



Formulation and Evaluation of Garlic Ointment and its Anti-Bacterial Activity against *Escherichia coli* and *Bacillus subtilis*

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ABSTRACT

Garlic (*Allium sativum* L. Fam. Liliaceae) has been used as medicine in ancient times and has long been known to have antibacterial, anti-fungal and anti-viral properties. The aim of present study to formulate the garlic ointment and evaluate its anti-microbial activity. The formulations were prepared by fusion method and evaluated for its physio chemical parameters like colour, odour, pH, spreadability, irritancy, stability study. The *in vitro* anti-bacterial activity of the garlic ointment was determined against *Escherichia coli* and *Bacillus subtilis* using the Muller hinton agar by cup plate method. This study shows the garlic ointment has greater anti-bacterial activity when compared to other synthetic ointment.

Keywords: Anti-bacterial activity, garlic ointment, *Escherichia coli* and *Bacillus subtilis*.

INTRODUCTION

The increasing mortality rate of infectious diseases is one of the most challenging public health problems faced by different countries worldwide. Numerous synthetic antibiotic agents have been used for the management of infectious diseases. Medicinal plants have been used for many years in the treatment of a vast number of human diseases by the community, specifically in traditional medicine. They are considered the main source of new natural and safe drugs to be utilizing in managing diseases as an effective and harmless alternative medicine¹. Garlic has been approved to reduce the risk of cancer, boosting the immune system and protecting against inflammation as well as infectious agent. It is the most important preventive remedy, a universal folk spice and food. In the past, garlic has been utilized as a remedy during the various diseases such as typhus, dysentery, cholera, and influenza. Garlic is one of those plants that were prevent blood clotting, and contains anti-tumor properties. It can also boost the immune system and maintain health. It can help to prevent some forms of cancer, heart disease, strokes and viral infections^{2,3}.

Garlic extract inhibits the growth of Gram positive and Gram negative bacteria, such as *Staphylococcus*, *Streptococcus*, *Micrococcus*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Lactobacillus*, *Pseudomonas*, *Shigella*, *Salmonella*, *Proteus*, and *Helicobacter pylori*^{4,5}. Its antibacterial activity is mainly due to the presence of allicin produced by the enzymatic activity of allinase on alliin. Allicin also makes garlic smell.

MATERIALS AND METHODS

Collection of Plant Material

Garlic bulbs were collected from the local market in madurai. Wool fat, cetostearyl alcohol, hard paraffin and

white soft paraffin were purchased from SD fine chem limited.

Method of Extraction

The 60g of shade dried and grinded bulb of garlic was filled a soxlet apparatus and poured water over a bulb. Then allow the extraction for about 72 hours, after extraction completed, the collected aqueous extract that should be removed using distillation. After distillation the garlic powder was collected for further experimental process⁶.

Preparation of garlic ointment

Table 1: Ointment formula

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
5	Garlic powder	0.1 mg

Initially ointment base was prepared by weighing accurately grated hard paraffin which was placed in china dish on water bath. After melting of hard paraffin remaining ingredients were added and stirred gently to aid melting and mixing homogenously followed by cooling of ointment base. To this added accurately weighed garlic powder aqueous extract to the ointment base by fusion method to form a smooth paste⁷.

Evaluation of garlic ointment⁸

Organoleptic Characters:

The garlic ointment was formulated and evaluated for its organoleptic characters (color, state, odour). The



appearance of the garlic ointment was analyzed by its color and roughness visually and by touch.

PH determination

The pH of the formulations was determined using digital pH meter. About 0.5 g of the ointment was weighed and dissolved in 50 ml of DMSO, the measurement of pH of formulation was done in triplicate and average values were calculated.

Spreadability

Spreadability of the ointment was done by using two sets of glass slides of standard dimensions. The garlic ointment formulation (3 g) was placed over one of the slides; the other slide was placed on the top of the ointment, such that the ointment was sandwiched between the two slides. Weight was placed upon the upper slides so that the ointment between the two slides was pressed uniformly to form a thin layer.

Skin Irritancy test:

This test was performed on 3 albino rats and weighing between 150 – 200 g. The animal skin was shaved and cleaned. After cleaning the animal skin, the 30 mg of garlic ointment was applied. Aqueous solution of 0.8% formalin was used as standard irritant. The animals were observed for 7 days for any sign of odema and erythema.

Accelerated stability studies

An accelerated stability study was performed on the formulation by maintaining at room temperature for 20 days with constant time interval. During the stability studies the parameters pH and physical changes were studied.

In vitro Anti-bacterial activity by using cup-plate method:

The Anti-bacterial activity of Garlic ointment (garlic ointment diluted by di methyl sulphoxide) diluted solution was carried out by cup-plate method by using test organism *Escherichia coli* and *Bacillus subtilis*. The sterilized Muller hinton agar plates were inoculated using spread plate technique in cup plate method. The experimental solution and standard solution were added to the petridish. The concentration of a diluted ointment and standard solution contain 50µl. the plates were placed in an incubator at 37°C for 48hrs⁹.

Table 2: Physicochemical parameters of garlic ointment formulation

S. No	Physicochemical parameters	Observation
1	Colour	Light brown
2	Odour	Pungent odour
3	pH	6.4± SD
4	Spreadability study	30 mg .cm/sec
5	Skin irritancy test	No irritancy
6	Stability study	Stable with pH 6.4 and 6.3

Measurement of Zone of Inhibition

After incubation period is over, the diameter of zone of inhibition was measured in Millimeter side of the ruler. The measurement was taken with ruler from the center of cup to edge of zone of inhibition. The diameter of zone of inhibition was determined by multiplying radius of zone of inhibition with 2. Finally, we get the diameter of zone of inhibition^{10, 11}.

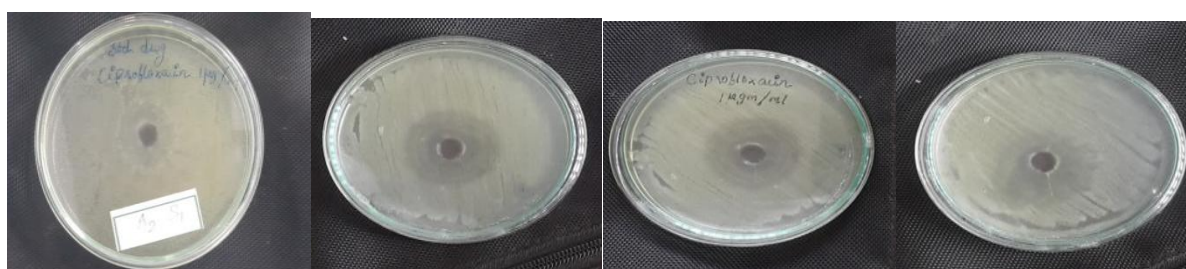


Figure 1

Figure 2

Figure 3

Figure 4

Figure 1: *Escherichia coli* against ciprofloxacin (0.1g) solution (50µl) (standard plate).

Figure 2: *Escherichia coli* against garlic ointment solution (50µl) (experimental plate).

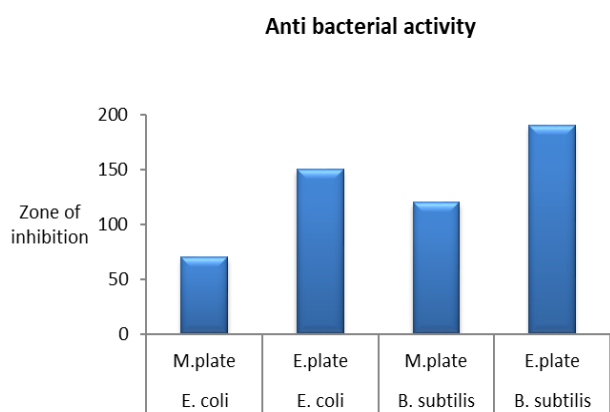
Figure 3: *Bacillus subtilis* against ciprofloxacin (0.1g) solution (50µl) (standard plate).

Figure 4: *Bacillus subtilis* against garlic ointment solution (50µl) (experimental plate).

Table 3: Measurement of zone of inhibition

Micro organism		Zone of inhibition (diameter/mm)
<i>Escherichia coli</i>	M.plate	70
	E.plate	150
<i>Bacillus subtilis</i>	M.plate	120
	E.plate	190

M.plate indicate Marketed plate, E.plate indicate experiment plate



Graph 1: Anti-bacterial activity

RESULTS & DISCUSSION

Preparation of garlic ointment

The ointment from garlic prepared by using the fusion method. The prepared ointment filled into the tube and stored at room temperature.

Evaluation of garlic ointment

The evaluation of ointment such as organoleptic parameters, spreadability, pH and stability studies are shown in Table 2.

In vitro Anti-bacterial activity:

Prepared formulation has shown good activity against both *Bacillus subtilis* and *Escherichia coli*. It was found that the formulation of garlic ointment was showing more zone of inhibition. The result of anti-bacterial activity of extracts reported in figure 2 and figure 4. The garlic ointment has high antibacterial potency and good skin penetration. Finally, garlic ointment against *Bacillus subtilis* shows significantly greater Anti-bacterial activity than *Escherichia coli*.

CONCLUSION

According to obtained results, garlic has potential to be used for topical antibacterial ointment can be a suitable alternative in medicine for prevention and treatment of many bacterial disease. The garlic ointment is required so that better, safe and cost-effective drugs for treating *Escherichia coli* and *Bacillus subtilis* causing diseases. This study shows that garlic ointment has high potential as antibacterial activity. When formulated as ointment for topical use and could therefore explain the successes claimed in the folk use of the plant in the treatment of common skin conditions. In experimental plate compared to the marketed plate against *Escherichia coli* and *Bacillus subtilis*, the experimental plate against *Bacillus subtilis*

shows significantly greater Anti-bacterial activity. The future work is being extended to perform the various activities.

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