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STUDIES ON ANTI-MICROBIAL TRAITS OF ESSENTIAL OIL OF *LIPPIA NODIFLORA* FRESH AND SHADE DRIED LEAVES FROM ANANTPUR DISTRICT, ANDHRA PRADESH, INDIA

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The leaves of *Lippia nodiflora* (Verbenaceae), a common weed abundantly available in Anantapur district, Andhra Pradesh, is reportedly used in the preparation of herbal drugs. The essential oil obtained from fresh and shade dried leaves has also been reported to be anti-microbial and anti-helmintic. In the present study it is found that fresh leaves yielded more oil than the shade dried leaves. The major components were found to be α–phellandrene (42.04%); longifolene (16.12%) and α–cedrene (14.67%). Further studies on anti-microbial properties of its essential oil obtained by hydrodistillaton (both fresh and dried leaves) showed more anti-microbial activity against *Escherichia coli, Streptococcus lactis; Streptococcus thermoacidophilus; Bacillus subtilis;* and *Lactobacillus bulgaricus*. The percentages of major constituents were studied by GC analysis.

Keywords : Anti-microbial; Essential oil; *Lippia nodiflora.*

Medicinal plants have played a vital role in the world health, since time immemorial. The traditional system of medicines in India prescribing various receipes of Indian herbs led to the evolution of 'Ayurveda'. For centuries essential oils from plants have been known in many different applications. Many of them were used in medicines, disinfectants, insect repellents, fragrances, etc. Plant products or medicines are nature based which make them harmless, without any side effects, biodegradable and non intervening in the balanced food chains prevalent in the ecosystem, thus nullifying any environmental hazards of pollution. In the present work, an attempt has been made to study the bactericidal activity of essential oil of *Lippia nodiflora* a common weed distributed mostly in Anantapur district of Andhra Pradesh.

The leaves of *Lippia nodiflora* were collected from different places in Anantapur District in the month of June 2002. The essential oil was collected by subjecting the leaves (Fresh and shade dried) separtely for hydrodistillation in OTRI, (oil technological research institute) Anantapur. The oil was dried over anhydrous sodium sulphate to remove the traces of moisture and subjected to GC analysis using shimadzu GC 17 A Gas chromatographic unit, and column coated with 0.25 µl 5% biphency dimethyl silicone. Helicum was used as the carrier gas at a flow rate of 1.5 ml per minute. Component separation was achieved following a linear temperature programme of 60-220°C and percentage composition was calculated using peak normalization method. Nutrient broth, nutrient agar, Lurea broth and sodium tauro cholate media were procured from Hi-media Ltd., Mumbai, India, and media were prepared based on manufacturers instructions. The media were supplemented with 2% sodium taurocholate and 2% Tween 20 to increase the miscibility of the oil in the medium.

Five bacterial strains *(Escherichia coli, Streptococcus lactis, S. thermoacidophilus, Bacillus subtilis,* and *Lactobacillus bulgaricus)* used in the study were procured from National Chemical Laboratory, Pune, India and anti-bacterial activity was determined by 'Disc Diffusion' method.

Disk Diffusion Method : The anti-bacterial activity of essential oil of *Lippia* was determined by the Disc Diffusion method of Bauer *et al.*[1] Pure cultures of the test organisms were prepared on Nutrient agar slants. The broth cultures were prepared by

Table 1. Screening and minimum inhibitory concentration results of *Lippia nodiflora* (Zone size in mm)

S.No.	Name of Organism	25µl	35µl	45µl	MIC value
1.	*Escherichia coli*	10.5	10.7	15	300µl
2.	*Streptococcus lactis*	11	11	139.80	41 µl
3.	*Streptococcus thermoacidophilus*	9	10	10	400µl
4.	*Bacillus subtilis*	8.9	8.9	10	450µl
5.	*Lactobacillus bulgaricus*	1.2	10	137.80	41µl

inoculating 4-5 colonies of strain from the fresh pure culture. Nutrient agar, and Lurea agar slants were prepared by pouring sterile nutrient agar into petri dishes. Plates were dried in electric incubator with relay thermostat at 37±^{0}C to remove excess moisture from the surface. A sterile cotton swab was dipped into dilute culture, excess fluied was removed from swab by rotating it on the inner side of the test tube wall, and it was spread on to agar surfaces of petridishes. Filter paper discs with different concentration of oil (25 µl, 35 µl and 45 µl) were mounted on the agar surfaces. Then the plates were incubated at 37±1^{0}C for over night and zone of inhibition were observed around the disc aganist five bacterial test isolates.

Determination of minimum inhibitory concentration values of the oil : The minimum inhibitory concentration of the oil was determined by the tube dilution techniques[2] against those bacteria found susceptible by the Disc diffusion method. In this procedure Nutrient broth and Lurea broth supplemented with 2% Tween 20 to increase the miscibility of the oil in the medium. The minimum inhibitory concentration of the oil was determined as the least concentration inhibiting the growth of the organism in the test tube. Subsequently sub-cultures were carried out from the diluted tubes into nutrient agar slants and Lurea agar slants in order to determine whether the activity of the oil was bacteriostatic or bactericidal in action.

From the preliminary screening by Disc diffusion method, it was observed that all the five strains of bacteria were inhibited by *Lippia* oil. But a very small degree of variation was observed in their zone sizes (Table 1). From the tube dilution technique, which was the method of evaluating the minimum inhibitory concentration, it was observed that the minimum inhibitory concentration value of *Escherichia coli* was 300 µl, for *Streptococcus thermoacidophilus* it was 400 µl, and for *Bacillus subtilis* it was 450 µl and for *Streptococcus lactis* and *Lactobacillus bulgaricus* it was found to be 41 µl.

It has also been observed that the yield of essential oil from fresh leaves of *Lippia* was more than 2% than the oil obtained from shade dried leaves. Further this yield was gradually reduced as per the increase in drying periods.

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References

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