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STUDIES ON PHYTOCHEMICAL COMPOSITION AND ANTIBACTERIAL POTENTIAL OF METHANOLIC LEAF EXTRACT OF *MENTHA ARVENSIS* .LINN

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ABSTRACT

The success of chemotherapy lies in the continuous search for new drugs to counter the challenge posed by resistant strains. The ethanol extract of the leaves of *Mentha arvensis* .Linn were investigated for their antimicrobial activities against pathogens namely two gram - positive (*Staphylococcus aureus* & *Bacillus subtilis*) and three gram negative (*Escherichia coli*, *Klebsiella pneumoniae* & *Proteus vulgaris*) and antibiotics were used as positive reference standards in the tests by disc diffusion method. The methanolic leaf extract showed maximum activity against gram positive bacteria. The slight variation is found between zone of inhibition in control (Ampicilin) and methanolic leaf extract. The zone of inhibition of extract is slightly lesser than that of the control. The phytochemical analysis carried out revealed the presence of Cardiac Glycosides, Flavonoids, Steroids and Tannins. Alkaloids, Glycosides and Saponins, were not detected from any of the plant extract under study. The results provide justification for the use of the plant in medicine to treat various infectious diseases.

KEYWORDS : antibacterial activity, antibiotics, *Escherichia coli*, methanol extract

INTRODUCTION

Nature has been a source of medicinal agents since times immemorial. The importance of herbs in the management of human ailments cannot be overemphasized. It is clear that the plant kingdom harbors an inexhaustible source of active ingredients invaluable in the management of many

intractable diseases. Furthermore, the active components of herbal remedies have the advantage of being combined with many other substances that appear to be inactive. However, these complementary components give the plant as a whole a safety and efficiency much superior to that of its isolated and pure active components (1).

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Medicinal plants represent rich antimicrobial agents. Plants are used as a source of many potent and powerful drugs medicinally (2). Plants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm the activity and to ascertain the parameters associated with it. The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world (3, 4). The increased prevalence of multi drug resistance strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the specter of untreatable bacterial infections and adds urgency to the search for new infections fighting strategies (5).

Mentha arvensis belongs to the family, Lamiaceae, which is popularly known as pudina, which is a common household remedy that has found its use in the Indian indigenous system of medicine against several ailments as an anaesthetic, anti-phlogistic, antidepressant, [6], antiseptic, anti-spasmodic, carminative, digestive, expectorant, nervine, stomachic, tonic and anti-fertility drug [7]. It has beta-galactosidase activity [8] and it protects against radiation-induced lethality [9]. It contains menthol (66%), (-)-menthyl acetate (15%) and (-)-menthone (8%) and it also has some phenolic content. Based on the above features the present study was assessed to evaluate the 'Studies on Phytochemical Composition and Antibacterial Potential of Methanolic leaf extract of *Mentha arvensis* .Linn' with the following objectives i) to study the various phyto compounds present in the *Mentha arvensis* and ii) to evaluate the antimicrobial property of the study plant.

MATERIALS AND METHODS

Collection of Plant Material

Mint was purchased from the local market. The leaves were separated and washed under running tap water. Thoroughly washed leaves were allowed for shade drying under room temperature in the laboratory. The dried leaves were ground to fine powder using a blender. The powder was preserved in an air tight bottle for further studies.

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Preparation of Solvent Extracts

10 grams of thoroughly grounded powder were then soaked in 100ml of organic solvent (methanol) in a conical flask, plugged with cotton and kept on a rotary shaker at 190- 220 rpm for 24 hours at 40°C. After 24 hours, it was filtered through a Whatmann filter paper No 1 and the supernatant was collected and the solvent was evaporated to make the final volume one fourth of the original volume.

Preliminary Phytochemical analysis

Methanolic mint leaf extract was preliminary qualitatively screened for phytochemicals as per standard biochemical procedures (10, 11). The crude extract was diluted with methanol to the concentration of 1mg/ml. The qualitative phytochemical analysis of crude methanolic mint leaf was performed to determine the presence of Flavonoids, Saponins, Tannins, Terpenoids and Cardio glycosides.

ANTIMICROBIAL ACTIVITY

TEST MICROORGANISM

In vitro antimicrobial studies were carried out on five bacterial strains, two gram - positive (*Staphylococcus aureus* & *Bacillus subtilis*) and three gram negative (*Escherichia coli*, *Klebsiella pneumoniae* & *Proteus vulgaris*) were obtained from KMCH, Coimbatore.

DISC DIFFUSION METHOD

The bioassay used was the standard Agar Disc Diffusion method (12). Mueller Hinton Agar was prepared for the study. Mueller Hinton agar plates were swabbed with a suspension of each bacterial species, using a sterile cotton swab. Subsequently, the sterilized filter paper disc was completely saturated with the test compound. The impregnated dried discs were placed on the surface of each inoculated plate. The plates were incubated overnight at 37° C. Each organism was tested against each organism in triplicate. Methanol was used as negative control. Standard discs of Ampicillin served as positive antibacterial control. The test materials having antimicrobial activity inhibited the growth of the microorganisms

and a clear, distinct zone of inhibition was visualized surrounding the disc. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition in mm.

RESULT AND DISCUSSION

Preliminary Phytochemical Activity

The methanolic leaf extract of *Mentha arvensis* was subjected to various phytochemical

test for determining the presence of secondary metabolites like Alkaloids, Cardiac Glycosides, Flavonoids, Glycosides, Saponins, Steroids and Tannins. (Table.1). Out of these compounds, Cardiac Glycosides, Flavonoids, Steroids and Tannins were present. The test of the compound such as Alkaloids, Glycosides, Saponins, were found to be absent. On the basis of observation, the presence and absence of above compounds were reported in table 1.

Table 1: The phytochemical & Biochemical Analysis of Methanolic leaf extract of *Mentha arvensis*

| Compound | Observation | Result |
|--------------------|--------------------------------|---------|
| Alkaloids | No precipitation | Absent |
| Cardiac Glycosides | Formation of coloured rings | Present |
| Flavanoids | Formation of yellow colour | Present |
| Glycocides | No colour change | Absent |
| Saponins | No frothing | Absent |
| Steroids | Presence of pink colour | Present |
| Tannins | Extract change into blue black | Present |

ANTIBACTERIAL ACTIVITY

The methanolic leaf extract of *Mentha arvensis* was tested against human pathogenic bacteria's like *Bacillus subtilis*, *E. coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Staphylococcus aureus*. Out of these five pathogenic selected for this study three were gram negative *E. coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, and two were gram positive (*Bacillus subtilis*, *Staphylococcus aureus*). The Ampicillin the commercial available antibiotic is used as control. The zone of inhibition was measured after 24 hours against all the bacteria's. The results were tabulated (Table 2)

The maximum activity (19 ± 0.06 mm) zone of inhibition was seen against *Staphylococcus aureus* (gram positive bacteria). The least activity was measured against *Proteus vulgaris* (11 ± 0.03 mm) a gram negative bacteria. The *E.coli*, *Klebsiella pneumoniae*, and *Bacillus subtilis* showed 13 ± 0.03 mm, 12 ± 0.05 mm and 18 ± 0.38 mm respectively. In general the methanolic leaf extract showed maximum activity against gram positive bacteria. The slight variation is found between zone of inhibition in control (Ampicillin) and methanolic leaf extract. The zone of inhibition of extract is slightly lesser than that of the control.

| BACTERIA | CONTROL AMPICILLIN (mm) | ZONE OF INHIBITON (mm) |
|------------------------------------|-------------------------|------------------------|
| <i>Bacillus subtilis</i> (+ve) | 18 ± 0.57 | 18 ± 0.38 |
| <i>Escherichia coli</i> (-ve) | 14 ± 0.03 | 13 ± 0.03 |
| <i>Klebsiella pneumoniae</i> (-ve) | 12 ± 0.01 | 12 ± 0.05 |
| <i>Proteus vulgaris</i> (-ve) | 12 ± 0.89 | 11 ± 0.03 |
| <i>Staphylococcus aureus</i> (+ve) | 19 ± 0.13 | 19 ± 0.06 |

Table 2: Antibacterial Activity of Methanolic Leaf Extract of *Mentha arvensis*

Values are mean \pm SD

CONCLUSION

This preliminary evaluation indicated that the methanolic leaf extract of *Mentha arvensis* has significant activity against the test bacterial strains used. Further studies are necessary to identify the main active constituents.

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