

FORMULATION AND EVALUATION OF ANTIMICROBIAL ACTIVITY OF HERBAL OINTMENT OF ETHANOLIC EXTRACT OF ACALYPHA INDICALINN

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Abstract- The antimicrobial activity of ethanolic extract of dried leaves of acalypha indica linn ointment is formulated and evaluated. The preliminary invitro antimicrobial activity of the extract at various concentration and those of their ointment were determined against some microorganism suing the agar cup plate method. The minimum inhibitory concentration (MIC) was also determined by agar dilution method. The physical properties of ointment formulated with extract were evaluated using standard procedure.

Keywords: Acalypha indica, herbal ointment, antimicrobial activity, Evaluation ointment.

INTRODUCTION

Introduction Many of the plants used today were known to the people of ancient cultures throughout the world and were highly considered their preservative and medicinal powers. Scientific experiments on the antimicrobial properties of plants and their components have been documented in the late 19th century.^[1] India has a rich flora that is widely distributed throughout the country. Herbal medicines have been the basis of treatment and cure for various diseases and physiological conditions in traditional methods practiced such as Ayurveda, Unani and Siddha. Medicinal components from plants play an important role in conventional as well as western medicine. Plant derived drugs have been a part of the evolution of human, healthcare for thousands of years. Plant based drugs were commonly used in India and China.^[2] At the same time, indigenous people of the rest of the planet were also utilizing plants for curing diseases. Today, nearly 88% of the global populations turn to plant derived medicines as their first line of defense for maintaining health and combacting diseases. One hundred and nineteen secondary plant metabolites derived from plants are used globally as drugs; 15% of all angiosperms have been investigated chemically and of that 74% of pharmacologically active plant derived components were discovered. Currently, people of Asia and India are utilizing plants as part of their routine health management.^[3] Acalypha indica L. (family: Euphorbiaceae) is a weed widely distributed throughout the plains of India. It has been reported to be useful in treating pneumoniae, asthma, rheumatism and several other ailments.^[4] The dried leaves of Acalypha indica was made into a poultice to treat bedsore and wounds and the juice of Acalypha indica is added to oil or lime and used to treat a variety of skin disorders. The leaves of Acalypha grandis have also been reported to possess contraceptive activity.^[5] Several chemical and biological investigations have been carried out on this plant. In the present study, an attempt has been made to enrich the knowledge of antibacterial activity of Acalypha indica plant extract against Grampositive and Gram-negative bacteria.^[6]

MATERIALS METHODS

Collection of Plant Material

The acalypha indica linnleaves were collected from in and around Perambalur. These are authenticated by botanist, department of botany, national college, Trichy. Then the leaves cleanedproperly and shade dried at room temperature.

Preparation of Acalypha Indica Extract

Leaves of the plant were collected and washed thoroughly with distilled water and shade dried for 10 days. Dried leaves were ground into powder form. 100gm powder was imbibed with 350ml of 90% ethanol for 3hrs. and transferred to percolator with addition of 150ml of 90% ethanol for maceration for 7 days with occasional stirring. Finallyethanolic extract was collected and concentrated to get blackish green residue. The extract was stored in the airtight container at cool and dark place.^[7]



Fig No:1 (Extract of acalypha indica)



Fig No:11 (Residue of acalypha indica extract)

FORMULATION OF OINTMENT**Table 3: Formulation of Ointment Bases**

S.NO	Name of ingredients	Quantity to be taken
1	Wool fat	0.5gm
2	Cetostearyl alcohol	0.5gm
3	Hard paraffin	0.5gm
4	Yellow soft paraffin	8.5gm

Table 4: Formulation of Herbal Ointment

S.NO	Name of ingredients	Quantity to be taken		
1	Prepared acalypha indicaextract	F1	F2	F3
		0.08gm	0.10gm	0.12gm
2	Ointment base q.s	10gm	10gm	10gm

Procedure for Preparation of Herbal Ointment

- a) 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 ingredients were added and stirred gently to aid melting and mixing homogeneously followed by cooling of ointment base.
- b) Herbal ointment was prepared by mixing accurately weighed *Acalypha indica* extract to the ointment base by levigation method to prepare a smooth paste with two or three times its weight of base, gradually incorporating more base until to form homogeneous ointment, finally transferred in a suitable container.^[7]

Evaluation of Antimicrobial Activity of Herbal Ointment Microorganism and Culture Media

Bacterial cultures such as *staphylococcus aureus*, *streptococcus*, *entrecoccus faecalis*, *E.coli*, *pseudomous aeruginosa*, *Klebsilla pneumoniae*. were obtained from Eumic analytical lab and Research Institute, Tiruchirapalli. Bacterial strains were maintained on nutrient agar slants at 40C

Inoculum Preparation

Bacterial cultures were subcultured in liquid medium (nutrient broth) at 37oC for 8hours and further used for the test (105-106CFU/ml). These suspensions were prepared immediately before the test was carried out.

Preparation of culture media Nutrient agar media

Nutrient agar medium is one of the most commonly used medium for several routine bacteriological purposes:

Table 5: Formulation of Agar Medium

INGREDIENTS	Grams /litre
peptone	5gm
Beef extract	3gm

Acar	15gm
Sodium chloride	5gm
Yeast extract	1.5gm
Ph	7.0

After adding all the ingredients into the distilled water it is boiled to dissolve the medium completely and sterilized by autoclaving at 15lb psi pressure (121°C) for 15 minutes.

NUTRIENT BROTH

The nutrient broth was prepared by the same composition without agar. At the adding all the ingredients into the distilled water it is boiled to dissolve the medium completely and sterilized by autoclaving at 15 lb psi pressure (121°C) for 15 minutes.

ASSAY OF ANTIMICROBIAL ACTIVITY

Microbial inoculum preparation:

The nutrient broth was prepared, then identified bacterial colonies were inoculated into the broth culture were used for antimicrobial activity.

KIRBY BAUER AGAR WELL DIFFUSION ASSAY

The nutrient agar medium was prepared and sterilized by autoclaving at 121°C 15 lbs pressure for 15 minutes then aseptically poured the medium into the sterile petriplates and allowed to solidify the Bacterial broth culture was swabbed on each petriplates using a sterile bud. Then wells were made by well cutter. The organic solvent used for extraction of plants is used as a negative control, gentamicin is used as a positive control. The samples (two concentration 50µg & 100µg) positive and the negative controls were added to each well aseptically.

This procedure was repeated for each Petri plates then the petriplates were incubated at 37°C for 24 hrs. After incubation the plates were observed for the zone of inhibition.

8.1. Screening of Antimicrobial Activity

The prepared herbal ointment of various concentrations and alcoholic extract of *acalypha indica linnare* exhibited for antimicrobial activity against various micro organisms such as Gram- Negative bacteria, viz *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsilla pneumoniae* Gram- positive bacteria, viz *staphylococcus*, *streptococcus*, *enterococcus*. The degree of response of the test sample was different for different selected microbes. The zone of inhibition ranged from 20 to 32 (mm/ ml).

SAMPLE :A, B, C, D, E Ethanol extract

CONTROL :Gentamicin antibiotic disc

Gram negative bacteria

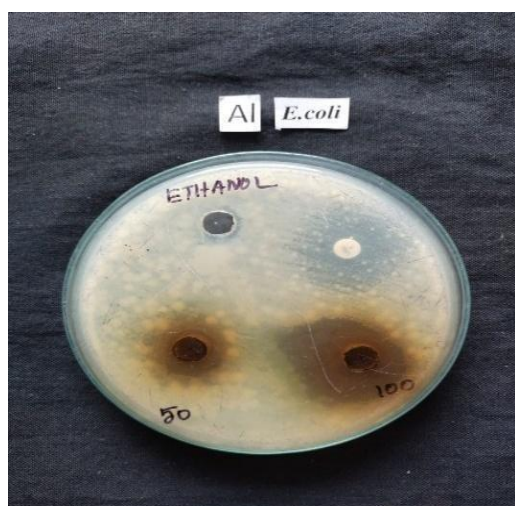
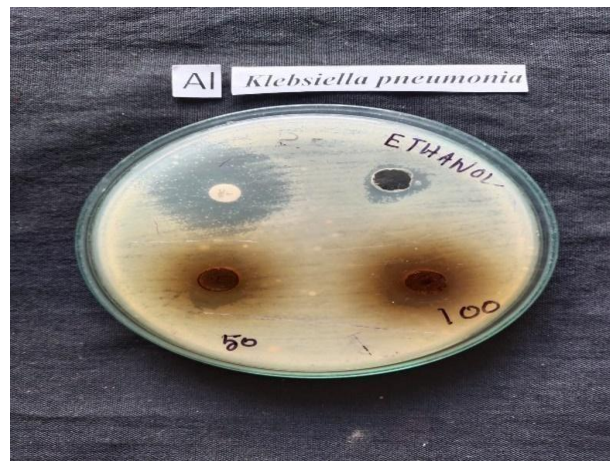


Figure:1



Figure:2

Figure:3



Gram positive bacteria

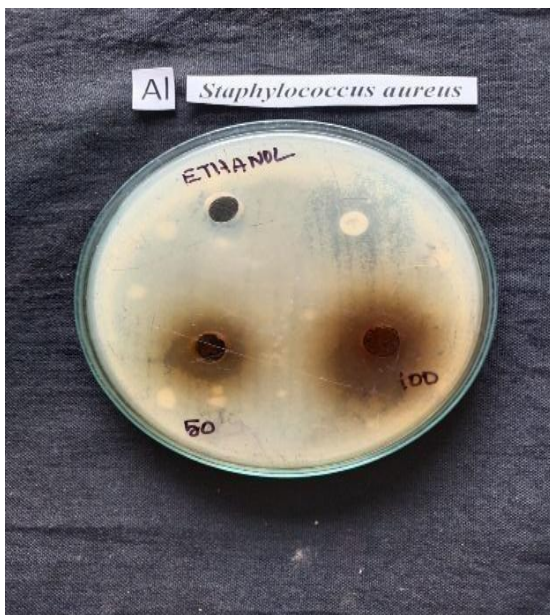


Figure:4



Figure:5

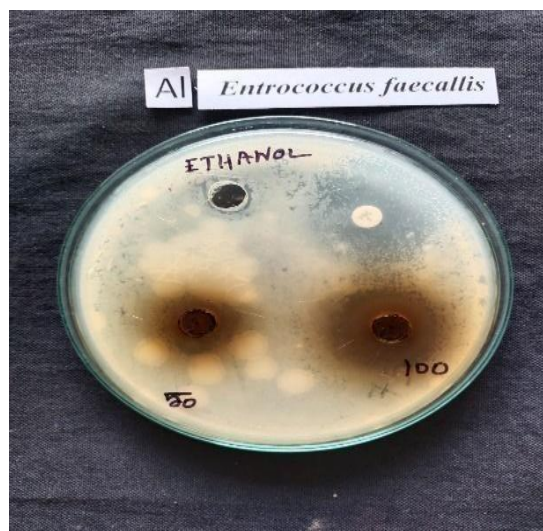


Figure:6

Zone inhibition of acalypha indica

SAMPLE	Extract 100 µl Added And Zone Of Inhibition (Mm/MI)			
	50µl	100µl	Negative Control	Positive Control
<i>Staphylococcus aureus</i>	25	35	0	25
<i>Streptococcus</i>	23	32	0	20
<i>Enterococcus faecalis</i>	22	31	0	25
<i>E.coli</i>	25	38	0	25
<i>Pseudomonas aeruginosa</i>	34	37	0	25
<i>Klebsiella pneumonia</i>	26	34	0	30

RESULT:**Screening of antimicrobial activity**

The prepared transdermal patch of various concentration and ethanolic extract of acalypha indica leaves are exhibited for anti microbial activity against various microorganism such as Gram negative bacterial, viz E.coli, Gram positive bacteria staphylococcus aureus

CONCLUSION

The study determine the good anti microbial activity of the ointment formulation containing the herbal extracts. These could make them potential anti microbial agents effective in the treatment of skin infections. The use of ethanolic extract produce a effective anti microbial property. The prepared formulation effective in bacteria. The zone of inhibition is more against bacteria agent. So the prepared ointment have better antimicrobial property.

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