

# FORMULATION AND EVALUATION OF HERBAL OINTMENT CONTAINING TINOSPORA CORDIFOLIA & PSIDIUM GUAJAVA AGAINST STAPHYLOCOCCUS AUREUS BACTERIA.

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## KEYWORDS

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## ABSTRACT

The present study aimed to develop and evaluate a herbal ointment containing Tinospora cordifolia and Psidium Guajava against Staphylococcus aureus. The ointment was formulated using a combination of the two plant extracts, which have been reported to possess antibacterial properties. The antibacterial activity of the ointment was evaluated using three different formulations, with Mupirocin as the standard drug. The results showed that the herbal ointment exhibited significant antibacterial activity against Staphylococcus aureus, with the most effective concentration showing a zone of inhibition of 19 mm. The study demonstrates the potential of Tinospora cordifolia and Psidium Guajava as natural antibacterial agents, and highlights the potential of the developed ointment as an alternative to conventional antibiotics.

## INTRODUCTION

Tinospora cordifolia (Giloy) is an Ayurveda climbing shrub used in Ayurveda as well as unani, belonging to the family Menispermaceae, it is commonly known as Guduchi, Heart leaved moonseeds, Amrita, Gurach. Antimicrobial activity of T. cordifolia with different solvents on different micro-organisms, showed good antifungal and antibacterial activity. It has been taken a methanolic extract of T. cordifolia against both bacterial groups. Singh et al., has reported silver nanoparticles from the stem of T. cordifolia, which possess antibacterial activity against the different strains of bacteria.

Psidium Guajava L., popularly known as guava, is a small tree belonging to the myrtle family (Myrtaceae). Aqueous and organic extracts of guava leaves have been demonstrated to have

antibacterial activity due to an inhibitory effect against antibiotic-resistant clinical isolates of Staphylococcus aureus strains.

A methanol extract exerted antibacterial effects, preventing the growth of different strains from several bacteria such as Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus spp., and Shigella spp. Four antibacterial flavonoids (morin-3-O-lyxoside, morin-3-O-arabinoside, Quercetin, and quercetin-3-O-arabinoside) of the leaf extract of Psidium Guajava are found to be effective against the pathogenic bacteria.

- **Materials:**
- **Ingredients:**

Table No .1. Name of Ingredients

Sr No	Name of Ingredients

1.	Ethanollic Extract of <i>Tinospora cordifolia</i> and <i>psidium</i> Guajava
2.	Wool fat
3	Cetosteryl Alcohol
4.	Hard paraffin
5.	Yellow Soft paraffin
6.	Ethanol

➤ **Method :- Extraction:**

Collect plant material and dry them in a shaded area. Grind the dried plant material into a fine powder using a grinder or mortar and pestle. Assemble the Soxhlet apparatus, which consists of a distillation flask, a condenser, and an extraction chamber. Ensure that all connections are secure and that the apparatus is clean and dry. Select a suitable solvent, such as methanol, ethanol, or water, depending on the desired compound to be extracted. Add the solvent to the distillation flask, ensuring that the level of the solvent is below the bottom of the condenser. Place the ground guava plant material in the extraction chamber.

Ensure that the plant material is evenly distributed and that the chamber is not overloaded. Heat the solvent in the distillation flask, causing it to evaporate and rise into the condenser. The condensed solvent will drip back into the extraction chamber, where it will extract the desired compounds from the plant material. Repeat the extraction process for several hours or overnight, depending on the desired level of extraction. The extracted compounds will be collected in the distillation flask. Remove the flask from the Soxhlet apparatus and allow the extract to cool. Concentrate the extract using a rotary evaporator or by allowing it to evaporate slowly. The resulting extract can be used for further analysis or purification.

➤ **Formulation of Ointment**

**Table No 2. Formula of ointment**

Sr.No	Name of Ingredients	Quantity to be taken		
		F1	F2	F3
1	<i>Tinospora cordifolia</i>	0.5 gm	1gm	1gm
2	<i>Psidium</i>	1 gm	0.5 gm	1Gm
3	Wool fat	0.5gm	0.5gm	0.5gm
4	Cetosteryl Alcohol	0.5 gm	0.5 gm	0.5 gm
5	Hard paraffin	0.5 gm	0.5 gm	0.5 gm
6	Yellow Soft paraffin	8.5gm	8.5gm	8.5gm
7	Wool fat	0.5gm	0.5gm	0.5gm

□ **Procedure for preparation of ointment**

- 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 ingredient's were added and stirred gently to aid melting and mixing homogeneously followed by cooling of ointment base.
- Prepare the herbal ointment by mixing accurately weighed *Tinospora cordifolia* and *Psidium* Guajava extract with two different concentrations. First concentration A is (1: 0.5) 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.

- Prepare the herbal ointment by mixing accurately weighed *Tinospora cordifolia* and *Psidium* Guajava extract with two different concentrations. Second concentration B is (0.5:1) 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.

➤ **Antibacterial Activity of Formulation:**

□ **Test Microorganisms and growth Condition:**

The microorganisms used in this study include, (*streptococcus aureus*) bacteria. These microorganism were taken from our laboratory collection. The bacteria were stored and activated on nutrient agar.

**Table No .3 Observation**

Sr.No.	Bacteria kill after 48 hours
Standard	23mm

Sample 1	16mm
Sample 2	17mm
Sample 3	19mm

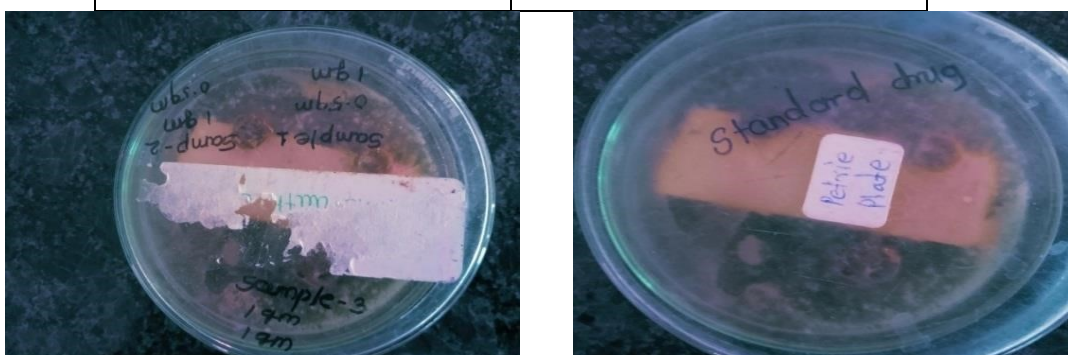
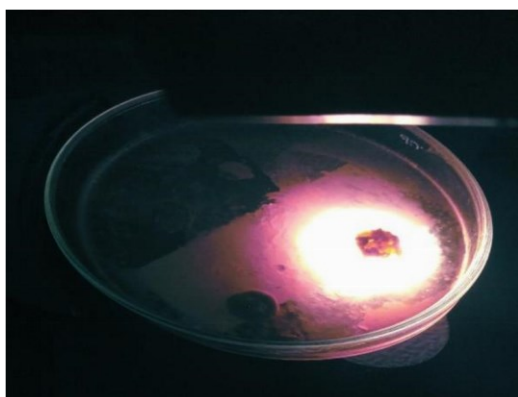
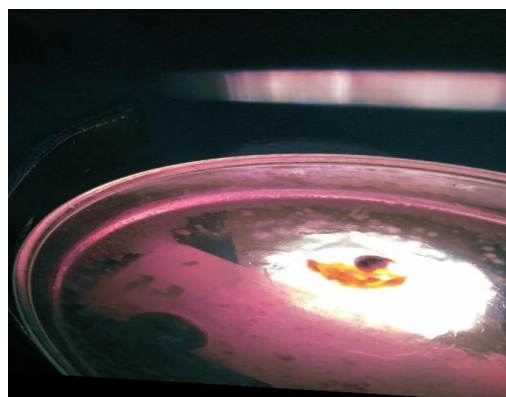


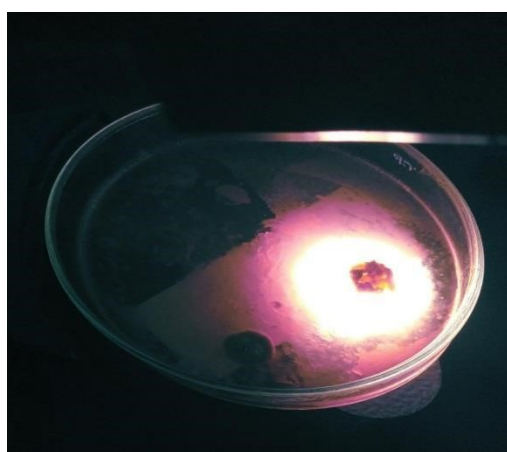
Fig No .1 Before 48 hours sample and standard



Sample 1



Sample 2



Sample 3



Standard drug mupirocin

Fig No 2 .After 48 hours Observation

## 7. RESULT:

### ➤ Phytochemical test:

Table No 4. Phytochemical Test for Hydroalcoholic Extract

Phytochemical tests/ reagents used	Hydroalcoholic Extract
Alkaloids	
1.Dragendroff's test	+++

2.Hager's test	++
3.Wagner's test	+++
<b>Physterols</b>	
Liebermann' s. burchard test	++
<b>Saponins</b>	
Foam test	++
<b>Phenolic compound and tannins</b>	
1.Ferric chloride test	+++
2.lead acetate	+++
<b>Protein and amino acid</b>	
1.Biuret test	+
2.ninhydrin test	+
<b>Flavonoids</b>	
Shinoda test	+++

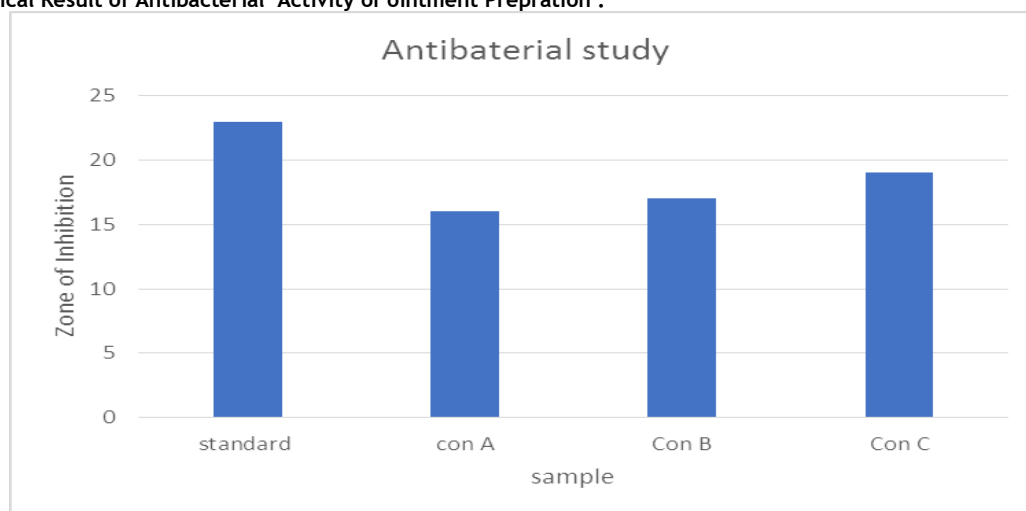
Mild :- +, Moderate:- ++, Strong:- +++

➤ **Evaluation Parameter of Ointment:**  
**Table No .5 Evaluation parameters:**

Parameter	Observation
Colour	Green
Odour	Characteristics
Consistensy	Smooth
PH	5.5
Spradiability ( Seconds)	6
Extrudability	10 cm
Loss of drying	40%
Solubility	Soluble in water , alcohol , chloroform
Washability	Good
Irritancy	Non - irritant

Stability study	Stable
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➤ Graphical Result of Antibacterial Activity of ointment Preparation :



Scale = on x axis sample , on y axis zone of inhibition

➤ Zone of inhibition:

Table No. 6 Zone of inhibition:

Sr. No.	Organism	Zone of Inhibition	
1]	Staphylococcus aureus	F1	Control
			No Inhibition
		F2	Standard
			23 mm
		F3	Sample
			16 mm
			Control
			No Inhibition
			Standard
			23 mm
			Sample
			17 mm
			Control
			No Inhibition
			Standard
			23 mm
			sample
			19 mm

Abrevisions:

Con. =Control, Sam. =Sample, Std. =Standard

## DISCUSSION

The formulated ointment was evaluated for its physical parameters, such as appearance, texture, and pH. The ointment was also evaluated for its antibacterial activity using the agar well diffusion method. The antibacterial activity of the formulated ointment was evaluated against a standard strain of bacteria. The standard sample showed a zone of inhibition of 23 mm, indicating effective antibacterial activity. The test samples, Conc.A, Conc.B, and Conc.C, showed zones of inhibition of 16 mm, 17 mm,

and 19 mm, respectively. The results of the antibacterial activity study indicate that the formulated ointment has moderate to good antibacterial activity. The zones of inhibition observed for the test samples were lower than that of the standard sample, but still significant. This suggests that the extracts of *Tinospora cordifolia* and *Psidium Guajava* have antibacterial properties, which can be attributed to the presence of bioactive compounds such as alkaloids, flavonoids, and phenolic acids.

## CONCLUSION

The present study was undertaken to formulate and evaluate an ointment using *Tinospora cordifolia* and *Psidium Guajava* for its antibacterial activity. The extracts of these plants were obtained using Soxhlet extraction and formulated into an ointment. The evaluation parameters included physical parameters, such as appearance, texture, and pH, as well as antibacterial activity. Based on the findings of this study, it can be concluded that the formulated ointment has potential as a topical application for skin infections. However, further studies are needed to optimize the formulation, evaluate its safety and efficacy in human subjects, and explore its potential as an alternative to conventional antibacterial agents.

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