

**FORMULATION AND EVALUATION OF HERBAL OINTMENT FOR
ANTIMICROBIAL ACTIVITY**Aravinda Nalla*¹ and Krishna Mohan Chinnala²¹Assistant Professor, Department of Pharmaceutics, School of Pharmacy, Nalla Narasimha Reddy Education Society's Group of Institutions, Hyderabad, Telangana, India.²Department of Pharmaceutics, School of Pharmacy, Nalla Narasimha Reddy Education Society's Group of Institutions, Hyderabad, Telangana, India.***Corresponding Author: Aravinda Nalla**

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ABSTRACT

The present work is to formulate and evaluate the ointment of garlic bulb extract for anti-microbial activity. The benzene extract was prepared by Soxhalation method. The ointment base was prepared and four formulations of ointments were done by incorporating the extract in the base by levigation method. All the formulations were evaluated for their physicochemical parameters like colour, odour, pH, spreadability, extrudability, consistency, diffusion study, solubility, washability. Also the formulation was evaluated for its stability at various temperature conditions which shows no change in the irritancy, spreadability and diffusion study. From four ointments, F4 was found to be the best formulation as it shows 98% drug release within 6hours, drug content 98.8% and it shows more zone of inhibition against Bacillus as compared to other three formulations.

KEYWORDS: The present work is to formulate and evaluate the ointment.**INTRODUCTION**

Garlic (*Allium sativum* L.) is one of those plants that were seriously investigated over several years and used for centuries to fight infectious diseases.^[1] The taxonomic position of garlic and related genera had been a matter of controversy for long period of time. The most recent classification scheme of garlic was class Liliopsida, subclass Liliidae, superorder Liliianae, order Amaryllidales, family Alliaceae, subfamily Allioideae, tribe Allieae and genus *Allium* which is mainly based on the sequences of nuclear ribosomal DNA.^[2]

Allium sativum, family Alliaceae has been reported to possess anti-oxidant, anti-microbial, anti-tumour, anti-mutagenic, anti-inflammatory, antiviral and antiulcer properties.^[3] Garlic and its extracts have been used to treat infections for thousands of years.^[4] Allicin (the name being derived from that of the garlic species *Allium sativum*) is considered to be the main biologically active antimicrobial phytochemical produced in garlic extracts, and was first recognized as such in 1944.^[5]

MATERIALS AND MATERIALS**Materials****Collection of Plant Material**

Garlic bulbs were collected from the local general store, Narapally, Hyderabad. Wool fat, cetostearyl alcohol,

hard paraffin and white soft paraffin were purchased from SD fine chem limited.

Methods**Extraction process- Soxhlet apparatus**

Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent (water, ethanol, benzene), and the impurity is insoluble in that solvent. If the desired compound has a high solubility in a solvent then a simple filtration can be used to separate the compound from the insoluble substance. Normally a solid material containing some of the desired compound is placed inside a thimble made from thick filter paper, which is loaded into the main chamber of the Soxhlet extractor. The Soxhlet extractor is placed onto a flask containing the extraction solvent. The Soxhlet is then equipped with a condenser. The solvent is heated (55°C) to reflux. The solvent vapour travels up a distillation arm and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapour cools, and drips back down into the chamber housing the solid material. The chamber containing the solid material slowly fills with warm solvent. Some of the desired compound will then dissolve in the warm solvent. When the Soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm, with the solvent running back down the distillation flask. This cycle may be allowed to repeat many times two days

during each cycle; a portion of the non-volatile compound dissolves in the solvent. After many cycles the desired compound is concentrated in the distillation flask. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled. After extraction the solvent is removed, typically by means of a rotary evaporator, yielding the extract compound. The non soluble portion of the extracted solid remains in the thimble, and is usually discarded.

Analytical Method Development for Garlic extract

Preparation of Phosphate buffer pH7.4: Dissolve 2.38 g of disodium hydrogen phosphate, 0.19 g of potassium dihydrogen phosphate and 8 gm of NaCl in sufficient distilled water to produce 1000ml buffer.

Determination of absorption maxima (λ_{max}) for garlic extract

1ml of stock solution B was taken in 10ml volumetric flask and volume was made up with solutions of pH 7.4. Concentration of 10 μ g/ml solution in PBS was prepared and scanned on a double beam spectrophotometer against Phosphate buffer pH7.4 as a blank in the absorbance range from 200-400nm. An absorption maxima (λ_{max}) of 220 nm was obtained. This λ_{max} was selected for preparation of standard curve of garlic extract in Phosphate buffer pH 7.4.

Preparation of stock solutions of the garlic extract

a) Stock solution A: Accurately weighed 100mg of garlic extract was taken in 100ml volumetric flask and volume was made up to 100 ml with phosphate buffer pH 7.4 to get a concentration of 1000 μ g/ml.

b) Stock solution B: From stock solution A, 10 ml was taken in 100ml volumetric flask and volume was made up to 100 ml with phosphate buffer pH 7.4 to get a concentration of 100 μ g/ml.

Preparation of Standard Curve for garlic extract

Standard curve in Phosphate Buffer pH 7.4

From the stock solution B-100 μ g/ml, take aliquots of 1,2,3,4 and 5ml solution and dilute up to 10ml to obtain concentrations from 10 to 50 μ g/ml with Phosphate Buffer pH 7.4. Then determine the absorbance at λ_{max} 220 nm against phosphate buffer pH 7.4 as blank. Repeat the experiment three times and plot a calibration curve from the mean value.

Preparation of ointment base

Table 1: Formulation of ointment base.

S. No.	Name of Ingredient	Quantity to be taken
1.	Wool fat	0.5g
2.	Cetostearyl alcohol	0.5g
3.	Hard paraffin	0.5g
4.	White soft paraffin	8.5g

Table 2: Formulation of Herbal ointment.

Formulation code	Prepared garlic extract (g)	Ointment base q.s. (g)
F1	0.5	10
F2	1	10
F3	1.5	10
F4	1.75	10

Procedure for preparation of herbal ointment^[6]

a) Initially prepare the ointment base by weighing accurately grated hard paraffin which is to be place in evaporating dish on water bath. After melting of hard paraffin add remaining ingredients and stir gently to aid melting and mixing homogeneously followed by cooling of ointment base.

b) Prepare the herbal ointment by mixing accurately weighed garlic extract 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 homogeneous ointment, finally transferred in a suitable container.

RESULTS AND DISCUSSION

Determination of absorption maxima

Maximum absorbance of the garlic extract was found to be 0.63 at 220 nm.

Table 3: Absorption maxima values.

Wave length (nm)	Absorbance
200	0.4772
210	0.1762
220	0.6369
230	0.4205
240	0.3621
250	0.3113
260	0.2659
270	0.2092
280	0.1969
290	0.1700
300	0.1606
310	0.1457
320	0.1328
330	0.1179
340	0.1090
350	0.0985
360	0.0901
370	0.0796
380	0.0426
390	0.0425
400	0.0434

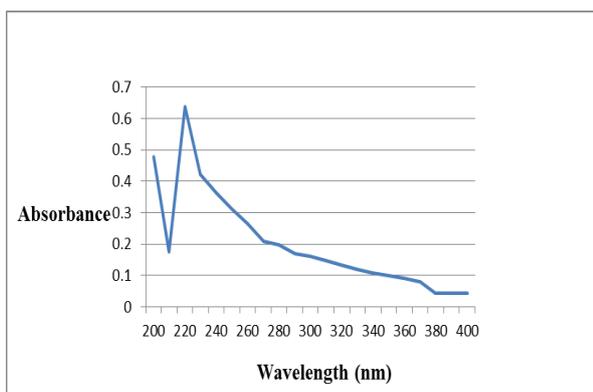


Figure 10: Absorption maxima of garlic.

Table 4: Standard curve in Phosphate Buffer pH 7.4.

Concentration ($\mu\text{g/ml}$)	Absorbance (nm)
10	0.19
20	0.42
30	0.63
40	0.83
50	0.98

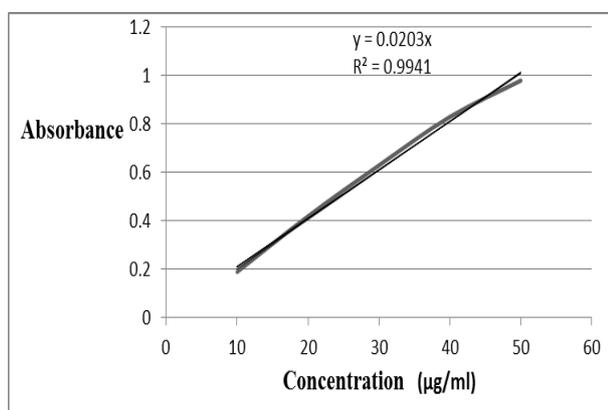


Figure 11: Standard curve in Phosphate Buffer pH 7.4.

Physicochemical parameters^[7,8]

Colour and Odour

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

Consistency

Smooth and no greediness was observed.

pH

pH of prepared herbal ointment was measured by using digital pH meter. The solution of ointment was prepared by using 100ml of distilled water and set aside for 2hrs. pH was determined in triplicate for the solution and average value was calculated. It was found to be 7.2.

Spreadability

The spreadability was determined by placing excess of sample in between two slides which was compressed to uniform thickness by placing a definite weight for 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:

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

Where

S= Spreadability

M= Weight tide to the upper slide

L= Length of glass slide

T= Time taken to separate the slides

It was found to be 5 seconds.

Extrudability

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

Diffusion study

In vitro drug release studies of samples were carried out by using Modified Franz diffusion cell. Dialysis membrane previously soaked in pH 7.4 phosphate buffer was taken and placed in between donor and receptor compartments. In the donor compartment 10mg of formulation was added. Volume of the diffusion medium was maintained 25 ml in receptor compartment and temperature maintained at $34 \pm 0.5^\circ\text{C}$, and rpm was maintained at 25 by using hot plate magnetic stirrer. Aliquots were withdrawn at intervals of 15, 30, 45, 1hr....up to 6hours and replaced by equal volumes of diffusion medium. Aliquots were suitably diluted with pH 7.4 and analyzed by UV Spectrophotometer at 220 nm. F4 shows 98% of drug release within 6 hours.^[9]

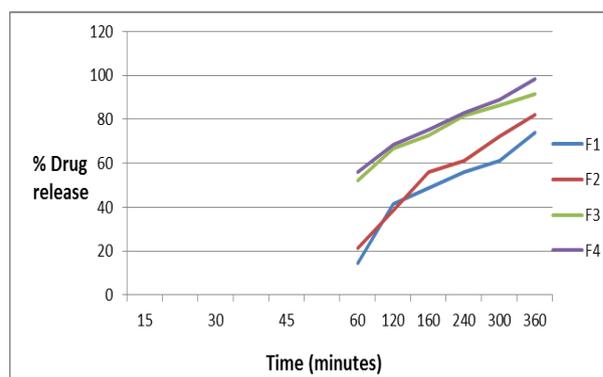


Figure12: % Drug release of F1, F2, F3, F4.

Drug Content

10mg of the ointment was taken and dissolved in distilled water. Then absorbance was measured at 220nm using UV-Visible spectrophotometer. Drug content of F4 was found to be 98%.

Table 5: Drug Content.

Formulation Code	% Drug Content
F1	90.31
F2	93.52
F3	95.84
F4	98.86

LOD

LOD was determined by placing the formulation in petri-dish on water bath and dried for the temperature 105^oC. It was found to be 20%.

Solubility

Soluble in water, alcohol and chloroform.

Washability

Formulation was applied on the skin and then ease extend of washing with water was checked.

Non irritancy Test

Prepared herbal ointment was applied to the skin of human being and observed for the effect.

Antimicrobial activity

Required quantity of agar was prepared and microorganism (*Bacillus*) was inoculated into it. Then agar solution was poured into the petri plates and allowed to stand for few minutes to get solidified. After solidification, required size of bores were made using a boarer. After that the prepared ointments (of different concentration) were filled into it. The whole procedure was carried out in aseptic laminar air flow chamber. Now the petri plates were placed into incubator and allow it for growth of micro organism for 24hrs. After 24hrs, zone of inhibition was checked to determine the antimicrobial activity of prepared ointment.

Table 6: Zone of inhibition of F1, F2, F3 and F4.

Formulation code	Zone of inhibition against <i>Bacillus</i>
F1	1cm
F2	1.5cm
F3	1.8cm
F4	2cm

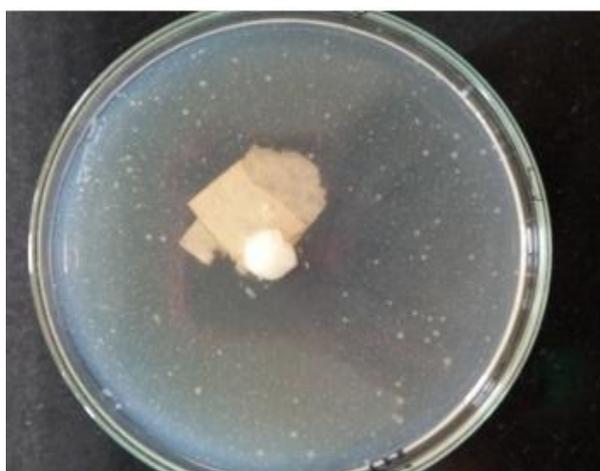


Figure 13: Zone of inhibition of F1.

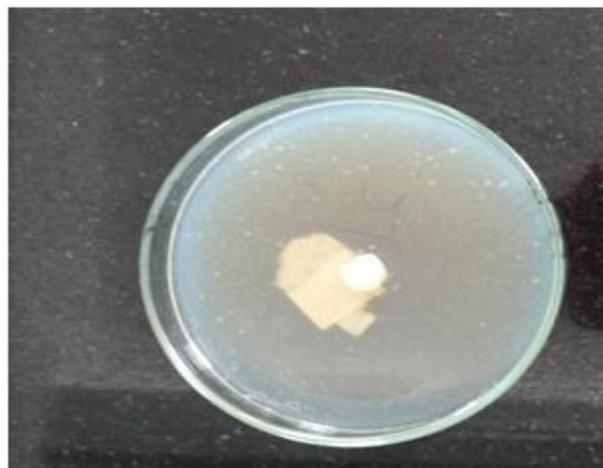


Figure 14: Zone of inhibition of F2.

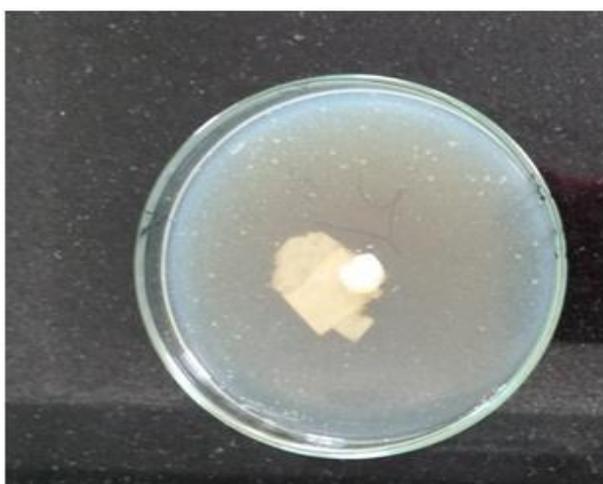


Figure 15: Zone of inhibition of F3.

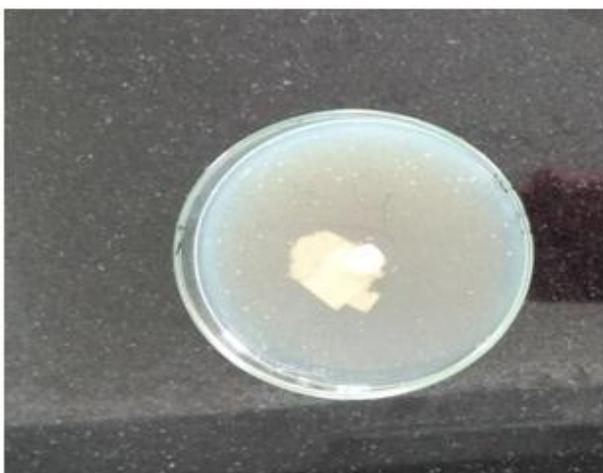


Figure 16: Zone of inhibition of F4.

Stability study

Physical stability test of the herbal ointment was carried out for four weeks at various temperature conditions like 2^oC, 25^oC. The herbal ointment was found to be physically stable at different temperature i.e. 2^oC, 25^oC and 35^oC within four weeks.

Table 7: Physicochemical parameters.

Physicochemical parameter	Observation
Colour	Pale white
Odour	Characteristic
Consistency	Smooth
pH	7.2
Spreadability (seconds)	5 seconds
Extrudability	0.5 g
Diffusion study (after 6 hours)	98%
Loss on drying	20%
Solubility	Soluble in water, alcohol and chloroform.
Washability	Good
Non irritancy	Non irritant
Stability study	Stable at 2 ⁰ C, 25 ⁰ C and 35 ⁰ C

SUMMARY

The present study was done to prepare and evaluate the herbal ointment. For this the herbal extracts were prepared by using Soxhlet extraction process to obtain a good yield of extract and there was no harm to the chemical constituents and their activity.

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

The physicochemical properties were studied which shows satisfactory results for spreadability, extrudability, washability, solubility, loss on drying and others. Also the formulation was placed for a stability study at different temperature conditions like 2⁰C, 25⁰C and 37⁰C within four weeks. There were no changes observed in spreading ability, diffusion study as well as irritant effect. From the above results, F4 was found to be the best formulation as it shows 98% drug release within 6hours, drug content 98.8% and it shows more zone of inhibition against Bacillus as compared to other three formulations.

CONCLUSION

From the ancient times garlic was using to treat cardiovascular diseases, viral diseases, hypertension, wounds, diabetic, cancer and atherosclerosis. It is also having anti-oxidant activity, anti-microbial activity. The present experimental study showed that it is possible to develop and evaluate garlic ointment with benzene extract of garlic bulbs for anti-microbial activity.

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