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COMPARATIVE STUDY OF ANTIMICROBIAL ACTIVITY OF *LEONOTIS NEPETIFOLIA* (L.) AND *HYPTIS SUAVEOLENS* (LABIATAE)

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ABSTRACT:

The Family Labiatae (Lamiaceae) is largest and widely spread in both temperate and tropical regions however; it is most abundant or cosmopolitan. It consists of about 220 genera and more than 3500 species. In Nasik 15 genera and 59 species are found. In the present study comparative antimicrobial activity of some species of Labiatae like *Leonotis nepetifolia* (L.) and *Hyptis suaveolens* is carried out. In the present study the aqueous, methanol, petroleum ether, acetone and ethyl alcohol extract of the aerial part of *Leonotis nepetaefolia* and *Hyptis suaveolens* was screened for the antimicrobial activity of bacterial strains *Escherichia coli*, *Klebsiella pneumonia* ,*Salmonella paratyphi*, *Staphylococcus aureus* and one fungal strain *Candida albicans*. During preliminary screening of some taxa may show many new constituents which have great medicinal values. Literature reveals that most of the members of this family possess bioactive compounds. Therefore in the present study antimicrobial study of species of Labiatae viz. *Leonotis nepetaefolia* and *Hyptis suaveolens* has been undertaken.

Key words: - *Antimicrobial, Extract.*

INTRODUCTION:

The Family Labiatae (Lamiaceae) is largest and widely spread in both temperate and tropical regions however; it is most abundant or cosmopolitan. It consists of about 220 genera and more than 3500 species. In Nasik 15 genera and 59 species are found. In the present study comparative antimicrobial activity of some species of Labiatae like Leonotis nepetifolia (L.) and Hyptis suaveolens is carried out. Leonotis nepetifolia (L.) is used in traditional medicine in therapy of bronchial asthma, diarrhoea, fever, malaria and as an analgesic agent in menstrual pains; also to treat common cold and to alleviate cough (Clement et al., 2005; Maregesi et al., 2007; Lans, 2007). In India, the flowers are used in case of hardly healing wounds, scars and burns. This plant exhibited various biological activities such as antifungal and antibacterial activities. Hyptis suaveolens is used for traditional medicine as an anticancer agent (Kingston et al., 1979), popularly used in the treatment of respiratory and gastrointestinal infections, indigestion, colds, pain, fever, cramps and skin diseases (Asekun, 1999, Oliveira et. al, 2005). The leaves are used as an anticancer and antifertility (in females) agent, while their aqueous extract has showed an antinociceptive effect and acute toxicity. To date, although few reports (Jain et. al,, 1974, Zollo et al., 2005) have found antibacterial and antifungal properties of H. suaveolens essential oil there has been a lack of studies emphasizing its anti-Aspergillus activity. In the present study the aqueous, methanol, petroleum ether, acetone and ethyl alcohol extract of the aerial part of Leonotis nepetaefolia and Hyptis suaveolens was screened for the antimicrobial activity of bacterial strains Escherichia coli, Klebsiella pneumonia ,Salmonella paratyphi, Staphylococcus aureus and one fungal strain Candida albicans. During preliminary screening of some taxa may show many new constituents which have great medicinal values. Literature reveals that most of the members of this family possess bioactive compounds. Therefore in the present study antimicrobial study of species of Labiatae viz. Leonotis nepetaefolia and Hyptis suaveolens has been undertaken.

MATERIAL AND METHODS :-

Collection, identification and processing of plant material Fresh plant material of Leonotis nepetaefolia and Hyptis suaveolens was collected from the Nasik and nearby areas of Nasik. Plants were correctly identified with the help of Flora of

Maharashtra and Flora of Nasik district .Plant material washed under running tap water and dried in the sun light. It was then homogenized to fine powder with electric blender and stored in airtight bottles. This sample was used for extraction of organic compound.

B) Extraction of organic crude material from plant: 50 gm sample (leaves, stem and inflorescence) weighed separately and used for soxhlation. On polarity the following solvents are selected

1) Petroleum ether

2) Ethanol

3) Methanol

4) Acetone

5) Distilled water

ANTIMICROBIAL TESTS

a) Antimicrobial activity of plant extract To study antimicrobial activity following four bacterial strains were used

1) *Escherichia coli* (ATCC25922)

2) *Klebsiella pneumonia* (ATCC15380)

3) *Salmonella paratyphi*

4) *Staphylococcus aureus* (ATCC 25923)

and one fungal strain was used i. e. *Candida albicans.*

The bacterial isolates were cultured on nutrient agar and incubated at 37o c for 24 hrs and the microorganisms were repeatedly sub- cultured in order to obtain pure isolation. Morphological and biochemical reactions were carried to ascertain proper identification. They were inoculated into nutrient agar slants and stored at 4o c. Overnight broth culture of the respective bacterial strains was adjusted to turbidity equivalent to 0.5 McFarland standards. (0.2 ml culture of the organisms was dispensed into 20 ml sterile nutrient broth and incubated for 24hrs and standardized at 1.5 x 10 CFU/ml by adjusting the optical density to 0.1at 600 nm PERKIN ELMER UV- spectrophotometer).

MEDIA PREPERATION

As per composition following media were prepared.

PDA MEDIUM (for fungi)

(Potato dextrose agar medium)

1) Potato - 200 gm

2) Dextrose – 20 gm

3) Agar – 15 gm

4) Distilled water – 1000 ml

NUTRIENT AGAR MEDIUM (for bacteria)

1) Yeast extract - 10 gm

2) NaCl - 05 gm

3) Peptone - 10 gm

4) Distilled water - 1000 ml

5) Agar - 20 gm

The antimicrobial assay was performed by following method:

Agar well diffusion method for solvent extract

Agar well diffusion method for solvent extract the media (Mueller Hinton Agar no. 2) along with the inoculums was poured into the Petri plate (Hi-media). For agar well diffusion method a well was prepared in the plates with the help of a cork borer, the freshly prepared inoculum was swabbed all over the surface of the MHA plate using sterile cotton swab. Four wells of 6mm diameter were bored in the medium with the help of sterile cork borer having 6 mm diameter and were labeled properly and fifty micro liters of the working suspension / solution of different medicinal plant extract and same volume of extraction solvent for control was filled in the well with the help of micropipette. Plates were left for some time till the extract diffuse in the medium with the lid closed and incubated at 37o c for 24 hour and measured using scale and mean were recorded after incubation plates were observed for zone of inhibition. Antimicrobial tests were done in triplicates and diameter of zone of inhibition (mm) is expressed as means. The determination of minimum inhibitory concentration (MIC) of crude extracts was conducted according to standard procedures (Dhar et al., 1968). The MIC method was applied on extracts that proved their high efficacy against microorganisms by the Disc diffusion method .The highest dilution of the plant extract that still retains an inhibitory effect against

the growth of a microorganism is known as MIC. Selected plant extracts were subjected to a serial dilution using sterile nutrient broth medium as diluents. In a series of test tubes a loopful of an individual microorganism was loaded and incubated at 370C for 24 hours. After incubation the MIC was determined by transferring a loopful of culture on agar surface; incubating the inoculated plates at 370C for 24 hours. The highest dilution of the plant extract retained its inhibitory effect resulting in no growth (absence of turbidity) of a microorganism is recorded as the MIC value of the extract.

RESULTS AND DISCUSSION

An antimicrobial is substance that kills or inhibits the growth of microorganisms such as bacteria fungi or protozoan. Antimicrobial drugs either kill microbes (microbicidal) or prevent the growth of microbes (microbistatic). Disinfectants are antimicrobial substances used on nonliving object. The history of antimicrobials begins with the observations of Pasteur and Joubert, who discovered that one type of bacteria could prevent the growth to another. They did not know at that time that the reason one bacterium failed to grow was that the other bacterium was producing an antibiotic. Technically, antibiotics are only those substances that are produced by one microorganism that kill, or prevent the growth of another microorganism. Of course, in today's common usage the term antibiotic is used to refer to almost any drug that attempts to rid your body of a bacterial infection. Antimicrobials include not just antibiotics, but synthetically formed compounds. The discovery of antimicrobials like penicillin and tetracycline gave the way for better health for millions around the world. The future effectiveness of antimicrobial therapy is somewhat in doubt. Microorganism, especially bacteria are becoming resistant to more and more antimicrobial agents. Bacteria found in hospitals appear to be especially resilient, and are causing increasing difficulty for the sickest patients those in the hospital. In the present study the aqueous, methanol, petroleum ether, acetone and ethyl alcohol extract of the aerial parts of selected plants were screened for the antibacterial and antifungal activity (Photo plate - 1). In Leonotis nepetaefolia highest zone of inhibition was shown by leaf acetone, ethanol and petroleum ether extract with S. aureus with 15, 16 and 12 mm respectively. Very poor response was observed with aqueous extract as 10 mm with K. pneumoniae and C. albicans. Petroleum ether extract showed zone of inhibition with only S. aureus (12 mm) (Plate – 1). MIC study Leonotis nepetaefolia (Plate – 1) revealed that no any aqueous concentration proved to be strongly susceptible for any of the pathogen. This might have resulted from the lack of solubility of the active constituents in aqueous solutions. 75 % and 100% concentration of ethyl alcohol stem extract found to be susceptible to S. paratyphi and S. aureus respectively. 100 % concentration of acetone found to be susceptible for K. pneumoniae, S. aureus and C. albicans respectively. Only 75 % and 100% concentration of petroleum ether found susceptible for K. pneumonia, S. aureus and other strains found strongly resistant to extract. 100 % methanol extract found susceptible to S. paratyphi. In the present study the antimicrobial activity of different solvents of crude extract of leaf, stem and inflorescence of H. suaveolens was evaluated (Photoplate - 2). The crude extract were screened for activity against bacterial strains like E. coli, , K. pneumoniae, S. paratyphi, S. aureus and a fungal strain C. albicans. Highest zone of inhibition was shown by inflorescence extract with methanol, acetone and ethanol extract with K. pneumoniae 14, 12 and 10 mm respectively. Very poor response was observed with aqueous extract in all bacterial and fungal stains. Petroleum ether stem extract showed zone of inhibition with only C. albicans (10 mm) (Plate – 1). Based on results described, we may conclude that the methanol extract of Hyptis suaveolens showed high antimicrobial activity against the bacteria Klebsiella pneumoniae, Salmonella paratyphi, & fungal strain Candida albican. The acetone extract of Hyptis suaveolens shows high antimicrobial activity against the klebsiella pneumonia, Salmonella paratyphi, Staphylococcus aureus and Candida albicans. This plant can be further subjected to isolation of therapeutic antimicrobials and carry out further pharmacological evaluation. MIC study of crude extracts of Hyptis suaveolens (Plate – 1) revealed that no any aqueous concentration proved to be strongly susceptible for any of the pathogen. This might have resulted from the lack of solubility of the active constituents in aqueous solutions. 25, 50 and 75% concentration of acetone stem extract found to be susceptible to K. pneumoniae. 50 and 75% concentration of inflorescence acetone extract found to be susceptible for K. pneumoniae, S. paratyphi and C. albicans respectively. 25 and 50% concentration of stem petroleum ether and ethanol found susceptible for C.albicans. 100 % leaf and inflorescence methanol extract found susceptible to. K. pneumoniae, C. albicans and S. paratyphi. Malele et al. (2003) reported a strong antifungal

activity for the oil of H. suaveolens leaves at 500 and 1000 µg/mL against Saccharomyces cerevisiae, Mucor sp. and Fusarium moniliforme, whereas Asekun et al. (1999) reported that the oil of H. suaveolens leaves (5 mg/L) displayed significant inhibitory activity against gram-positive (Staphylococcus aureus and Bacillus cereus) and gram-negative (Escherichia coli and Pseudomonas aeruginosa) bacteria and yeast (Candida albicans). The antimicrobial activity results shown that plant extracts were effective against both gram negative and gram positive bacteria and for fungal strain also. The demonstration of antibacterial activity and antifungal activity against bacteria and fungi may be indicative of presence of broad spectrum antibiotic compounds. The present investigation brings out adequate data on the antimicrobial potential of different solvent extracts of various crude extracts. The study of compounds with antibacterial activity have targeted plants with a history of ethno bacterial uses (Joyle, 1996 and Sindabiwae, 1999), while Herrera et al., (1996) reported that only a few studies have targeted on randomly collected plants with localized distribution patterns. Findings of Araujo et al., (2003) demonstrated that antimicrobial properties of substances on desirable tools in the control of undesirable micro-organisms especially in the treatment of infection and in food spoilage. From the research of De Boer et al., (2005) it was noted that successful prediction of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The traditional or practitioners make use of water primarily as a solvent. The result from the present study revealed that ethanol extracts of the selected plant was much better and powerful mainly due to the better solubility of the active compounds in organic solvents. This findings leads to the support of De Boer et al., (2005) who demonstrated the better solubility of the active compounds in organic solvent. The presence of antimicrobial substances in the higher plants is well established. Plants have provided a source of inspiration for novel drug compound's as plants derived medicines have made significant contribution towards human health. The current scenario of antibiotics is very threatening with significant emergence of resistance among bacterial pathogens against available antibiotics. Based on the results obtained in this study, we conclude that aerial parts of all the selected plants of Lamiaceae possess significant amount of antimicrobial property against a wide range of microorganisms. The present study indicates that these plants might serve as important medicinal plant. Further phytochemical and pharmacological investigation is very essential to prove the efficacy of this rare medicinal herb.

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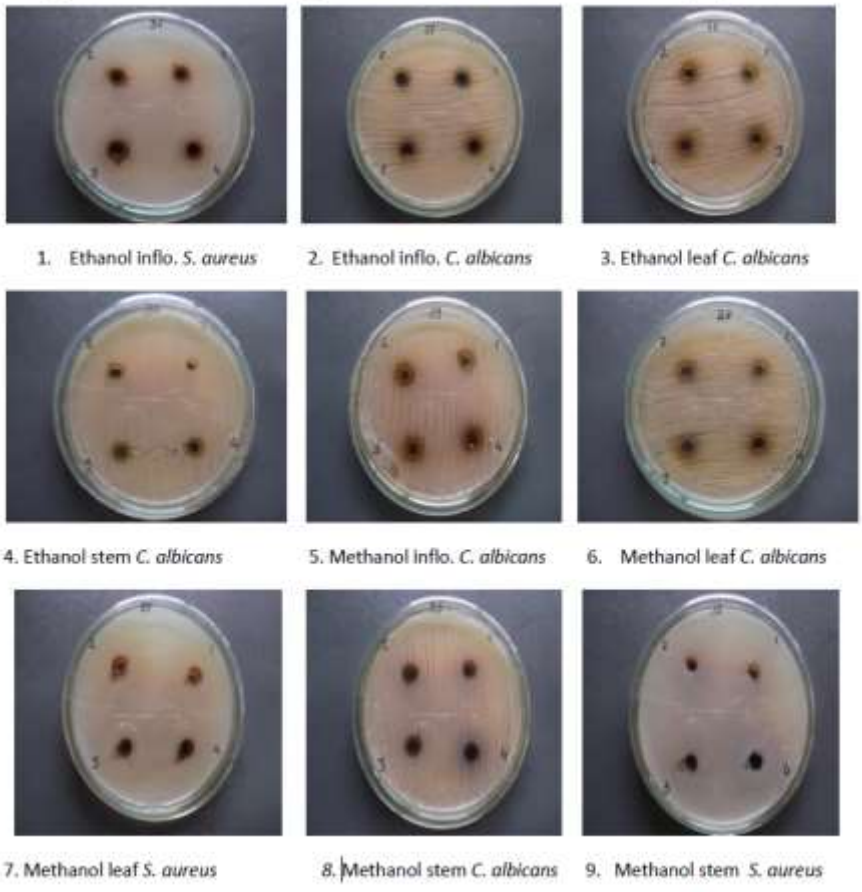
Photoplate – 1 Antimicrobial activity of different solvent extracts of *Leonotis nepetaefolia*

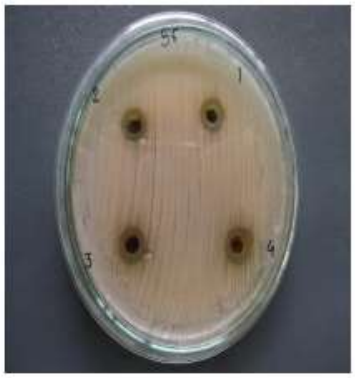


1. Ethanol inflo. *S. aureus* 2. Ethanol inflo. *C. albicans* 3. Ethanol leaf *C. albicans*

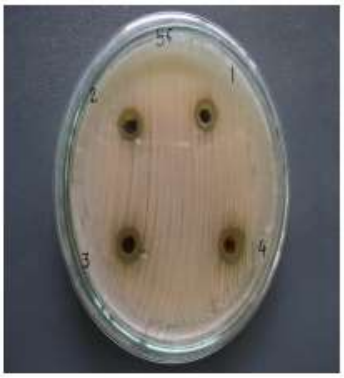
4. Ethanol stem *C. albicans* 5. Methanol inflo. *C. albicans* 6. Methanol leaf *C. albicans*

7. Methanol leaf *S. aureus* 8. Methanol stem *C. albicans* 9. Methanol stem *S. aureus*

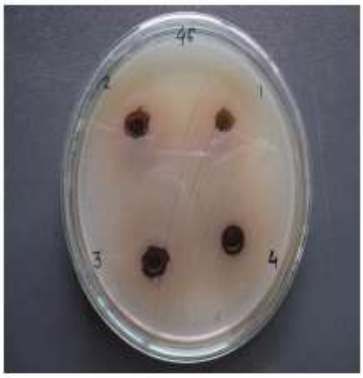
Photoplate – 2 Antimicrobial activity of different solvent extracts of *Hyptis suaveolens*



1. Acetone inflo. S. paratyphi



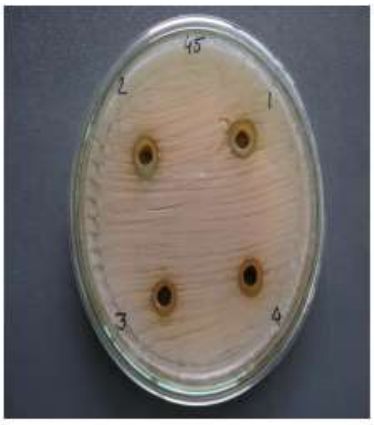
2. Acetone stem C. albicans



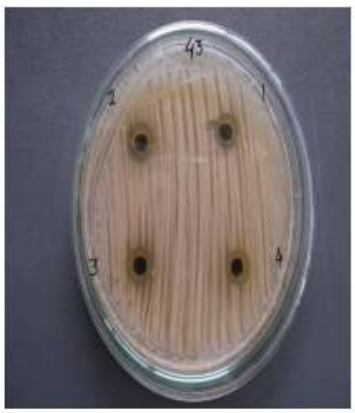
3. Ethanol stem S. areus

4. Ethanol inflo. C. albicans

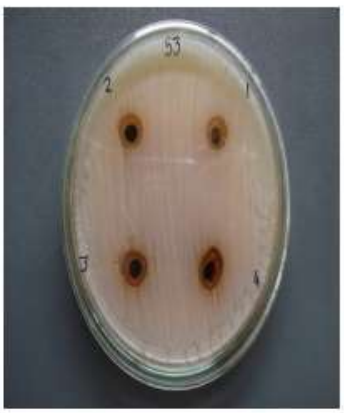
5. Ethanol leaf C. albicans



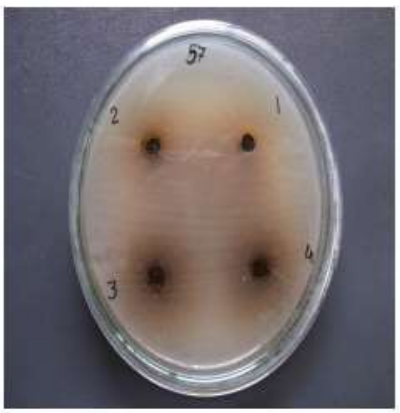
6. Ethanol stem C. albicans



7. Methanol leaf C. albicans



8. Methanol Inflo. C. albicans



9. Acetone Inflo. K. pneumoniae

Plate – 1 In-vitro antimicrobial activity of different extracts of four species of Lamiaceae