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Development and Evaluation of Anti Acne Cream Using Extracts of Vitex Negundo and Hibiscus Rosa-Sinensis

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ABSTRACT

Acne vulgaris is the most common chronic skin disease of the world. The bacteria responsible for acne are Staphylococcus aureus. So many synthetic products are available in the market for acne treatment including antibiotics, but serious side effects arises due to the long-term use of synthetically prepared anti-acne preparations. Bacterial resistance is the major problem that occurs due to the irrational use of antibiotics, in addition to this skin problem, such as erythema, allergy, sunburn and melanin pigmentation. As from ancient times, natural plant substances have been shown to be promising candidates for acne treatment without side effects. Therefore in the present study, the anti acne cream formulation has been prepared using *Vitex negundo* (leaves), *Hibiscus rosa-sinensis* (flower) and Tea Tree Oil (*Melaleuca alternifoli*). The prepared formulation was evaluated on the basis of greasiness, spreadability, homogeneity, skin irritancy, viscosity, pH, emolliency and stability. The antibacterial study was also performed using a well diffusion technique and results showed that formulation possess sufficient anti bacterial activity. This showed that the optimized formulation has anti-acne properties.

Keywords: Anti acne, *Staphylococcus aureus*, *Vitex negundo*, Hibiscus *rosa-sinensis*, *Melaleuca alternifolia.*

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INTRODUCTION

Acne vulgaris is the formation of comedones, papules, pustules, nodules, and cysts occurs as a result of obstruction and inflammation of pilosebaceous units (hair follicles and their accompanying sebaceous gland). It can also be present as non-inflammatory lesions, inflammatory lesions, or a mixture of both, affecting mostly the face but may come out on back and chest as well. The most common bacteria responsible for acne is Staphylococcus aureus but some studies report that Propionibacterium acne has also been isolated from acne patient. [1]

Etiology

Development of acne occurs due to blockage of follicles, hyper keratinization and the formation of keratin and sebum (microcomedo). Increase in production of androgen, increases the size of sebaceous glands and hence shows increase in the production of sebum. The microcomedo can be expanded to form an open blackhead or a closed comedo. Comedones occur due to the obstruction of the sebaceous glands with sebum, natural oil and dead skin cells. The natural commensal bacterium Propionibacterium acnes can cause inflammation and inflammatory lesions such as pustules or nodules and papules infected in the dermis around the microcomedo or comedone, which cause redness, scarring or hyperpigmentation.

Table 1: Factors Responsible for Acne Development [1]

Medications	Drugs that causes acne are Phenytoin, Isoniazid, Phenobarbital, Lithium, Ethionamide, Steroids, Azathioprine, Quinine and Rifampin
Hormonal	Menstrual cycles and puberty may causes acne because of increase in androgens level and increased sebum production
Psychological	Studies shows that increased stress levels are associated with increased acne severity
Genetic	There is a tendency for acne to run in families, and specific genetic mutations may increase your risk of developing acne
Infectious	P. acnes are anaerobic bacterium species that mainly causes acne. However Staphylococcus aureus has also been discovered to play an important role
Diet	Certain dietary factors, including skim milk and carbohydrate-rich foods such as bread, bagels and chips may worsen acne. Chocolate has long been suspected of making acne worse

Acne is classified according to predominance of specific skin lesion.

- Comedonal acne: Presence of open and closed comedones but usually no inflammatory papules or nodules.

- Mild acne: Presence of comedones and a few papulopustules.
- Moderate acne: Presence of comedones, inflammatory papules, and pustules; a greater number of lesions are present than in milder inflammatory acne.
- Nodulocystic acne: Presence of comedones, inflammatory lesions, and large nodules greater than 5 mm in diameter; scarring is often evident.

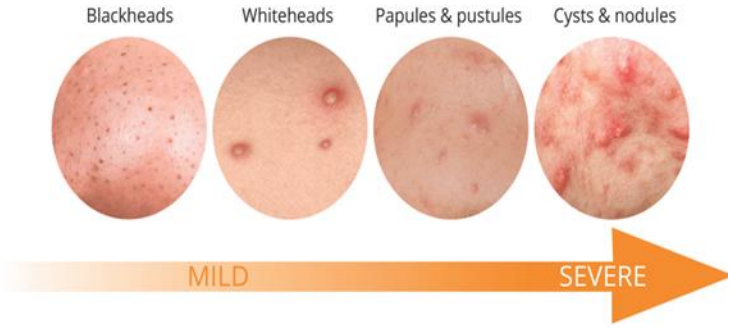


Figure 1: Phases of Acne

Thus, acne is classified as the lesions in comedonica (level I), papular- pustular (level II) and nodular (levels III, IV and V) and in severity in mild, moderate, moderate to severe, severe and serious (Table 1). The lesions are located predominantly on the face, neck, chest and back, places with more follicle

Table 2: Clinical Classification of Acne

z	**Types of Acne**	**Injury Type**	**Degree**	**Gravity**
Non Inflammatory	Comedonica	Blackhead	I	Mild
Inflammatory	Papular-Pustular	Pimple Pustules	II	Moderate
	Nodular	Nodule	III	Moderate to Severe
		Cysts	IV or conglobata Severe Scar	Severe
		Scar	V or fulminant	Serious

Various available treatments include drugs like benzoyl peroxide, clindamycin, salicylic acid, adapalene etc. But because of their serious side effects such as increased resistance towards bacteria, dryness, skin irritation, burning etc. herbal remedies is replacing them. [2]

Among the alternate system of medicine, the herbal topical therapeutic agents are gaining more attention because of its convenience and lesser side effects. Vitex negundo, commonly known as Nirgundi and also known as Chinese chaste tree and horseshoe vitex, is native to tropical Eastern and Southern Africa and Asia. Studies have reported that fruits, flowers and leaves posses antibacterial activity found against E. coli, P. aeruginosa and S. aureus.[3]

Hibiscus rosa-sinensis known colloquially as china rose is a bushy, evergreen shrub or small tree bearing flower. Reports have confirmed the presence of flavonoids, tannins and triterpenoids responsible for its antibacterial effects. Tea Tree oil has also been added for its additional antimicrobial properties. Thus, in the present study anti acne cream is formulated using extracts of *Vitex negundo* and *Hibiscus rosa-sinensis.* [4]

MATERIALS AND METHOD:

Collection and authentication:

The leaves of *Vitex negundo*, flowers of *Hibiscus Rosa sinesis* and tea tree oil were purchased from local market and authenticated by Dr. Narendra Vyas, Department of Pharmacognosy, Modern Institute of Pharmaceutical Sciences, Indore (HERB/MIPS/2018/0031). All the other ingredients were of analytical grades.

Preparation of extracts:

The leaves of *Vitex negundo* and flowers of *Rosa sinensis* were air dried and powder was used for extraction.

- **For *Vitex negundo* leaves extract:-**

  50 gm of dried powder was taken and used for extraction using soxhlet apparatus with 150 ml of ethanol and was run upto 4 days. The ethanolic extract was collected and evaporated to get dried extract.(19)

- **For *Rosa sinensis* extract:-**

  50gm of dried powder was taken and macerated with 150 ml of hydroalcoholic mixture and was run upto7days. Then the filtrate was collected and evaporated to get dried powder.

PHYTOCHEMICAL SCREENING: [4]

Detection of Saponin:

Foam Test: Small quantity of extract was shaken with 2ml of water. If foam production persists for 10 min, it indicated the presence of saponins.

Detection of Carbohydrate:

Benedict's test: Extract was boiled with small amount of benedict solution, if color changes from blue to yellow and finally to red, it indicated the presence of carbohydrate.

Detection of Tannins:

Galactic Test: 1% gelatin solution containing sodium chloride was added in formulation, white precipitation indicated the presence of tannins.

Detection of Flavonoids:

Aluminum Chloride Test: The filtrates were shaken with 1 ml of 1% aluminum chloride solution. The light yellow color indicated the presence of flavonoid. On addition of dilute NaOH and HCl, the yellow solution turned to colorless confirming the presence of flavonoids.

Detection of Alkaloids:

Mayer's Test: To 2-3 ml of extract, few drops of Mayer's reagent were added by side of the test tube and white yellowish precipitate was produced indicating the test as positive. It varies in the precipitation from a neutral or slightly acidic solution.

Formulation of Herbal Anti Acne Cream[5]:

- The composition of anti-acne cream is shown in Table.
- The formulation was prepared as water in oil emulsion using extracts of *Vitex Negundo* leaves & *Hibiscus Rosa Sinensis* flower.
- The oil soluble components stearic acid, tea tree oil and others such as cetyl alcohol, lanolin wax liquid paraffin and vitamin E were mixed to form oil phase (Part A).
- The phase was prepared by dissolving the components in the beaker on water bath at a temperature of 80°C.
- The water soluble components glycerin, triethanolamine and extracts in different proportions were dissolved in the purified water to form Part B by heating on water bath at same temperature up to 80ºC.
- Water phase (Part B) was then mixed in oil phase with constant stirring until cream was formed.
- Preservative (methyl paraben) was then added with continuous trituration.

Table 3: Composition of Anti Acne Cream (100 gm)

S.NO.	Ingredients	F1 (%)	F2 (%)
1	*Vitex negundo*	1%	2%
2	*Hibiscus Rosa sinesis*	2%	1%
3	Tea Tree oil	2%	2%
4	Stearic acid	10%	10%
5	Cetyl alcohol	4%	4%
6	Lanolin wax	4%	4%
7	Liquid paraffin	5%	5%
8	Glycerin	5%	5%
9	Methyl paraben	0.05%	0.05%
10	Thiethanolamine	0.05%	0.05%
11	Vitamin E	0.5%	0.5%
12	Purified water	Up to 100%	Up to 100%

EVALUATION OF FORMULATION:

The following parameters were assessed to evaluate the prepared anti acne cream:

General appearance:

The general appearance included color, odor and pearlescence evaluation, which was checked by visual inspection.

pH measurement:

The pH of various formulations was determined by Digital pH meter. One gram of each formulation (cream) was dissolved in 100 ml of distilled water (i.e. 1% aqueous solution) and pH was measured. The measurement of pH of each formulation was done three time and average values were mentioned.

Homogeneity:

Homogeneity and texture were tested by pressing a small amount of formulation between thumb and finger.

Grittiness:

The preparations were subjected to immediate skin feel by applying it on skin so as to test the presence of any gritty particles.

Spreadability:

Spreadability may be expressed by the extent of the area to which the topical application spreads when applied to the affected parts on the skin. The therapeutic efficiency of the formulation also depends upon its spreading value.

The spreadability was determined by taking about 3gm sample and applying it between two glass slides. The slides were pressed together to obtain a film of uniform thickness by placing 1000 gm weight for 5 minutes. Thereafter a weight (10gm) was added to the pan and the top plate was subjected to pull with the help of string attached to the hook. The time in which the upper glass slide moves over the lower plate to cover a distance of 10 cm is noted.

The spreadability (S) can be calculated using the formula

$$S = M \times L / T$$

Where, S= Spreadability, M = Weight in the pan (tied to the upper slide), L = Length glass slide, T = Time (in sec.) taken to separate the slides.

The determinations were carried out in triplicate and the average of three readings was recorded.

Type of smear:

The type of film or smear formed on the skin after application of cream was checked.

Emolliency:

Emolliency, slipperiness and amount of residue left after the application of fixed amount of cream was observed.

Skin Irritancy test:

Area of 1sq.cm was marked on the left hand dorsal surface. The cream was applied to the specified area and time for occurrence of any sensitivity or reaction was noted. Development of irritancy, erythema, and edema was checked if any for regular intervals up to 24 hrs and reported.

Stability Study:

The stability study was carried out by storing the anti acne cream in air tight container at three different temperatures which are 8ºC, 27ºC and 40ºC for 2 month. The preparations were evaluated after prescribed duration for color, homogeneity and pH.

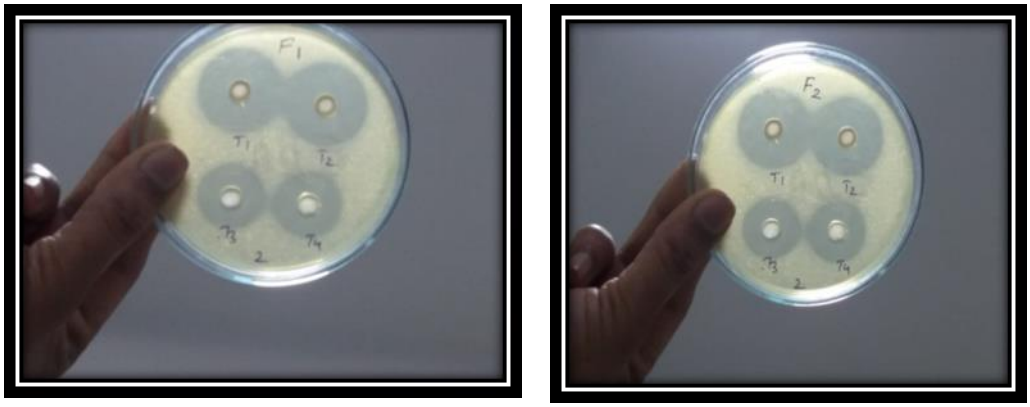
ANTI-BACTERIAL TESTING:

Microorganism:

The gram positive bacterial strain of *Staphylococcus aureus* was selected for study and obtained from the Department of Microbiology,(Modern Laboratory Pvt Ltd. Sanwer Road, Indore). The test organisms were further subculture at 37ºC for 24 hours. The culture of bacteria were maintained in their appropriated ager slant at 4ºC throughout the study and used as stock cultures**.**

Well - diffusion Method:

The nutrient media selected for study was Nutrient Agar (Hi media)**.** The formulations were subjected to antibacterial activity using agar well diffusion method. Fucidin cream was used as standard. Plates were sterilized in hot air oven at 160ºC for 2 hrs and were used for preparation of plates. The bacterial suspension was spread uniformly on the solid agar using cotton swabs and plates were incubated at 37^{o}C for 24 hrs for bacterial growth. A sterile borer of 8mm was then used to make wells of equidistance in each of the plates. The samples prepared with different concentration of formulated creams (10 and 100mg/ml) were introduced into wells and plates were incubated at 37ºCfor 24 hours. The antibacterial activity was evaluated by measuring the zone of inhibition (in mm) and was expressed as Mean ± Standard Error Mean.



F1 **F2**

Figure 2: Antibacterial Activity of Anti-acne Formulations

RESULTS AND DISCUSSION

The formulations were prepared from leaves of *Vitex negundo* and flowers of Hibiscus rosasinesis. The results of phytochemical screening confirmed the presence of active constituents responsible for anti bacterial activity.

The herbal anti-acne cream was prepared and evaluated on various physicochemical parameters and the results are shown in table. Both the preparations gave satisfactory results on all the defined parameters. Results concluded that Formulation 1 showed much greater anti bacterial effect, hence was chosen for stability study.

After two months of stability study, the tested physicochemical parameters were maintained and results of study showed no change in appearance of cream during the accelerated stability studies at temperatures 8°C

The type of smear formed on the skin was not greasy after the application of both creams. The creams were easy to remove after application by washing with water. The formulations were able to produce uniform distribution of extracts in the cream. This was confirmed by visual examination and by touch. There were no changes in term of colour of the cream even it was kept for a long period of time. After feel test showed that the creams were emollient and slipperiness in incubator for 8 weeks. The results of accelerated stability test showed that there were no any changes in the color of the cream.

PHYTOCHEMICAL SCREENING

Table 4: Phytochemical Screening of *Vitex negundo, Hibiscus rosa-sinensis* and Tea Tree Oil

Chemical Test	*Vitex negundo*	*Hibiscus rosa-sinensis*	*Tea Tree Oil*
Saponins	+	-	+
Carbohydrate	+	+	-
Tannins	+	+	+

Flavonoids	+	+	+
Alkaloids	-	+	+

EVALUATION OF PREAPRED FORMULATIONS

Table 5: Physicochemical Evaluation of Formulated Anti-acne Cream

Parameters	Results	
	Formulation 1	Formulation 2
Color	Light brown	Light brown
Odor	Pleasant	Pleasant
Pearlescence	Present	Present
pH	6.0±0.03	6.6±0.05
Homogeneity	Smooth and consistent	Smooth and consistent
Grittiness	Non gritty	Non gritty
Spreadability(g-cm/sec)	4.03	3.28
Type of smear	Non greasy	Non greasy
Emolliency	No residue left	No residue left
Skin irritation	Non irritating	Non irritating

Table 6: Anti Bacterial Activity of Anti-acne Formulation

Concentration (mg/ml)	Zone of Inhibition (mm)	
	F1	F2
10	4.83±0.75	3.15±1.54
100	15.12±1.02	14.95±0.23
Positive Control	16.08±0.17	15.78±0.61

Table 7: Stability Study of Formulation F1

Parameters	Before Stability Study	After Stability Study		
		8ºC	27ºC	40ºC
Color	Light brown	Light brown	Light brown	Light brown
Homogeneity	Smooth and consistent	Smooth and consistent	Smooth and consistent	Smooth and consistent
pH	6.0±0.03	6.1±0.54	6.2±0.23	6.4±0.12

The present study was undertaken to develop herbal formulation in the management of acne caused by bacterial infection. The extract of *Vitex negundo* and *Rosa sinesis* leaves had earlier scientifically proven for its antibacterial activity. Preliminary phytochemical screening of ethanolic extract of *Vitex nigundo* and hydroalcoholic extract of *Rosa sinesis* showed the presence of constitutent's flavonoids and tannins that are presponsible gives anti-bacterial activity.

Herbal cream has been developed containing extract of *Vitex nigundo* and *Rosa sinesis* leaves. The developed cream was evaluated using various physicochemical parameters. The pH measurement of the formulation ranges from 6.4 to 6.9, which lie in the normal pH range of the human skin. Spreadability values showed that formulation spread with an ease and the type of

smear is non greasy. From the stability studies, creams showed no changes in color, appearance and homogeneity.

The results of antibacterial activity revealed that between the two prepared formulations containing extract of *Vitex nigundo* and *Rosa sinesis* leaves, F1 exhibited more significant antibacterial activity when compared with standard (Fucidin cream).

CONCLUSION:

Anti acne formulation that are being widely used are synthetic and are associated with many side effects such as microbial resistance etc. Moreover allopathic drugs are also frequently used to treat acne vulgaris and results in adverse side effects. For this reason herbal remedies are considered as safe and their demand is increasing in global market. In the present study, the formulation is prepared with extracts of herbal plants i.e. *Vitex negundo* and *Rosa sinesis* and evaluated at various parameters. The prepared formulations showed significant effects against acne causing organism. So the study concluded that the prepared anti acne cream is effective in treating acne vulgaris and is safe for long term use. Thus, it was concluded that the antiacne gel of fruit juice of C. aurantifolia with carbopol as a gelling agent could produce the effective and stable gel of anti-acne product. Thus, it was concluded that the antiacne gel of fruit juice of C. aurantifolia with carbopol as a gelling agent could produce the effective and stable gel of anti-acne product. Thus, it was concluded that the antiacne gel of fruit juice of C. aurantifolia with carbopol as a gelling agent could produce the effective and stable gel of anti-acne product. Thus, it was concluded that the antiacne gel of fruit juice of C. aurantifolia with carbopol as a gelling agent could produce the effective and stable gel of anti-acne product.

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