ANTIMICROBIAL ACTIVITY OF HEARTWOOD OF TECOMA STANS.

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Received: 19 Jan 2012, Revised and Accepted: 09 Mar 2012

ABSTRACT

The aim of the present study was to evaluate the antimicrobial activity of various extracts from heartwood of Tecoma stans. The different solvent extracts of Tecoma stans effective against tested bacteria (Pseudomonas fluorescens, Clavibacter michiganensis sub sp. michiganensis, Xanthomonas axanopodis pv. malvacearum, Staphylococcus aureus, E. coli, Pseudomonas aeruginosa and Klebsiella pneumonia) and fungi (all species of Aspergillus and Alternaria). In phytochemical screening showed that the different solvent extracts of Tecoma stans the tannin, flavonoids, phenol, alkaloids, steroids, anthraquinones and saponins were present in all solvent extracts. The Strong antimicrobial activities were observed in the ethanolic and methanolic extracts of T. stans than that of water extract. These findings provide scientific evidence to support traditional medicinal uses and indicate a promising potential for the development of an antimicrobial agent from T. stans plant. This medicinal plant by in vitro results appears as interesting and promising and may be effective as potential sources of novel antimicrobial drugs.

Keywords: Tecoma stans, Antimicrobial, Phytochemicals.

INTRODUCTION

The increase in prevalence of multiple drug resistance has showed downs the development of new synthetic antimicrobial drugs and the new drug is necessary to search for new antimicrobial from alternative sources. Phytochemicals from medicinal plants showing antimicrobial properties have the potential of filling this need because of structures are different from those of the more studied and their more action may too very likely differ1. In this growing interest, many of the Phytochemical bioactive compounds from a medicinal plants have shown many pharmacological activities 1. Screening of various bioactive compounds from plants has lead to the discovery of new medicinal drug which have efficient protection and treatment roles in against various diseases3.

The rapid emergence of multiple drug resistance strains of pathogen to current antimicrobial agents has generated an urgent intensive for new antibiotics from medicinal plants. Many medicinal plants have been screened extensively for their antimicrobial potential worldwide 4. Free radicals which have one or more unpaired electrons (superoxide, hydroxyl, peroxyl) are produced in normal or pathological cell metabolism and the compounds that can scavenge free radicals have great potential in ameliorating the diseases and pathological cells 5.

Tecoma stans (Bignoniaceae) known as yellow elder is an erect shrub or small tree. The plant has been used for a variety of purposes in herbal medicine, treating diabetes and digestive problems. Extracts from Tecoma stans leaves have been found to be inhibit the growth of the yeast infection. Senthilkumar et al. (2010) have reported that the extracts having antibacterial activity on human pathogenic bacteria. In present study was aimed to examine the total Phenolic content and Phytochemical analysis of ethanol, methanol and water extract of Tecoma stans were screened for antimicrobial properties using standard methods. The findings from this work may add to the overall value of the medicinal potential of the plant.

MATERIALS AND METHODS

Plant material

The heartwood of Tecoma stans were collected from the medicinal garden of the college of Pharmacy Shahada, In August and were air dried at room temperature for 4 weeks and were authenticated by Botanical survey of India, Koregong road, Pune.

Preparation of extracts

Heartwood of Tecoma stans Plant were air dried at room temperature for 4 weeks to get consistent weight. The dried parts were later ground to powder. 100 g of wet and dried samples were extracted with distilled water, ethanol, and methanol separately for 2 days in water both with a shaking attachment. The extract was lyophilized under 5 μm Hg pressure and stored at -200C. The experimental were carried out using an appropriate amount of lyophilized material.

Phytochemical analysis

Phytochemical analysis was carried out for saponins, flavonoids, steroids, phenol, anthroquinone, alkaloids and tannins. Wagner's and Heger's reagents was used for alkaloid foam test for saponins, Mg: HCl and Zn-HCl for flavonoids, acetic anhydride and sulphuric acid for steroids, chloride and gelatin for tannins, ferric chloride for phenol, hexane and diluted ammonia for anthraquinones test. All these experiments were carried out for distilled water, ethanol and methanol extracts individually.

Determination of Antimicrobial Activity

Antimicrobial assay

Pseudomonas fluorescens, Clavibacter michiganensis sub sp. michiganensis, Xanthomonas oryzae pv. oryzae, anthenomas axanopodis pv. malvacearum and strains of Staphylococcus aureus, E. coli, Pseudomonas aeruginosa and Klebsiella pneumonia bacteria were obtained from stock cultures presented at -80°C at P.S.G.V.P.Mandal’s Department of Microbiology, Shahada, dist-Nandubbar, Maharashtra. Two Gram positive bacteria tested were Clavibacter michiganensis sub sp. michiganensis, Staphylococcus aureus and six Gram negative bacteria tested were Pseudomonas fluorescens, Xanthomonas oryzae pv. oryzae, Xanthomonas axanopodis pv. malvacearum, E. coli, Pseudomonas aeruginosa and Klebsiella pneumonia. All bacteria were grown on nutrient agar media. Fungi (Aspergillus flavus, Aspergillus niger, Alternaria carthami, Alternaria helianthi, Cercospora carthami, Fusarium solani, Fusarium oxysporum, Fusarium verticilloides and Nigrospora oryzae were obtained from P.S.G.V.P. Mandal’s Department of Microbiology, Shahada, dist-Nandubbar,Maharashtra. All fungi were grown on potato dextrose agar medium.

Paper disc method

Diameter of zone of inhibition was determined using the paper disc diffusion method as described 7. A swab of the bacteria or fungi suspension containing 1x108 CFU/ml was spread on to Petri plates containing nutrient agar media separately. Each solvent extracts
were dissolved in each solvent to final concentration of 10mg/ml. Sterile filter paper discs (6mm in diameter) impregnated with 1mg of plant extracts were placed on culture plates separately for bacteria and fungi. The plates were incubated at 37°C for 24h. The standard chloramphenicol (10μg) for bacteria and carbendazim for fungi discs were used as positive controls. Antimicrobial activity was indicated by the presence of clear inhibition zone around the discs. The assay was repeated thrice and mean of three experiments was recorded.

RESULTS

Phytochemical analysis

The phytochemical screening showed that the different solvent extracts of _Tecoma stans_, the tannin, flavonoids, phenol, alkaloids, steroids, saponins and phenol were present in all solvent extracts. The phytochemicals strongly present in the ethanol and methanol extracts. But the water extract yielded less quantity of phytochemicals (Table-1).

Table 1: Phytochemical analysis for the different solvent extracts of heartwood of _T. stans_

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Alkaloids</th>
<th>Flavonoids</th>
<th>Saponins</th>
<th>Tannins</th>
<th>Phenols</th>
<th>Steroids</th>
<th>Anthraquinones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Ethanol</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Water</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+++ Strong; ++ medium; +poor Presence; -: absence, repeated the experiments three times for each replicates, Classification was based on observation of color intensity and amount of precipitate.

Antimicrobial assay

The antimicrobial activities of methanol and ethanol extracts of _T. stans_ gave different zones of inhibition on the organisms tested (Table 2). The methanolic and ethanolic extracts inhibited the growth of all most all the isolates of bacteria and fungi. The methanol and ethanol extract showed more potent against _E. coli_, _Xanthomonas axanopodis pv. malvacearum_, _Clavibacter michiganensis sub sp. michiganensis_, _Staphylococcus aureus_, _Pseudomonas aeruginosa_, _Pseudomonas fluorescens_ and moderate activity observed in _Xanthomonas oryzae pv. oryzae_. All the solvent extracts exhibited high activity on all species of _Aspergillus_ and _Alternaria_. All the extracts did not showed any effect on species of _Fusarium_ and _Nigrospora oryzae_.

Table 2: Zone of inhibition (in mm) of antimicrobial activity y disc diffusion method using different solvent extract of _Tecoma stans_.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Samples</th>
<th>Methanol</th>
<th>Ethanol</th>
<th>water</th>
<th>chloramphenicol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial pathogens</td>
<td>9±1</td>
<td>8±1</td>
<td>6±1</td>
<td>18±</td>
<td></td>
</tr>
<tr>
<td><em>Klebsiella pneumonia</em></td>
<td>13±2</td>
<td>12±2</td>
<td>3±1</td>
<td>20±</td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>10±1</td>
<td>9±1</td>
<td>1±1</td>
<td>18±2</td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>9±1</td>
<td>4±1</td>
<td>4±1</td>
<td>16±</td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>8±1</td>
<td>7±1</td>
<td>3±1</td>
<td>21±</td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas fluorescens</em></td>
<td>5±1</td>
<td>4±1</td>
<td>2±1</td>
<td>16±</td>
<td></td>
</tr>
<tr>
<td><em>Xanthomonas oryzae pv. oryzae</em></td>
<td>13±2</td>
<td>14±2</td>
<td>5±1</td>
<td>22±</td>
<td></td>
</tr>
<tr>
<td>Fungal pathogens</td>
<td>14±2</td>
<td>12±2</td>
<td>4±1</td>
<td>21±</td>
<td></td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>15±2</td>
<td>10±2</td>
<td>2±1</td>
<td>21±</td>
<td></td>
</tr>
<tr>
<td><em>Alternaria carthami</em></td>
<td>8±1</td>
<td>7±1</td>
<td>3±1</td>
<td>19±</td>
<td></td>
</tr>
<tr>
<td><em>Alternaria helianthi</em></td>
<td>5±1</td>
<td>5±1</td>
<td>2±1</td>
<td>21±</td>
<td></td>
</tr>
<tr>
<td><em>Cercospora carthami</em></td>
<td>7±1</td>
<td>5±1</td>
<td>3±1</td>
<td>21±</td>
<td></td>
</tr>
<tr>
<td><em>Nigrospora oryzae</em></td>
<td>5±1</td>
<td>4±1</td>
<td>2±1</td>
<td>20±</td>
<td></td>
</tr>
</tbody>
</table>

+: Presence; -: absence, repeated the each experiments three times for each replicates

DISCUSSION

In recent years, the search for phytochemicals possessing antimicrobial properties has been on the rise due to their potential use in the therapy of various chronic and infectious diseases. Due to risk of adverse effects encountered with the use of synthetic antibiotics, medicinal plants may offer an alternative source for antimicrobial agent with significant activity against pathogenic and infective microorganisms. In addition, a number of antibiotics have lost their effectiveness due to the development of resistant strains, mostly through the expression of resistance genes. Results of our findings confirmed the use of _T. stans_ as traditional medicine. We found strong antimicrobial and antioxidant activities specifically in the ethanolic and methanolic extracts of _T. stans_. High TPC values found in ethanolic and methanolic extracts (11.32 and 11.64 mg GAE/g extract) imply the role of phenolic compounds in contributing these activities.

Plant phenolic compounds have been found to possess potent antimicrobial11. The flavonoids from plant extracts have already been found to possess antimicrobial properties in various studies9, 10, 11. The presence of alkaloids has shown as antimicrobial activity12. Plant based steroids also possess as antimicrobial extracts13. The anthraquinones have also shown as antimicrobial properties14. Strong presence of tannins in all extracts may explain its potent bioactivities are known to possess potent antimicrobial activities9. The Saponins from plant extracts have already reported as potent antimicrobial agents15. The present investigation has shown that the ethanol and methanol extract of _T. stans_ have active phytochemicals which are able to inhibit plant and animal pathogenic bacteria and fungi. The ethanol and methanol extract fractions showed significantly antimicrobial activity against all Gram-positive and Gram-negative bacteria and different fungi tested. These findings provide scientific evidence to support traditional medicinal uses and indicate a promising potential for the development of an antimicrobial agent from _T. stans_ plant. This medicinal plant by _in vitro_ results appear as interesting and promising and may be effective as potential sources of novel antimicrobial drugs.
ACKNOWLEDGEMENT

The author feels to express sincere thanks to the Management, Principal and staff of PSGVP Mandal's college of Pharmacy, Shahada, The department of Pharmacology Karpagam University, Coimbatore, for their valuable support and co-operation during the research work.

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