inhibitory effect on the

S. aureus, but the combination of extracts (F3) showed more zone of inhibition as compared to individual extracts (F1&F2).

Table No.2: Evaluation of Gels

Formulation Batch	Colour	Consistency	pH	Spreadability gm.cm/sec	Extrudability	Drug content	Zone of inhibition (mm)
F1	Brownish	Semisolid	6.67	11.76	Good	96.66%	14
F2	Slight yellowish	Semisolid	6.58	11.50	Good	95.65%	12
F3	Brownish	Semisolid	6.79	12.03	Excellent	97.38%	17



Figure No. 6: Zone of Inhibition of Combination of Neem and Garlic against *S. Aureus*



Figure No. 7: Zone Of Inhibition against *S. Aureus*

CONCLUSION

Natural remedies are boon to any disease. In the world market, herbal formulations are in great demand. Herbal medicines are believed to be safer than allopathic medicines. All the formulations were optimized based on evaluation parameters such as Physical appearance, Washability, pH, Spreadability, Extrudability, Drug Content, Antiacne activity. After

evaluation, this study concludes that formulation batch F3 i.e. the gel which contains a combination of both extracts was comparatively better than other formulations F1 and F2.

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