

Formulation and evaluation of anti-bacterial and anti-fungal activity of poly herbal ointment containing *Allium sativum*, *Allium cepa*, *Azadirachta indica*, *Menthapiperita*

Dr.M. Senthil Kumar, Dr.S. Valarmathi*, R. Balaji, S. Aravind

*Department of Pharmaceutics, AnnaiVeilankanni's College of Pharmacy,
Chennai 600015, Tamilnadu, India.*

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ABSTRACT

Herbal medicines are also called as botanical medicines play an important role in modern medicines used to alleviate human illness and for general health, the medicinal plants are the richest source of bioactive compounds uses in traditional and modern medicines. The demand for plant based medicine is increased in both developing and developed countries, due to their non-toxicity, less side effect and easy availability at affordable prizes. The scientific evidence has brought about the possibility of utilization of herbal plant in the treatment of fungal and bacterial infections and the development of anti-bacterial and anti-fungal products. In present study, poly herbal ointment containing herbals like *Allium sativum* (Garlic), *Allium cepa* (Onion), *Azadirachta indica* (Neem), *Menthapiperita* (Peppermint) was formulated and evaluated for the study of anti-bacterial and anti-fungal activity. The evaluation is done by using cup plate method for zone of inhibition. This formulation had been then tested for its physicochemical properties like pH, loss on drying, spreadability, extrudability, diffusion study, solubility, non-irritant and washability given satisfactory results. This poly herbal ointment can be used in the treatment of rashes, burns, wounds and other fungal infections like superficial mycoses.

Key words: *Allium sativum*, *Allium cepa*, *Azadirachta indica*, *Menthapiperita*, Anti-bacterial and anti-fungal activity, ointment.

I. INTRODUCTION

Herbal plants are the oldest source of pharmacologically active compounds and have provided human kind with medicinally useful compounds from centuries, refers to the use of any plants morphology for medicinal purpose. Long practiced outside of conventional medicine, herbalism is becoming more mainstream as up-to-

date analysis and research show their value in the treatment and prevention of disease. Recently, the World Health Organization estimate that 90% people worldwide rely on herbal medicines for some aspect of their primary healthcare. Along with other dosage forms, herbal drugs are also formulated in form of ointment. An ointment is a viscous semisolid preparation used topically on variety of body surface. Ointments are used topically for several purpose, e.g. as protectants, antiseptic, emollients, etc.

Numerous studies have been conducted with the various plants, screening anti-fungal and anti-bacterial activity as well as discovery of new, antibacterial and antifungal compounds. *Allium sativum* (family-Amaryllidaceae) is bulbous flowering plant known as garlic, it contain large number of sulfur compounds and these compounds being a powerful antibiotic and antifungal activity in a low μM range against Gram-positive and negative bacteria also effective against acid fast bacteria such as mycobacterium tuberculosis and *H.pylori*, that cause of gastric ulcers. *Allium cepa* (family- Amaryllidaceae) known as onion, it contains allium thiosulfinate compounds it possess several biological properties, such as antibacterial, antifungal, antimicrobial, antimutagenic, antioxidant activities. *Azadirachta indica* (family-Meliaceae) known as Neem is well known for medicinal properties. Its leaves possess broad spectrum of activity against Gram- positive and negative bacteria including *M.tuberculosis*, *Vibrio cholera*. *Menthapiperita* (family- Lamiaceae) known as peppermint it exhibits antimicrobial, antiviral, and antifungal activities against various types of bacteria and yeasts. The antimicrobial activity of peppermint are mainly due to the combined effects of major compounds such as I-menthol, menthone, menthyl acetate, and limonene. This poly herbal ointment can be used in the

treatment of rashes, burns, wounds and other skin infections like superficial mycoses.

II. MATERIALS AND METHOD

Collection of materials:

Garlic, Onion, Neem, Peppermint fine powders were purchased on online website Amazon. All other chemicals used were of analytical grade and purchased from Merck Ltd. India.

Method of preparation of herbal ointment:

a) All the dried herbs were finely powdered in electronic mixer and then passed through a sieve(no.120). The powder should be very fine or else there will be a problem in the binding.

b) Initially ointment base was prepared by weighing accurately grated hard paraffin which was placed in evaporating dish on water bath. After melting of hard paraffin remaining wool fat, cetostearyl alcohol, white soft paraffin added and stirred gently to aid melting and mixing homogeneously followed by cooling of ointment base.

c) Herbal ointment was prepared by mixing accurately weighed herbal fine powders with Glycerin used as levigation agent, then added to the ointment base by levigation method to prepare a smooth paste with 2 or 3 times its weight of base. Gradually incorporating more base until to form homogenous ointment. Finally transferred in a suitable container.

Table: 1 Formulation of ointment base

S.NO	Ingredients	Quantity to be taken
1	Wool fat	0.5 gm
2	Cetostearyl alcohol	0.5 gm
3	Hard paraffin	0.5 gm
4	White soft paraffin	8.5 gm

Table: 2 Formulation of ointments

S.No	Ingredients	Formulation			
		F1	F2	F3	F4
1	Garlic powder	1 gm	-	-	0.2 gm
2	Onion powder	-	1 gm	-	0.2 gm
3	Neem powder	-	-	1 gm	0.2 gm
4	Peppermint powder	0.2 gm	0.2 gm	0.2 gm	0.2 gm
5	Ointment base	9 gm	9 gm	9 gm	9 gm

Table: 3 Excipients used in the formulation

S.NO	Ingredients	Uses
1	Wool fat	Emollient
2	Cetostearyl alcohol	Emulsion stabilizer
3	Hard paraffin	Lubricant
4	White soft paraffin	Moisturiser
5	Glycerin	Levigating agent and emollient

EVALUATION

Determination of Colour and Odour:

Physical parameters like colour and odour were examined by the visual examination.

Determination of Consistency:

Consistency of the formulated product was tested for the smoothness and greediness

Determination of thepH:

pH of prepared herbal ointment was measured by using digital pH meter. The solution of ointment was prepared by using 100ml distilled water and set aside for 24hrs. pH was determined in triplicate for the solution and average value was calculated.

Determination of Spreadability:

The spreadability was determined by placing excess of sample in between two slides which was compressed to uniform thickness by placing a definite time. The time required to separate the two slides was measured as spreadability. Lesser the time taken for separation of two slides results better spreadability. Spreadability was calculated by following formula

$$S = m \times \frac{1}{t} \quad (1)$$

Where,

S = Spreadability

M = Weight tide to upper slide

L = Length of glass slide

T = Time taken to separate the slides

Determination of Extrudability:

The herbal ointment of 5 gm filled in collapsible tube. The extrudability was determined in terms of weight of ointment required to extrude 0.5cm of ribbon of ointment in 10 seconds.

Determination of presence of foreign particles:

To observe the presence of foreign particle, spread small amount of ointment on clean glass slide and

observe the sample against diffused light to check presence of foreign particles.

Determination of homogeneity:

Homogeneity can be checked by centrifugation techniques. Centrifuge the ointment using centrifuge machine and suitable speed. The rate of phase separation can be observed by visual appearance.

Diffusion study:

The diffusion study is carried out by preparing nutrient agar medium. A hole bored at the center of the agar medium and then herbal ointment was placed in it. The time taken by the ointment to get diffused through agar nutrient medium was noted after 60 minutes.

Determination of Loss on drying:

Loss on drying was determined by placing 0.5 gm formulation in petri-dish on water bath 30 minutes and dried

$$\%LOD = \frac{\text{wt. of moisture content in sample}}{\text{(Total wt. of sample)} \times 100}$$

Determination of Solubility:

The solubility of the ointment was checked using different solvents like water, alcohol, ether and chloroform.

Determination of Washability:

Herbal ointment was applied on the skin and easy washing with water was checked.

Accelerated stability testing:

Perform accelerated stability testing of ointment for 7 days at room temperature. Then, keep the formulation at 40°C ± 1°C for 1 month. During the period, withdraw the sample periodically every week and test for all evaluation parameters.

Non irritancy test:

Non irritancy test were taken from randomly 4 volunteers and requested to evaluate the all 4 (F1) (F2) (F3) (F4) formulations.

Anti-bacterial and Anti-fungal activity:

Determination of zone of inhibition:

Anti-microbial activity was checked by agar diffusion method. The culture were grown in nutrient broth and incubated at 37°C, for 24 hrs. After incubation periods was over, 0.1 ml of culture was seeded in 25 ml molten nutrient agar butts, mixed and poured into sterile petri plates and allowed to solidify. The well was bored with 6 mm borer in seeded agar. 0.1 g of each ointment sample was added in each well. Plates were kept at 10°C as a period of pre diffusion for 30 minutes. After it normalized to room temperature; the plates were incubated at 37°C for 24 hrs in case of bacteria and at 27°C for 48 hrs in case of fungi. After incubation period was over, the zone of inhibition was measured with help of Hi-antibiotic zone reader.

Determination of Stability study:

Physical stability test of the poly herbal ointment was carried out for four weeks at various

temperature conditions like 2°C, 25°C and 37°C. The herbal ointment was found to be physically

III. RESULT AND DISCUSSION:

The present study was done to prepare and evaluate the poly herbal ointment. For this the herbal powders were prepared by using simple grinding and sieving process to obtain a good yield of raw material and there was no any harm to the chemical activity

The levigation method was used to prepare ointment so that the uniform mixing of the herbal powders with the ointment base was occurred which was stable during the storage.

The physicochemical parameters were studied which shows satisfactory results and also the poly herbal ointment was placed for a stability study at different temperature conditions for four weeks and observed.

Table:4 Physicochemical evaluation of formulated poly herbal ointment

Physicochemical parameters	Observation			
	F1	F2	F3	F4
Colour	Light green	Light green	Light green	Light green
Odour	Characteristic	Characteristic	Characteristic	Characteristic
Consistency	Smooth and no greediness was observed.	Smooth and no greediness was observed.	Smooth and no greediness was observed.	Smooth and no greediness was observed.
Spreadability (seconds)	8	7.8	7.9	7.6
Extrudability (cm)	0.4	0.4	0.2	0.3
Presence of foreign particles	No presence	No presence	No presence	No presence
Homogeneity	Smooth, opaque and greasy on application	Smooth, opaque and greasy on application	Smooth, opaque and greasy on application	Smooth, opaque and greasy on application
Diffusion study	0.5 cm	0.4 cm	0.6 cm	0.4 cm
LOD	7%	7%	9%	6%
Solubility	Soluble in boiling water, slightly soluble in alcohol and miscible with ether and chloroform	Soluble in boiling water, slightly soluble in alcohol and miscible with ether and chloroform	Soluble in boiling water, slightly soluble in alcohol and miscible with ether and chloroform	Soluble in boiling water, slightly soluble in alcohol and miscible with ether and chloroform

Washability	Good	Good	Good	Good
Non-irritancy test	Non-irritant	Non-irritant	Non-irritant	Non-irritant

Table: 5 Determination of zone of inhibition:

SI.NO	Ointment	Zone of inhibition(mm)			
		E.coli	S.aureus	A.niger	P.notatum
1	Garlic & peppermint	20	17	23	24
2	Onion & peppermint	20	15	20	20
3	Neem & peppermint	20	22	22	30
4	Poly herbal (Mixture)	20	22	23	28

Table: 6 Determination of Stability study of poly herbal ointment for one month

SI.NO	Parameters	Accelerated stability condition	1 st Week	2 nd Week	3 rd Week	4 th Week	Result
1	Odour	40°C ± 1°C	Characteristic	Characteristic	Characteristic	Characteristic	Stable
2	Colour	40°C ± 1°C	Light green	Light green	Light green	Light green	Stable
3	Consistency	40°C ± 1°C	Smooth	Smooth	Smooth	Smooth	Stable
4	pH	40°C ± 1°C	5.3	5.2	5.4	5.4	Stable
5	Spreadability (seconds)	40°C ± 1°C	7.6	7.4	7.8	7.5	Stable
6	Extrudability (cm)	40°C ± 1°C	0.3	0.4	0.4	0.4	Stable
7	Presence of foreign particles	40°C ± 1°C	No presence	No presence	No presence	No presence	No Presence of foreign particles
8	Homogeneity	40°C ± 1°C	Smooth, opaque and	Smooth, opaque and	Smooth, opaque and	Smooth, opaque and greasy on	Homogenous

			greasy on application	greasy on application	greasy on application	application	
9	Diffusion study	40°C ± 1°C	0.4 cm	0.4 cm	0.5 cm	0.3 cm	Stable
10	LOD	40°C ± 1°C	6%	6%	6.2%	6.2%	Stable
11	Solubility	40°C ± 1°C	Soluble in boiling water, slightly soluble in alcohol and miscible with ether and chloroform	Soluble in boiling water, slightly soluble in alcohol and miscible with ether and chloroform	Soluble in boiling water, slightly soluble in alcohol and miscible with ether and chloroform	Soluble in boiling water, slightly soluble in alcohol and miscible with ether and chloroform	Stable
12	Washability	40°C ± 1°C	Good	Good	Good	Good	Stable
13	Non-irritancy test	40°C ± 1°C	Non-irritant	Non-irritant	Non-irritant	Non-irritant	Stable

IV. CONCLUSION

The antibacterial and antifungal herbal ointment was prepared by using herbs exhibits broad spectrum antibacterial and antifungal activity against the tested micro-organisms. Among all four formulations, F4 poly herbal formulation ointment shows better antibacterial and antifungal activity against tested micro-organisms. The results of different physical and chemical tests of F4 ointment showed that it best to use topically in order to protect against skin infections caused by fungus and bacteria. Herbal medicines are less toxic and minimal side effects. Herbs exhibits antibacterial properties against wide range of gram-positive and gram-negative bacteria and antifungal medication, also known as anti mycotic medication, is a pharmaceutical fungicide or fungistatic used treat and prevent mycosis caused by fungus.

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CONFLICT OF INTEREST:

There are no conflict of interest.

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