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Research Article

**A COMPARATIVE STUDY OF DIFFERENT HERBAL
OINTMENT FOR ANTIMICROBIAL ACTIVITY****P.Baskar^{1a}, P.Kathirvel^{1a}, M.Kural oviya^{1a}, G.Priyadharshini^{1a*}, P.Vaishnavi^{1a}, J.karthi^{1b},
M.Deepthi^{1c}**^{1a*} B.Pharm, Pallavan Pharmacy College, Kanchipuram, Tamil Nadu, India.^{1b} Principal, Dept of Pharmacognosy, Pallavan Pharmacy College, Kanchipuram,
Tamil Nadu, India.^{1c} Assistant professor, Dept of Pharmacy Practice, Pallavan Pharmacy College, Kanchipuram,
Tamil Nadu, India.**Article Received:** January 2023**Accepted:** February 2023**Published:** March 2023**Abstract:**

*The aim of this work was to formulate four different herbal ointments with ethanolic extract of *Achyranthes aspera* linn, *Cymbopogon citaratus*, *Euphorbia hirta*, *Thyme vulgaris*; (ii) investigate the in vitro antimicrobial activity of the formulated herbs using disc diffusion method. The antibacterial screening was done against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Aspergillus niger* using sterile distilled water. Further, the physicochemical properties like colour, pH, spreadability, washability, skin irritancy, solubility, diffusion study loss of drying, and stability study were evaluated. The results showed that the ethanol extract of herbs possess activity even at low concentration and also shows maximum zone of inhibition comparable to the standard, ciprofloxacin (anti-bacterial), clotrimazole (antifungal). The solubilities and stability studies were good for all herbs.*

KEYWORDS: *Achyranthes aspera* linn, *Cymbopogon citaratus*, *Euphorbia hirta* linn, *Thyme vulgaris*, Antimicrobial activity, herbal ointment, ethanolic extract,

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INTRODUCTION:

Throughout Africa, there are more than 50,000 different species of medicinal plants, many of which are used in traditional medicine for illness prevention and treatment. Natural goods are thought to be the source of 50% of contemporary medications, and they are crucial to the process of developing new medications. Although modern medicine has made significant strides, plants continue to play a significant role in healthcare. Herbalism has been increasingly popular in recent years. Due to either poverty or a lack of access to modern treatment, the majority of the population still relies on medicinal plants for their main healthcare. Furthermore, it plays a significant role in African culture. The World Health Organization (WHO) has advocated the global authentication of therapeutic plants because it is apparent that these facts and their significance are true. Also, these serve as raw materials for the industrial production of pure chemical compounds. Research and development on medicinal plants are of interest for these and many other reasons. These well-known herbal plants include *Achyranthes aspera* linn, *Cymbopogon citaratus*, *Euphorbia hirta* linn, and *Thyme vulgaris*. *Achyranthes aspera* linn. is a species of plant belongs to the family Amaranthaceae^[1,2]. The plant is distributed as weed throughout India, tropical Asia and other parts of the world. It grows best in a fertile soil. It is erect, sometimes sprawling, long lived herb which can grow up to 2cm tall. Different parts of the plant like seeds, shoots, roots leaves are used for medicinal purpose. *Cymbopogon citaratus* is one of the best known species of the genus *Cymbopogon* with the synonyms *Andropogon nardus* subvar. It is native to asia (Indochina, Indonesia, and Malasiya) africa, but are widely cultivated in temperate and tropical regions of the world. *Cymbopogon ctiratus* is also known as west indian lemon grass or lemon grass^[8,9,10]. It comprises about 180 species. *Euphorbia* genus is one of the largest euphorbiaceae family of the "giant genera" of flowering plants with over 2000 currently recognized species. There are mostly originated from Africa or Madagascar. Ninety-one species of euphorbia grow in turkey or spurge in English are used in traditionally medicine. *Euphorbia hirta* is a small annual, branched prostrate herb with branches reaching 60cm in height, reddish or purple

with abundant latex and is hairy^[14,15,16]. The *Thymus* species of herbs, all of which are indigenous to Europe and Asia, are collectively referred to as thyme. Thyme commonly known as "common" or "garden" is the main variety that is grown for flowering and aesthetic uses in the commercial sector. The Western Mediterranean region, which includes southern Italy, is where *thyme* is indigenous. Based on these numerous claims. Four different herbal ointment's was formulated and evaluated *in vitro* to see if its antimicrobial properties are retained in the ointment formulation^[21,22]. Simple ointment (ointment base) consists of hydrocarbons (hard and soft paraffin) and absorption (wool fat and cetostearyl alcohol) bases which combine occlusive properties for longer action and emollient effect the specific objectives of this work were to: (i) Formulate four different herbal ointment with ethanolic extract of *Achyranthes aspera* linn, *Cymbopogon citaratus*, *Euphorbia hirta* linn, *Thyme vulgaris*; (ii) Investigate the *in vitro* activity of the ointment of *Achyranthes aspera* linn, *cymbopogon citaratus*, *Euphorbia hirta* linn, *Thyme vulgaris* on *B. subtilis*, *S. aureus*, *Escherichia coli* and *A. niger*^[23,24].

MATERIALS AND METHODS:**Materials**

Fresh leaves parts of *Achyranthes aspera* linn were collected from thenkodi village Tamilnadu, India. The *cymbopogon citaratus* leaves are collected from Pallavan pharamcy college, Iyyengarkulam, Kanchipuram district, Tamilnadu, India in the month of July2022. The whole plant *Euphorbia hirta* linn was collected from Thenkodi village, Chengalpattu district, Tamilnadu, India, in the month of June2022. *Thyme vulgaris* leaves are collected from Thiruvadisuam village, Chengalpattu district, Tamilnadu, India in the month of July 2022. The four different plants are authenticated by State drug testing laboratory, Chennai, Tamilnadu. The collected plant materials were shade-dried for one week and ground into fine powder using a mechanical grinder. Wool fat, hard paraffin, cetostearyl alcohol, white soft paraffin, ciprofloxacin, Ethanol, Other reagents used were of analytical grade and were used without further purification^[15,16,21].

Figure:1 collection of plant materials



Methods

Extraction

The dried leaves were pulverized into a coarse powder with the mechanical grinder. A 25 g quantity of the powder was extracted with 250 mL of ethanol using Soxhlet apparatus (continuous hot percolation). After further evaporation, the extraction process carried out 5-6 hours at $> 78^{\circ}\text{C}$ ^[22,23].

Figure :2 Extraction process



Preparation of ointments

Simple ointment was formulated according to the formula in Table 1. *Achyranthes aspera* linn, *Cymbopogon citaratus*, *Euphorbia hirta*, *Thyme vulgaris* four different ointment's was prepared by melting 9.75 g quantity of simple ointment in a china dish over hot water bath maintained at 80°C . The molten ointment was removed from the hot water bath and 0.25g each extract was added and triturated until cold. The formulation was transferred into an ointment jar and stored pending further investigations^[24,25].

Table 1: Formula for the preparation of Simple ointment

| SI.No | Name of ingredient | Quantity |
|-------|---------------------|----------|
| 1 | Wool fat | 0.5 |
| 2 | Cetostearyl alcohol | 0.5 |
| 3 | Hard paraffin | 0.5 |
| 4 | White soft paraffin | 0.5 |

Table 2: Composition of herbal ointment

| SI.No | Name of the ingredient | Quantity to be taken for (10g) |
|-------|---|--------------------------------|
| 1 | Prepared extract <i>Achyranthes aspera</i> linn | 0.25g |
| | Ointment base | 9.75g |
| 2 | Prepared extract <i>Cymbopogon citaratus</i> | 0.25g |
| | Ointment base | 9.75g |
| 3 | Prepared extract <i>Euphorbia hirta</i> linn | 0.25g |
| | Ointment base | 9.75g |
| 4 | Prepared extract <i>Thyme vulgaris</i> | 0.25g |
| | Ointment base | 9.75g |

Figure: 3 Herbal ointment preparations



Ointment spreadability test

One gram of the ointment was placed on one side of a slide and covered with a second slide. An object of known weight was placed on top of the second slide for 5 min. The second slide was allowed to slide over the first slide under gravity in a vertical position and the time taken noted. This procedure was done in triplicate and average value recorded [25].

Evaluation of pH of ointment

The pH of various formulations was determined using a Digital pH meter. One gram of the weighed formulation was dispersed in 100 mL of hot water and stay for 1 hour and the pH was determined directly, triplicate and average value is recorded.

Loss of drying:

Loss on drying was determined by placing ointment in petridish on water bath and dried for 105°C.

$$\text{Percentage Loss on Drying} = (\text{Weight - Molecular Weight}) / \text{Weight} \times 100$$

Washability:

Ointment formulations were applied on the skin and then ease extend of washing with water was checked. Washability was checked by keeping applied skin area under the tap water for about 10 mins.

Non irritancy test:

Ointment prepared was applied to the skin of human being and observed for the effect.

Diffusion study

The diffusion study was carried out by preparing agar nutrient medium of any concentration. It was poured into petridish. A hole board at the center of medium and ointment was placed in it. The time taken by

ointment to get diffused was noted. (After 60 minutes)

Solubility

Boiling water soluble, miscible with alcohol, ether, and chloroform.

Stability study

The stability studies are carried out for the prepared ointment at temperature of 37°C for 2 months.

Comparative study of different herbal ointment for antimicrobial activity**(a) Collection of microorganisms**

The antibacterial screening was done with different herbal ointment, against four bacteria isolates (*Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli*) using disc diffusion method.

(b) Antifungal screening

Active culture of the fungi made into slant was shaken vigorously to make fungal spores suspension. *A. niger* suspension was made in buffer peptone water with 24 h of incubation at 25 ± 0.5 °C. The suspension was mixed with sterile potato dextrose broth (PDB) poured into petri dishes and allowed to solidify. A sterile cork borer was then used to bore wells into the agar. Equal amount of the *different herbal ointment and ciprofloxacin* was dispensed into the agar, which was later observed for zone of inhibition [26,27].

(c) Control experiment

Control experiment was set up by using sterile distilled water in herbal ointment ciprofloxacin and clotrimazole was used as control.

The observations are tabulated**Table: 3 Antibacterial screening by disc diffusion method**

| Standard / control /Ethanol extract of herbal ointment | Diameter of Zone of inhibition(mm) | | |
|---|------------------------------------|------------------|-------------------|
| | Gram +ve | Bacteria | Gram -ve Bacteria |
| | <i>B. subtilis</i> | <i>S. aureus</i> | <i>E. coli</i> |
| Ciprofloxacin | 3.5 | 3.2 | 3 |
| control | 0.8 | 0.7 | 0.9 |
| <i>Achyranthesaspera</i> Linn | 1.7 | 2 | 2.3 |
| <i>Cymbopogoncitratu</i> s | 1.5 | 1.7 | 1.9 |
| <i>Euphorbiahirta</i> Linn | 0.1 | 0.8 | 1.1 |
| <i>Thymusvulgaris</i> | 3.3 | 3 | 2.7 |

Ethanol extract of herbal ointment

Table: 3 Antibacterial screening by disc diffusion method

| Standard / control /Ethanolic extract of herbal ointment | Diameter of Zone of inhibition(mm) | | |
|--|---------------------------------------|------------------|-------------------|
| | Gram +ve | Bacteria | Gram -ve Bacteria |
| | <i>B. subtilis</i> | <i>S. aureus</i> | <i>E. coli</i> |
| Ciprofloxacin | 3.5 | 3.2 | 3 |
| control | 0.8 | 0.7 | 0.9 |
| <i>Achyranthes aspera Linn</i> | 1.7 | 2 | 2.3 |
| <i>Cymbopogon citratius</i> | 1.5 | 1.7 | 1.9 |
| <i>Euphorbia hirta Linn</i> | 0.1 | 0.8 | 1.1 |
| <i>Thymus vulgaris</i> | 3.3 | 3 | 2.7 |

Ethanolic extract of herbal ointment

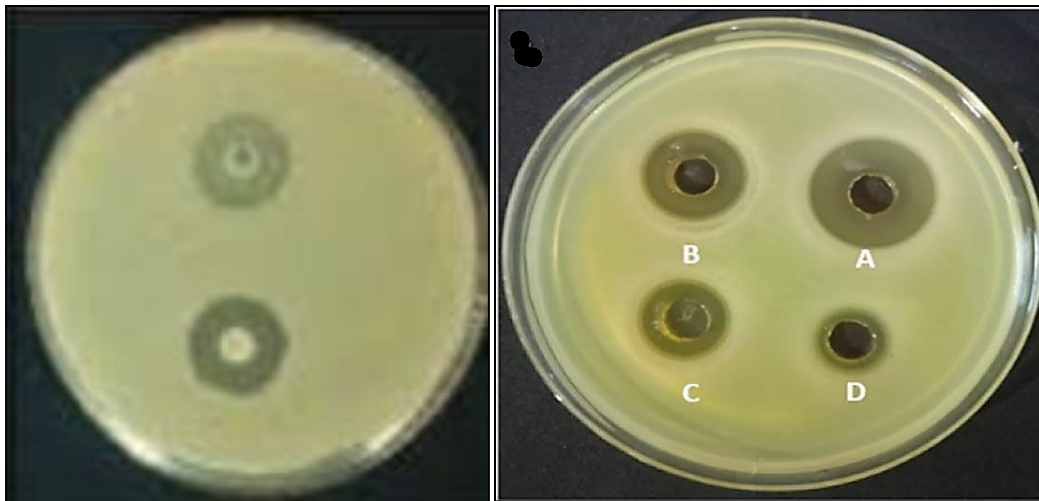
The observations are tabulated

Table: 19 Antifungal screening by disc diffusion method

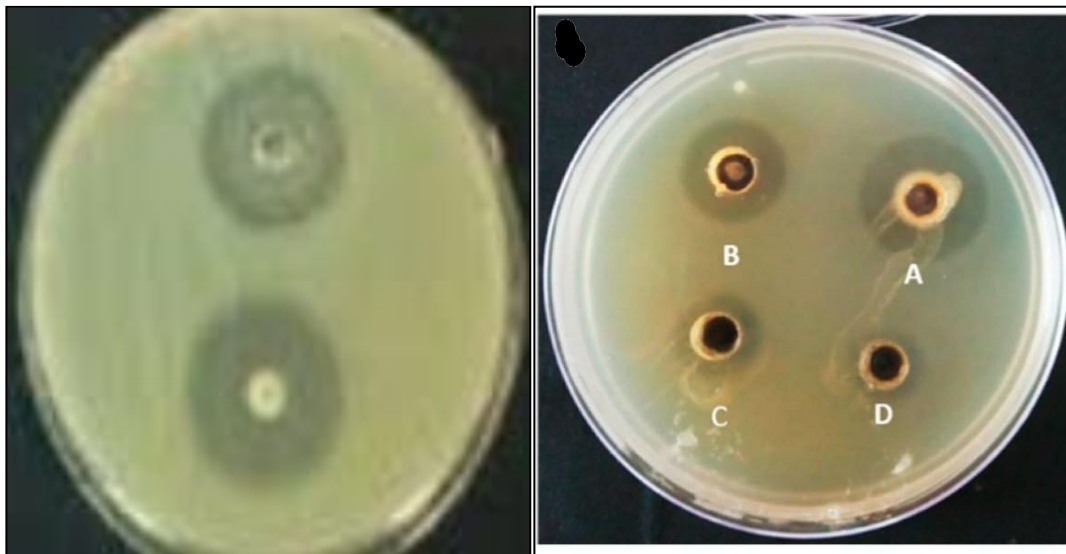
| Standard/ control/Ethanolic extract of herbal ointment | Diameter of zone of inhibition (mm) |
|---|-------------------------------------|
| | <i>A. niger</i> |
| clotrimazole | 3.5 |
| control | 0.8 |
| <i>Achyranthes aspera Linn</i> | 3 |
| <i>Cymbopogon citratius</i> | 1.5 |
| <i>Euphorbia hirta Linn</i> | 0.9 |
| <i>Thymus vulgaris</i> | 3.2 |

Ethanolic extract of herbal ointment

Figure: 4 Zone of inhibition against gram-positive bacteria

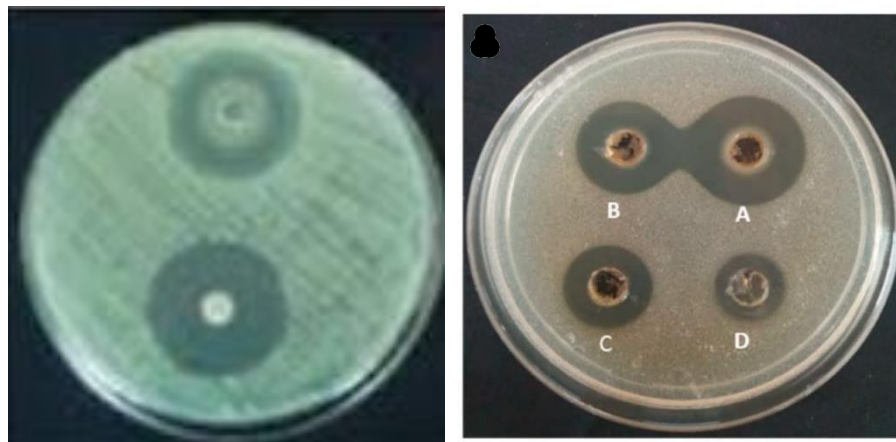
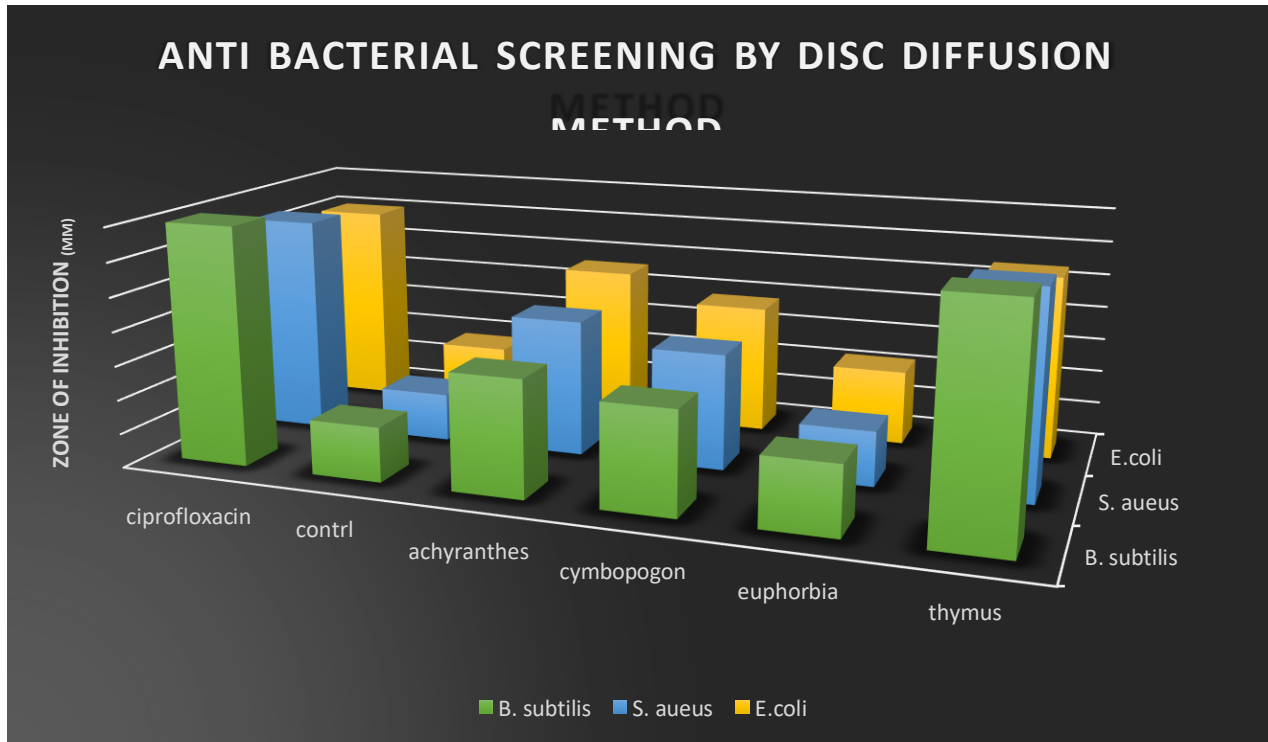


Bacillus subtilis



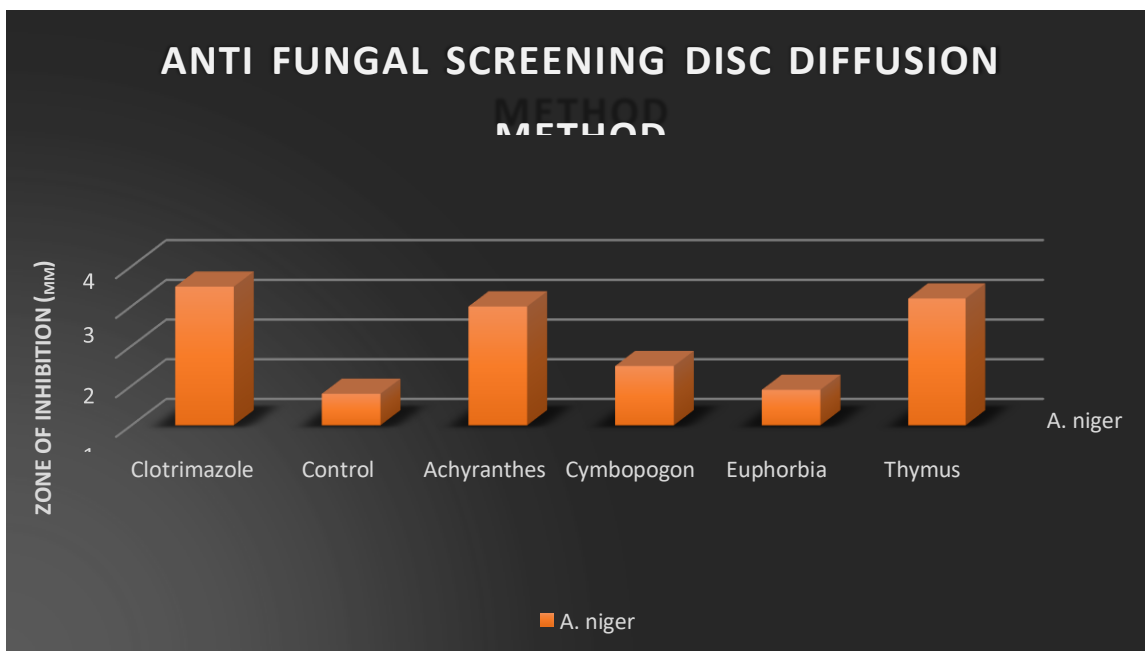
Staphylococcus aureus

Figure: 5 Graphical Representation of Antibacterial Activity of Ethanolic Extract



Aspergillus niger

Figure: 7 Graphical Representation of Antifungal Activity of Ethanolic Extract



RESULT AND DISCUSSION:

The ethanolic extract of herbal ointment was evaluated for its in-vitro antimicrobial activity using disc diffusion method respectively. The results suggested that the ethanolic extract of herbal ointments possess activity even at low concentration and also shows maximum zone of inhibition comparable to the standard, ciprofloxacin (anti-bacterial), clotrimazole (anti-fungal). The long term usage of drug causes serious adverse reactions. So herbal drugs are safe, since the adverse effect is mild or nil and lower the cost of therapy as comparable to the allopathic medicine. Further, the formulated herbal ointments was evaluated for their physicochemical properties like colour, pH, spreadability, washability, skin irritancy, solubility, diffusion study loss of drying, stability study. Therefore the colour of the *Eurphorbia hirta linn* is good when compared to *Cymbopogen citrurus*, *Thymus vulgaris*, *Achyranthes aspera linn*. The loss of drying, pH, and washability of the *Thymus vulgaris* shows better when compared to other herbal ointments. The solubility and stability studies, non-irritancy is good for all herbal ointment. Spreadability is good for *Thymus vulgaris* and *Achyranthes aspera linn*. Diffusion study is good for *Eurphorbia hirta linn*, *Thymus vulgaris*.

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