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Original Research Paper

# Phytochemical Analysis and Antibacterial Activity of *Euphorbia hirta* Linn. and *Tiliacora acuminata* Miers.

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## ABSTRACT

Methanol and water extracts of leaf, stem and root of *Euphorbia hirta* Linn. and *Tiliacora acuminata* Miers. were screened for their phytochemical compounds and *in vitro* antibacterial activity against two medically important pathogenic bacteria namely *Staphylococcus aureus* and *Pseudomonas fluorescens*. Phytochemical screening reveals the presence of several phytoconstituents in both the plant species and many of these have been investigated scientifically for antimicrobial activity. *In vitro* antibacterial study was done using Agar Well Diffusion method, and the study reveals the methanol leaf extracts of *E. hirta* and methanol root extracts of *T. acuminata* recorded highest activity against both the test organisms.

## INTRODUCTION

Medicinal plants are of local heritage with global importance. A large number of medicinal plants are exploited from natural flora for the production of drugs. Infectious diseases are the leading cause of death world wide. Antibiotic resistance has become a global concern (Westh et al. 2004) as the clinical efficiency of many existing antibiotics is being threatened by the emergence of multi-drug resistant pathogens (Bandow et al. 2003). The increasing failures of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents have led to the screening of several medicinal plants for their potential antimicrobial activity (Benkeblia 2004). Many infectious diseases have been known to be treated with herbal medicines throughout the history of mankind. Herbs are safer than modern day drugs because they are naturally rich in both biologically active and inert substances. They are mild in action and lack many side effects at normal dosage and are relatively inexpensive compared to most of the synthetic drugs.

According to World Health Organization, medicinal plants are the best source to obtain a variety of newer herbal drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy. The present study is aimed to carry out the preliminary phytochemical analysis and to screen *in vitro* antibacterial activity of leaf, stem and root extracts of *Euphorbia hirta* Linn. and *Tiliacora acuminata* Miers. against two bacterial pathogens namely *Staphylococcus aureus* and *Pseudomonas fluorescens.*

## MATERIALS AND METHODS

**Plant material:** Both the plant species *Euphorbia hirta* and *Tiliacora acuminata* were collected from the Thrissur district of Kerala. The plants were identified, confirmed and authenticated by the taxonomist of Department of Botany, University of Calicut, Kerala, India. The collected plants were dried. Since certain compounds get denatured in sunlight, they were dried under shade to avoid decomposition.

**Preparation of plant extracts:** The dried plant material such as leaf, stem and root was powdered with the help of grinding machine separately. Three grammes of each powdered plant material was extracted separately with respective solvent in Soxhlet extractor for 24 hours. The solvents used were methanol and water. The respective extracts were then condensed and collected in separate vessel. This was followed by the evaporation of respective solvents to make higher concentration of the extracts and stored at 4°C in airtight bottles for further use.

**Test organisms:** Cultures of two pathogenic bacteria namely *Staphylococcus aureus* and *Pseudomonas fluorescens* were obtained from Amala Ayurvedic Hospital and Research Centre, Amalanagar, Kerala. The bacterial cultures were subcultured and maintained on nutrient agar at 4°C.

**Qualitative phytochemical analysis:** The extracts of leaf, stem and root of both the plant species were subjected to preliminary phytochemical screening so as to detect the presence or absence of major phytoconstituents. Phytoconstituents analysed included alkaloids, steroids, tannins, phenols, glycosides, terpenoids, flavanoids, anthraquinone,

saponins and cardiac glycosides.

**Antibacterial activity assay:** The extracts obtained from different components of the plants were studied for their antibacterial activity using Agar Well Diffusion Method (Cole 1994, Espinel-Ingroff et al. 1995, Okeke et al. 2001). Three wells each having 5 mm diameter were bored in each plate with an aseptic cork borer after the nutrient media had solidified. The test bacterial strains obtained from overnight broth culture were seeded separately on sterile solidified agar medium by swab plate technique using sterile cotton swabs. After a 10 minutes setting, 200 µL of each respective extract was introduced into each of the respective wells with the aid of a Pasteur pipette. After holding the plates at room temperature for about 2 hours to allow diffusion of the extracts into the agar, the plates were incubated at 37°C ± 2°C for 24 hrs. The antibacterial activity was calculated by measuring the diameter (mm) of the inhibition zone around the well. Methanol and water used for preparing plant extracts were chosen as control for both the bacterial species tested. A commercially available standard antibiotic ampicillin was chosen as the reference. All extracts were tested in triplicates and the average values were taken.

## RESULTS AND DISCUSSION

It has been widely observed and accepted that the medicinal value of plants lies in the bioactive phytocomponents present in the plants (Veeramuthu et al. 2008). Preliminary phytochemical analysis of different extracts of *E. hirta* and *T. acuminata* revealed the presence or absence of some phytoconstituents and are presented in Tables 1 and 2.

The data show the presence of alkaloids, tannins, phenols, glycosides and terpenoids in both methanol and water extracts of leaf and root component of *T. acuminata*. However, in stem component, the constituent tannin is absent and steroid is present. In *E. hirta*, the methanol and water extracts show the presence of tannins, flavanoids, steroids, phenols, glycosides, anthraquinone and terpenoids in leaf component. Besides this, the presence of alkaloid is also noticed in methanol extract. With respect to the stem and root component, the methanol extract shows the presence of all the constituents that are present in the leaf component, with the exception of alkaloid in stem and flavonoid in root. However, with the water extracts, glycoside, tannin, terpenoid and anthraquinone in root and glycosides and anthraquinone in stem were only detected. The study indicates methanol and water have difference in solubility for different phytoconstituents present in both the plant species (Majorie 1999).

The data presented in Tables 3 and 4 clearly indicate the zone of inhibition (ZOI) produced by the methanol and water extracts of both *E. hirta* and *T. acuminata* respectively against *S. aureus* and *P. fluorescens*.

The data reveal that the methanol extracts of leaf, stem and root demonstrated better antibacterial activity against both the test bacteria, with certain exception in water extracts of *T. acuminata* against *P. fluorescens*. Among the different extracts of *E. hirta* tried in the present study, the highest ZOI against *S. aureus* was noticed in methanol leaf extract (14.75 mm diameter) whereas the same was in methanol root extracts in *T. acuminata* (18.00 mm diameter). With respect to the bacteria *P. fluorescens*, the highest ZOI was obtained in methanol leaf extracts (15.00 mm diameter) of *E. hirta* and water extracts of root in *T. acuminata*. Considering the entire investigation, it is clear that methanol root extract of *T. acuminata* against *S. aureus* (Fig. 1) and the methanol leaf extracts of *E. hirta* against *P. fluorescens* (Fig. 2) demonstrated the maximum antibacterial activity. The study noticed that both the control treatments did not exhibit any ZOI against the test bacteria. However, the ZOI produced by the standard reference ampicillin was considerably higher compared to the different extracts studied. In support of the present observation there are many plants which were tried before for their phytochemical screening and antimicrobial properties against antibiotic susceptible

Table 1: Phytochemical analysis of different parts of *Tiliacora acuminata.*

| Phytoconstituent | Methanol Extract | | | Water Extract | | |
|---|---|---|---|---|---|---|
| | Leaf | Stem | Root | Leaf | Stem | Root |
| Alkaloids | + | + | + | + | + | + |
| Tannins | + | - | + | + | - | + |
| Flavanoids | - | - | - | - | - | - |
| Steroids | - | + | - | - | + | - |
| Phenols | + | + | + | + | + | + |
| Glycosides | + | + | + | + | + | + |
| Terpenoids | + | + | + | + | + | + |
| Anthroquinone | + | - | - | - | - | - |

+ indicates presence and – indicates absence

Table 2: Phytochemical analysis of different parts of *Euphorbia hirta.*

| Phytoconstituent | Methanol Extract | | | Water Extract | | |
|---|---|---|---|---|---|---|
| | Leaf | Stem | Root | Leaf | Stem | Root |
| Alkaloids | + | - | + | - | - | - |
| Tannins | + | + | + | + | - | + |
| Flavanoids | + | + | - | + | - | - |
| Steroids | + | + | + | + | - | - |
| Phenols | + | + | + | + | - | - |
| Glycosides | + | + | + | + | + | + |
| Terpenoids | + | + | + | + | - | + |
| Anthroquinone | + | + | + | + | + | + |

+ indicates presence and – indicates absence

Table 3: Zone of Inhibition produced by different extracts of *Euphorbia hirta* against *Staphylococcus aureus* and *Pseudomonas fluorescens*

| Plant | Bacteria | Zone of Inhibition in Methanol extract (mm) | | | Zone of Inhibition in Water extract (mm) | | |
|---|---|---|---|---|---|---|---|
| | | Leaf | Stem | Root | Leaf | Stem | Root |
| *Euphorbia hirta* | *Staphylococcus aureus* | 14.75 | 11.75 | 8 | 12.5 | 10.25 | 0 |
| | *Pseudomonas fluorescens* | 15 | 8 | 0 | 13 | 0 | 0 |

Each value presented in the Table is an average of 3 replicates.

Table 4: Zone of inhibition produced by different extracts of *Tiliacora acuminata* against *Staphylococcus aureus* and *Pseudomonas fluorescens.*

| Plant | Bacteria | Zone of Inhibition in Methanol extract (mm) | | | Zone of Inhibition in Water extract (mm) | | |
|---|---|---|---|---|---|---|---|
| | | Leaf | Stem | Root | Leaf | Stem | Root |
| *Tiliacora acuminata* | *Staphylococcus aureus* | 11.5 | 12.5 | 18 | 0 | 10.25 | 14.25 |
| | *Pseudomonas fluorescens* | 10 | 7.5 | 10.5 | 0 | 8.5 | 13.75 |

Each value presented in the Table is an average of 3 replicates.

and resistant microorganisms (Nascimento 2000). The presence of phytochemicals and significant antibacterial activity were reported in the extracts of *Cocculus hirsutus* and *Hyptis sauveolens* (Satish et al. 2010).

According to Prusti et al. (2008), the potent bioactive components present in plant extracts might be responsible for antibacterial activity. The phytoconstituents identified in the present investigation are also known to be bactericidal, pesticidal or fungicidal in nature thus confirming the antimicrobial property (Lutterodt et al. 1999, Pretorius et al. 2001, El astal et al. 2005). It is clear from the present study that the isolation of antimicrobial principles present in the plant material are largely dependent on the type of solvent used in the extraction procedure. The traditional healers use primarily water as the solvent media for preparing plant extracts but in the present study, methanol extracts showed more consistent antibacterial property with certain exceptions in *T. acuminata* on P. *fluorescens* (Table 4). The higher antimicrobial activity exhibited by methanol extracts compared to water extracts may be due the greater ability of methanol to make soluble more number of antimicrobial principles in higher degree of concentration compared to water (Majorie 1999).

## CONCLUSION

The results obtained in the present investigation recommend the use of *E. hirta* and *T. acuminata* in human protection from pathogenic bacteria *P. fluorescens* and *S. aureus*. The investigation is also suggesting that, for the application, the preparation of the extracts should be done using the solvent methanol. It is further suggested that bioactive substances from these plants can be employed in the formulation of newer antimicrobial agents for the control of various bacterial diseases.

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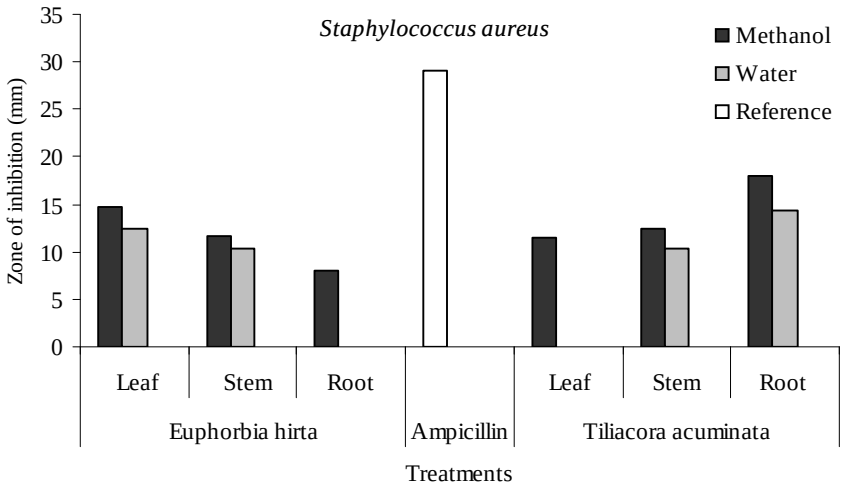


Fig. 1: Antibacterial activity of leaf, stem and root extracts of *Euphorbia hirta* and *Tiliacora acuminata* against the bacterial pathogen *Staphylococcus aureus.*

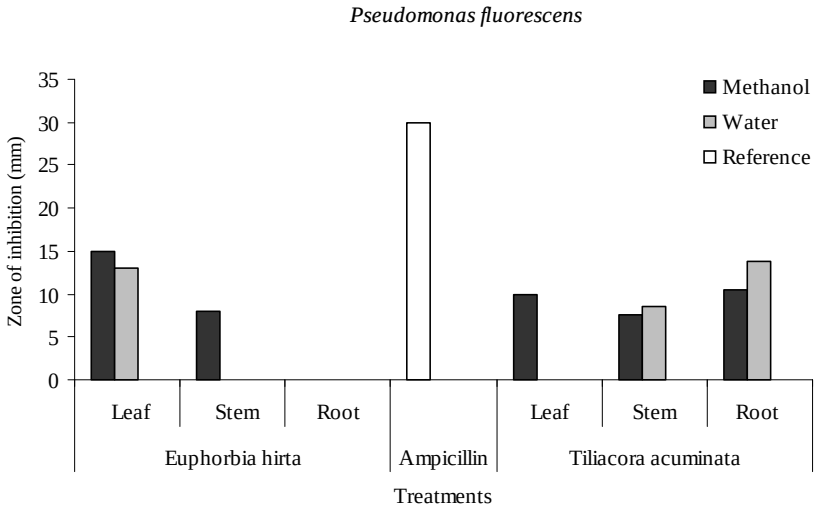


Fig. 2: Antibacterial activity of leaf, stem and root extracts of *Euphorbia hirta* and *Tiliacora acuminata* against the bacterial pathogen *Pseudomonas fluorescens.*

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