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# Antimicrobial and anthelmintic investigations on the leaf extracts and leaf essential oil of *Mikania scandens* (L) Willd

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## ABSTRACT

The present study is concentrated on anthelmintic and antimicrobial studies of *Mikania scandens* (L.)Willd., belongs to the family Asteraceae. Adult earthworms *(Pheretima posthuma*) were used to evaluate anthelmintic activity. The non-polar solvents showed most significant activity than the polar one. Antibacterial activity of plant extracts were checked against both gram positive and gram negative bacteria (*Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli*). The essential oil showed better antimicrobial activity than the leaf extracts and the standard Ampicillin in the case of *Staphylococcus aureus.* The methanol extracts was more effective as an antifungal agent than the hexane extract. On the otherhand , the essential oil was a very promising antifungal agent than the polar and non polar leaf extracts.

**Key words:** *Pheretima posthuma,* anthelmintic activity**,** Asteraceae, anthelmintic, Ampicillin

## INTRODUCTION

Microbial and helminth infections are among the most common infections in man, affecting a large proportion of the world's population. In developing countries they pose a large threat to public health and contribute to the prevalence of malnutrition, anaemia, eosinophilia and pneumonia. These infections can affect most populations in endemic areas with major economic and social consequences [1]. The gastro-intestinal helminthes become resistant to currently

available anthelmintic drugs therefore there is a foremost problem in treatment of helminthes diseases. Hence, there is an increasing demand towards natural anthelmintics[2].

The use of herbal remedies has risen in the developed countries in the last few decades. In this connection, plants continue to be a rich source of therapeutic agents. The active constituents of many drugs are found in plants or are produced as secondary metabolites. Secondary metabolites are biosynthesized in plants for different purposes including growth regulation, inter and intra-specific interactions and defense against predators and infections. Many of these natural products have been shown to present interesting biological and pharmacological activities and are used as chemotherapeutic agents or serve as the starting point in the development of modern medicines[3]. The remarkable contribution of plants to the drug industry has been possible, because of the large number of phytochemical and biological studies all over the world. In fact, plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times4. Over 50% of all modern clinical drugs are of natural product origin[5] .

The present study is concentrated on anthelmintic and antimicrobial studies of *Mikania scandens* (L.)Willd. belongs to the family Asteraceae. *Mikania scandens* (L.)Willd. Commonly known as climbing Hempweed, climbing Hempvine and Guaco. It prefers moist sites particularly swamps, near water edges. Forms dense mat over thicket of brush and small trees. This plant is commonly called 'Dhrtharastra pacha' as its mode of growth is similar to the Hindu mythological king, Dhrtharastar, the father of Kaurava. When this climber grows over the plants for a while the entire vegetation underneath are destroyed due to the lack of air and light. So, type of growth habit is similar that of the blind king 'Dhrtharastar' whose "embrace", called the notorious "Dhrtharastralinganam". As this plant also embraces and kill the entire vegetation underneath.

## MATERIALS AND METHODS

*Mikania scandens* (L.)Willd. was collected from Avanavanchery of Thiruvananthapuram district. A voucher specimen was identified and deposited in the herbarium of University College, Thiruvananthapuram(PCL. No.10003).

### Anthelmintic Assay

The Indian earth worm *Pheretima posthuma* (Annelida) was collected from Botanical garden , Department of Botany , University College, Trivandrum. The anthelmintic assay was carried as per the method of Ajaiyeoba, *et al*., (2001)[6] with necessary modification. Formulation (50 ml) containing different concentrations of crude extracts (1,3 and 5ml in distilled water) were prepared and six worms (same type) were placed in them. Time taken by worms for paralysis was noted when no movement could be observed except when the worm were shaken vigorously. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water ($50^0$ C). Albendazole (1 mg / ml) was used as reference standard while distilled water as control.

### Antimicrobial studies of plant extracts

The whole plant extracts prepared in different solvents by soxhlet apparatus and oil extraction by hydro distillation method using Clevenger apparatus were used for the antimicrobial studies.

### Antibacterial studies

The antibacterial effects of plant has been analysed by using different bacterial strains *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* All glassware including petriplates, test tubes, conical flask etc. were sterilized by autoclaving at $121^0$ C for 10 minutes. The nutrient agar medium was prepared by adding 28 g of nutrient agar in 1000 ml distilled water. For the solidification of the medium 10 g of agar was dissolved by placing it in the boiling water bath. Media was autoclaved at $121^0$ C for 15 minutes. Required volume of molten agar in to medium was poured into the sterile petridishes under aseptic condition . Peptone water was prepared by adding 13.6g of peptone in 1000 ml distilled water[7]. Then it is poured into test tubes. Then the test tubes autoclaved $121^0$ C for 15 minutes. From pure culture, 1-3 spores of bacteria was transferred to test tubes containing peptone water. Then this was incubated for one hour at $37^0$ C for regeneration. After one hour this was taken out and swabbed into

the nutrient agar plate. Then it was kept aside. Inoculation was done under flame in high aseptic condition, under laminar air flow chamber.

Disc diffusion method was used to study the antibacterial effects of plant extracts. Discs diameter (6mm) were cut form Whatman No.1 filter paper. They were sterilized by autoclaving $121^0$ C for 15 minutes. Then it was stored in aseptic condition. The disc were treated with different concentrations, 1 m/g, 2 m/g and 3m/g of each extracts. About 10 filter paper disc placed into the petridish and cover it with a glass cover. After 20 minutes the filter paper disc will be fully saturated with the extracts. With the help of a forceps one disc from each petridish were taken. Discs were placed on petridish containing nutrient media possessing bacterial spores. Control disc was prepared by treating with solvents and standard by antibacterial drug, Ampicillin. The prepared disc containing different concentrations of extracts, control disc and standard disc were placed on each petriplates containing pure culture of bacteria. Then it was kept for incubation at $37^0$C for 3 days.

## Antifungal Activity

Different fungal strains used for the present study are *Aspergillus niger* and *Pencillium sps.* Fungal media was prepared by dissolving 3.9g Potato Dextrose agar and 1 g agar in 100 ml distilled water and then autoclaved $121^0$ C for 15 minutes. Then media was poured into sterile properties. Pure culture of fungal strains was inoculated into fungal medium. Whatman No.1 filter paper with different concentrations, 1 m/g, 2 m/g and 3m/g of each extracts and standard (Bavistin) were placed in the agar medium contains fungal inoculums. Then petriplates were incubated for 2 days.

# RESULTS AND DISCUSSION

## Anthelmintic activity

The result of anthelmintic activity revealed that methanol and hexane plant extracts showed varing degree of activity against the worm (*Pheretima posthuma*) at different concentrations(1,3,5 mg/ml). The extracts showed anthelmintic activity in dose dependent manner giving shortest time of paralysis and death with 5 mg/ml. Hexane extract showed better anthelmintic activity. These extracts required the least time for causing paralysis and death of the earthworm when compared to methanol extracts. The results were depicted in Table 1 Hexane and methanol extracts of *Mikania scandens* (L.)Willd. Caused paralysis of 25.6, 45.1, min. and time of death14.20 , 58.37, min. respectively at concentration of 5 mg/ml. Of these analysis hexane extracts showed significant anthelmintic activity when compared with standard Albendazole.( Table 1).

## Antimicrobial activity of essential oil

Three economically important bacterial strains, *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* and fungal strains, *Penicillium* species and *Aspergillus niger* were used for the present study.

## Antibacterial activity

The results reavaled that the essential oil showed significant activity with varying magnitude. The zone of inhibition of different concentration of oil against different bacteria ranging from 9-21mm.( Table 2) Pure oil showed the inhibition zone diameter of of 11mm against *Pseudomonas aeuroginosa* where as other concentration of oil such as 2:1 and 1:1 showed 11 and 9 mm against *Pseudomonas aeuroginosa.* The zone of diameter of pure oil against *Staphylococcus aureus* is 21mm and the zone of inhibition diameter at 2:1 and 1:1 are 16mm and 12mm respectively. In Escherichia *coli* pure oil inhibition is 18mm followed by 15 mm (in 2:1) and 11mm in 1:1 concentration. The pure oil concentration showed inhibition diameter of about 21mm in the case of *Staphylococcus aureus,* 11mm in *Pseudomonas aeruginosa* and 18mm in *Escherichia coli* respectively (Table 2). Thus from the result it is revealed that pure oil concentration with inhibition zone of diameter 21mm shows significant antibacterial activity against *Staphylococcus aureus* than standard Ampicillin.

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## Antifungal activity

The pure oil concentration of *Mikania scandens* (L.) Willd. showed inhibition zone diameter of about 21mm and 17 mm against *Penicillium* species and *Aspergillus niger* respectively. At the concentration of 2:1, the inhibition zone was maximum of 15mm and 13mm and the concentration of 1:1,the maximum inhibition zone of 9 mm against *Penicillium* species and as 7 mm against *Aspergillus niger* as compared with standard Bavistin were 16mm and 14mm respectively(Table 3).

## Antimicrobial activity of plant extracts.

## Antibacterial activity

The Methanol extracts showed inhibition zone diameter of 12mm,18mm,13mm at a concentration of 3mg/ml against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* (Table 4) where as the zone of inhibitions were less in 2 mg and 1 mg concentration. The hexane extracts of plant showed inhibition zone diameter of plant showed inhibition zone diameter of 11mm, 15mm and 12mm at a concentration of 3mg/ml against same bacterial culture.1 mg/disc showed very low inhibition than the 2 mg/disc(Table 5).

*Staphylococcus aureus* showed 14mm, 13mm zone of inhibition at concentration of 2mg/ml in methanol and hexane respectively. *Escherichia coli* showed 13mm and 12mm zone inhibition in both extracts at a concentration of 3mg/ml.

## Antifungal activity

The antifungal activity of plant extracts given in the Table 6&7. The extracts were found to be effective against *Penicillium* species and *Aspergillus niger* showing inhibition zone ranging from 4-13mm. The methanol extracts showed inhibition zone diameter (IZD) of 13mm and 11mm at a concentration of 3 mg/ ml while standard gave 25mm and 21mm against *Penicillium* species respectively (Table 6). The hexane extract showed least activity against *Aspergillus niger*. In the 3mg/ml treatment *Penicillium* species showed 11mm which is the largest IZD when compared with standard drug Bavistin with IZD varing from 21 and 25mm in case of *Aspergillus niger* and *Penicillium* species respectively (Table 7).

**Table.1. Anthelmintic activity of extracts of *Mikania scandens* (L.)Willd.**

| **Extracts** | **Concentration (Mg/ml)** | ***Pheritima posthuma*** | |
|---|---|---|---|
| | | **Paralysis time (min.)** | **Death time (min.)** |
| **control** | | Nil | Nil |
| **methanol** | 1 | 65.15 | 125.6 |
| | 3 | 52.2 | 63.1 |
| | 5 | 45.10 | 58.37 |
| **Hexane** | 1 | 40.3 | 57.10 |
| | 3 | 35.5 | 46.12 |
| | 5 | 25.6 | 14.2 |
| **Standard - albendazole** | 5 mg | 70.6 | 90.2 |

**Table.2. Antibacterial activity of essential oil of *Mikania scandens*(L.)Willd**

| Oil concentration | Bacterial strain | | | Control |
|---|---|---|---|---|
| | ***Pseudomonas aureuginosa* (mm)** | ***Staphylococcus aureus* (mm)** | **Escherichia *coli* (mm)** | |
| **Pure oil** | **11** | **21** | **18** | **0** |
| **2:1** | **11** | **16** | **15** | **0** |
| **1:1** | **9** | **12** | **11** | **0** |
| **Standard (Ampicillin)** | **14** | **18** | **13** | |

**Table.3. Antifungal activity of essential oil of *Mikania scandens* (L.)Willd.**

| Oil concentration | Fungal strain | | control |
|---|---|---|---|
| | ***Penicillium* species (mm)** | ***Aspergillus niger* (mm)** | |
| Pure oil | 21 | 17 | 0 |
| 2:1 | 15 | 13 | 0 |
| 1:1 | 9 | 7 | 0 |
| **Standard (Bavistin)** | 16 | 14 | |

**Table.4. Antibacterial activity of methanol extracts of *Mikania scandens* (L.)Willd.**

| Extract | Concentration in mg/ml | Bacterial strains | Inhibition zone (mm) | Standard (Ampicillin) | Control |
|---|---|---|---|---|---|
| **Methanol** | **1mg/ml** | *Pseudomonas aeruginosa* | 8 | 14 | - |
| | | *Staphylococcus aureus* | 12 | 18 | - |
| | | *Escherichia coli* | 7 | 13 | - |
| | **2mg/ml** | *Pseudomonas aeuruginosa* | 11 | 14 | - |
| | | *Staphylococcus aureus* | 14 | 18 | - |
| | | *Escherichia coli* | 9 | 13 | - |
| | **3mg/ml** | *Pseudomonas aeuruginosa* | 12 | 14 | - |
| | | *Staphylococcus aureus* | 18 | 18 | - |
| | | *Escherichia coli* | 13 | 13 | - |

**Table:5 Antibacterial activity of Hexane extracts of *Mikania scnadens* (L.)Willd.**

| Extract | Concentration in mg/ml | Bacterial strains | Inhibition zone (mm) | Standard (Ampicillin) | Control |
|---|---|---|---|---|---|
| Hexane | 1mg/ml | *Pseudomonas aeruginosa* | 7 | 14 | - |
| | | *Staphylococcus aureus* | 9 | 18 | - |
| | | *Escherichia coli* | 6 | 13 | - |
| | 2mg/ml | *Pseudomonas aeuruginosa* | 8 | 14 | - |
| | | *Staphylococcus aureus* | 13 | 18 | - |
| | | *Escherichia coli* | 10 | 13 | - |
| | 3mg/ml | *Pseudomonas aeuruginosa* | 11 | 14 | - |
| | | *Staphylococcus aureus* | 15 | 18 | - |
| | | *Escherichia coli* | 12 | 13 | - |

**Table: 6. Antifungal activity of methanol extracts of *Mikania scandens* (L.)Willd.**

| Extract | Concentration in mg/ml | Fungal strain | Inhibition zone (mm) | Standard (Bavistin) | Control |
|---|---|---|---|---|---|
| Methanol | 1mg/ml | *Penicillium* species | 6 | 25 | 0 |
| | | *Aspergillus niger* | 4 | 21 | 0 |
| | 2mg/ml | *Penicillium* species | 10 | 25 | 0 |
| | | *Aspergillus niger* | 7 | 21 | 0 |
| | 3mg/ml | *Penicillium* species | 13 | 25 | 0 |
| | | *Aspergillus niger* | 11 | 21 | 0 |

**Table.7.Antifungal activity of hexane extracts of *Mikania scandens* (L.)Willd.**

| Extract | Concentration in mg/ml | Fungal strain | Inhibition zone (mm) | Standard (Bavistin) | Control |
|---|---|---|---|---|---|
| Hexane | 1mg/ml | *Penicillium* species | 5 | 25 | 0 |
| | | *Aspergillus niger* | 4 | 21 | 0 |
| | 2mg/ml | *Penicillium* species | 9 | 25 | 0 |
| | | *Aspergillus niger* | 8 | 21 | 0 |
| | 3mg/ml | *Penicillium* species | 11 | 25 | 0 |
| | | *Aspergillus niger* | 9 | 21 | 0 |

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