

Formulation and Optimisation of *Allium sativum* Anti-microbial ointment and Effect of ointment towards *E. Coli* and *Staphylococcus aureus*

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Date of Submission: 01-05-2025

Date of Acceptance: 10-05-2025

ABSTRACT: Anti-microbial ointment kills effectively and heals the wound by inhibits the growth of microorganism and bacteria that caused by any injuries. The optimum formulation was selected by after many evaluation tests of organoleptic characterization, homogeneity, p^H, melting point, loss on drying, diffusion, extrudability. *Allium sativum* and *Zingiber officinalis* were used in the formulations by single and combinedly with higher and lower quantities. Four different formulations were prepared on that ASO-2 was shows the optimum values. The ASO-2 formulation contains *Allium sativum* was used as an anti-microbial agent in this research work.

KEY WORDS: Anti-microbial agent, *Allium sativum*

I. INTRODUCTION

The skin is a relatively inhospitable environment for the growth of most pathogenic microorganisms. The hostility of the skin environment is attributed to relative dryness of cutaneous surface that provides in-sufficient amount of moisture that could support significant growth of pathogens and colonization with resident microflora that produces metabolites that are inhibitory to the growth of invading pathogens (Abbas et al., 2016). Eradication of resident flora greatly enhances the survival of *Staphylococcus aureus* and the subsequent development of infection. Skin infections may be either primary or secondary. Primary infections have characteristic morphologies and are initiated by single organisms, and usually occur in normal skin. Secondary infections originate in diseased by inflammation, burn or exfoliation, absorption is further increased (Adu et al., 2022). Ointments are the semi solid preparations used for external application to the skin. It consists of medicament or mixture of medicaments dispersed in a suitable anhydrous

base. Ointments are used as protectants, emollients or lubricants for physical effects (Michael E. Aulton et al., 2013), (B.M. MITHAL, 1997). Anti-microbial is an agent that destroys, or prevents the growth of microorganisms. It including antibiotics, antivirals, antifungals, and antiparasitic are medicines used to prevent and treat skin infections caused by bacteria and reduces the discomfort of various skin conditions (Andrewset al., 2001). Anti-microbial agents are categorised into 2 types based on their effect on bacteria. One type of antibiotic is Bactericidal that kills the bacteria, another type of antibiotic is Bacteriostatic that prevents the growth of bacteria. Mechanism of action antimicrobial agents: a)

Inhibition of cell wall synthesis (penicillins, cephalosporins, vancomycin) b) Inhibition of functions of cellulose membrane (polymyocins) c) Inhibition of protein synthesis. (Chloramphenicol, macrolides & clindamycin tetracyclines & aminoglycosides) d) Inhibition of nucleic acid synthesis. (Quindines & rifampicin) e) Inhibition of folic acid synthesis (sulphonamide & trimethoprim) (Ma Yet et al., 2016), (Dr. G. GANDHI MATHI et al., 2020). Tetracyclines are a class of antibiotics that treat several kinds of bacterial infections. Tetracyclines are octahedra naphthalene derivatives which are bacteriostatic and broad-spectrum antibiotics (Bezruket et al., 2017). Tetracyclines are directly derived from a bacterium known as *Streptomyces* genus of Actinobacteria. It inhibits protein synthesis by blocking the attachment of charged aminoacyl - tRNA to the A site on the ribosome. Tetracycline binds to the 30s subunit of microbial ribosomes. Thus, it prevents introduction of new amino acids to the nascent peptide chain. The action is usually inhibitory and reversible upon withdrawal of the drug. Tetracycline is used in topical infections, acne treatment, wound care, burn care, eye and ear infections, skin conditions and

preventing infections (Chandler et al., 2010). Garlic (*Allium sativum*) has a greater antimicrobial compound. Allicin is a characteristic sulphur-containing compound found in raw garlic produced from Allin and exhibits antimicrobial activity against both Gram-positive and Gram-negative bacteria. It has an inhibitory effect on bacterial biofilm formation. Allicin is unstable and tends to be converted into various compounds such as ajoene's and diallyl polysulfides, which have been reported to exhibit antimicrobial activity (Nakamoto et al., 2020). Ginger (*Zingiber officinale*) is a herb with thick tuberous rhizomes. Antimicrobial activity of the oil against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Candida albicans*, *Trichoderma* spp, *Aspergillus niger*, *Penicillium* spp and *Saccharomyces cerevisiae*. Ginger mostly acts upon anti-inflammatory, antibiotic, radiotherapy and chemotherapy (Islam et al., 2014). Tea tree oil (*Melaleuca alternifolia*) is also used as essential oil. Tea tree oil has anti-septic and anti-inflammatory actions. It is used as active

ingredient in many topical formulations and also used to treat cutaneous infections. The crushed leaves of tea trees were inhaled to treat cough and cold and sprinkled on wounds. Tea tree leaves were soaked to make an infusion to treat sore throats. Broad spectrum activity of tea tree oil includes Anti-bacterial, Anti-fungal, Anti-viral and Anti-protozoal activities. Tea tree oil can kill the fungus on the skin. It is mainly used as a flavouring agent to overcome the pungent odour of the formulations. It is used in many ways such as skin care, hair care, nail fungus, wound healing, acne, athlete's foot etc (Ninomiya et al., 2013).

II. MATERIALS AND METHODS

Bees wax, hard paraffin, ceto steryl alcohol, white soft paraffin, beef extract, peptone, NaCl, agar, distilled water were purchased from Yucca enterprises, Mumbai by St. Ann's college of pharmacy, garlic, ginger were purchased from local market and tea tree oil from super market.

Formulation of Anti-Microbial Ointment:

Table no.1: Formulation composition of Anti-Microbial ointment

Ingredients	Formulations			
	ASO-1	ASO-2	ASO-3	ASO-4
White bees wax	0.74	0.74	0.74	0.74
Hard paraffin	0.74	0.74	0.74	0.74
Ceto steryl alcohol	1.25	1.25	1.25	1.25
White soft paraffin	23.88	23.88	23.88	22.88
Oil of <i>Allium sativum</i>	1	2	-	2
Oil of <i>Zingiber officinalis</i>	1	-	2	1
Tetracycline	0.9	0.9	0.9	0.9
Tea tree oil	0.49	0.49	0.49	0.49

Total weight of Ointment: 30gr

Preparation of oils:

Take the fresh garlic and ginger cut into small pieces. Now crush the pieces with the help of mortar and pestle. Mix the coconut oil to ginger and olive oil to garlic. Boil the mixtures until the crushed pieces turn into brown colour. Filter the oils and store the oil extract in a glass (or) plastic container.

formulation, add ginger oil to the ointment base and for ASO-4 formulation, add required quantities of garlic and ginger oil to the ointment base. Finally add tea tree oil and active ingredient to all the formulations. The products were stored in a neatly cleaned container with proper lid (El-Gied et al., 2015), (Krishanu S, 2021).

Preparation of anti-microbial ointment:

Melt the beeswax, hard paraffin, cetosteryl alcohol, white soft paraffin separately. Add melted ingredients one by one with continuous stirring. For ASO-1 formulation, add garlic and ginger oil to the ointment base, for ASO-2 formulation, add garlic oil to the ointment base, for ASO-3



Fig.no.1: Anti-microbial ointments

EVALUATION TESTS

Physical characterization:Examine ointment for their colour, odour, homogeneity.

p^H test:Dissolve 1gr of ointment in a 50ml of distilled water. Observe the pH of ointment using pH meter (Maru ADet al., 2019).

Melting point test: Take required amount of ointment in a China dish. Place it on a water bath. Note the melting temperature of the ointment.

Loss on drying:Weigh the required quantity of ointment in an empty petridish. Place it in a hot air oven. Weigh the petridish for every 15min until it gets constant weight.

Diffusion study:Pour the agar nutrient medium into a petridish. A hole bored at the center and place the ointment in it. Kept in a hot air oven at a

suitable temperature limit until ointment to get diffuse and note the readings.

Extrudability:Fill the ointment in an empty aluminium tube at one end and close the other end. Apply pressure on ointment tube. Collect the amount of ointment has passed out from the tube.

Stability study:The stability study is carried out for the prepared ointment at temperature of 40°C for one month (Chavan RSet al., 2023).

Anti-microbialactivity test:

Staphylococcus aureus and Escherichia coli used for the determination of the antimicrobial activity of various ointment formulations and standard cup plate methods used for the study. The nutrient agar solution poured into the previously sterilized Petri dishes up to 5 mm thickness. Each microorganism added to Petri dishes with a sterilized loop and plates were allowed to solidify for 5 min. On each Petri dish, five perforations made with a metal tube with a 4 mm diameter to receive test materials. The standard (Tetracycline ointment) and previously prepared test materials were obtained from Pharmaceuticals laboratory prepared formulations added immediately into the wells and kept for incubation at 37°C for 24 hr to allow the microorganism to grow and reagents to diffuse through the culture medium. At the end of the incubation, the zone diameter measured with the help of a zone reader. All the experiments repeated 3 times to get accurate results (Ferdous. Aet al., 202), (Grooveet al., 1955).

III. RESULTS AND DISCUSSION

Table no.2: Evaluation test results

TEST	ASO-1	ASO-2	ASO-3	ASO-4
Colour	Lemon yellow	Lemon yellow	Lemon yellow	Lemon yellow
Odour	Aromatic	Aromatic	Aromatic	Aromatic
Homogeneity	Uniform	Uniform	Uniform	Uniform
p ^H	8.5	8	9	8.5
Melting point	38°C	38°C	38°C	38°C
Loss on drying	26.30%	23.93 %	35.41 %	26.19%
Diffusion study	1.2 cm	1.5 cm	1cm	0.9 cm
Extrudability	Good	Good	Good	Good
Stability	Stable	Stable	Stable	Stable

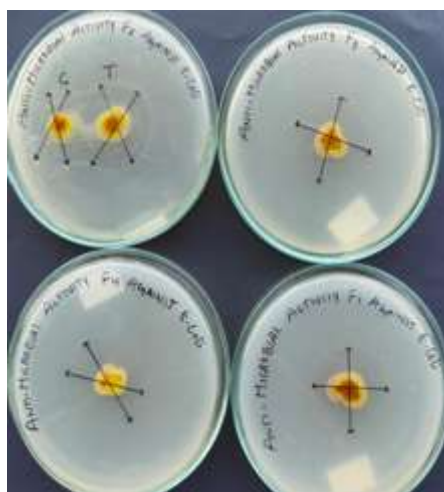


Fig.no.2: Anti-microbial activity against E.coli

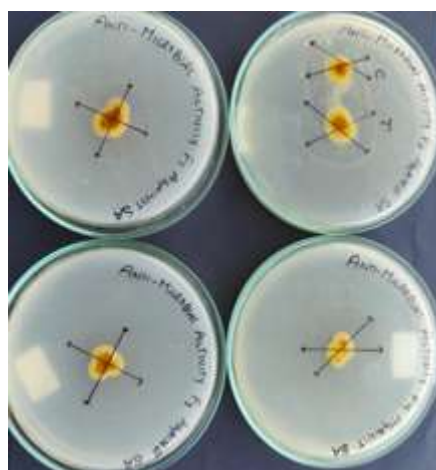


Fig.no.3: Anti-microbial activity against S.aureus

Table no.3: Antimicrobial activity of four formulation ointments

S.NO	NAME OF THE ORGANISM	Diameter				
		ASO-1	ASO-2 TEST	ASO-4 CONTROL	ASO-3	ASO-4
1.	Escherichia coli	2.45	2.8	2.2	2.7	2.5
2.	Staphylococcus aureus	2.45	2.8	2.2	2.7	2.5

The antibacterial activity of four different formulation ointments were determined by the cup plate method, and results were shown in table 3 and figures 2 & 3 respectively. Formulation ASO-2 shown highest zone of inhibition against both bacterial isolates Escherichia coli and staphylococcus aureus at different concentrations when to commercial antimicrobial ointment(control).The formulations showed

promising activity of antibacterial activity and these could be used for further studies.

DISCUSSION:

Different compositions of anti-microbial ointments were formulated by using anti-microbial properties of Allium sativum and Zingiber officinalis and pharmaceutical active ingredient. Among the four formulations ASO-2 formulation shows optimised values.8 is the p^H of optimised

formulation and P^H of remaining formulations were 8.5, 9, 8.5 of ASO-1, 3, 4 respectively. All the formulations melted at 37 °C that was tested by melting point test. The product drying capability was determined by the experiment of loss on drying. 23.93 % at least value of % loss on drying of ASO-2 formulation and the remaining formulations of % loss on drying values were 26.30, 35.41, 26.19 % respectively. The diffusability of the ointment was tested by preparing agar media and place the ointment in a bored area and the study was carried out at optimum temperature condition. By this diffusability study we confirmed ASO-2 formulation was more diffusible than other formulations. 1.5cm of ASO-2, 1.2cm of ASO-1, 1cm of ASO-3 and 0.9cm of ASO-4 formulation. The extrudability characteristic of all the formulations show the better flow. Stability studies of all the formulations were stable at 40°C were proved by stability studies up to 30 days.

IV. CONCLUSION:

In this study, various ointments were formulated with extracted ginger oil, garlic oil and tetracycline have a synergistic antibacterial activity. Anti-microbial ointment was used to heal the wounds, inhibits the growth of microorganism. During the study of antimicrobial activity of all ointment formulations were showed better antibacterial activity. It was lemon yellow colour, aromatic fragrance and very smooth texture. Garlic composed formulation shows the best results and it was selected as optimised formulation. The evaluation test results were as follows pH of ASO-2 was 8, good extrudability, 37 °C of melting point temperature, 23.93 % of Loss on drying, 1.5 cm of diffusability and stable at 40 °C respectively. And this could be a new source for various topical infections and these could be used for further studies.

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