PRELIMINARY PHYTOCHEMICAL SCREENING AND In Vitro ANTIMICROBIAL ACTIVITIES OF MEDICINAL PLANT EXTRACTS

Minocheherhomji Farida P.* and Vyas Bharat M.

1. Department of Microbiology, B. P. Baria Science Institute, Navsari, Gujarat (INDIA)
2. Department of Health, Ahmedabad Municipal Corporation, Ahmedabad (INDIA)

Received October 10, 2014 Accepted February 07, 2015

ABSTRACT
Medicinal plants are used for various ailments by Ayurvedic and Unani pharmacy and also by traditional healers. This study was aimed at carrying out phytochemical analysis and antimicrobial investigation of different plant extracts in water, acetone and methanol. The medicinal plants used for the purpose are Tinospora cordifolia, Withania sominifera, Asparagus racemosus and Ocimum sanctum against a panel of clinically significant bacterial, fungal and yeast strains. Phytochemical screening revealed the presence of alkaloids, tannins, saponins, flavonoids, carbohydrates, proteins, steroids, terpenoids and cardiologycosides. Presence of anthraquinone, anthrocyanins were observed in a few samples whereas phenolic flavonoids and phlobatannins were totally absent. Susceptibility by disc diffusion assay revealed significant antimicrobial activity of methanol and acetone extracts of these medicinal plants against gram positive bacteria such as Bacillus subtilis, Staphylococcus aureus and gram negative bacteria such as Proteus vulgaris, Salmonella typhi and Escherichia coli. The extracts obtained in methanol showed marked effect as antimicrobial agent than the extracts obtained in acetone and water.

Key Words: Antimicrobial activity, Phytochemical constituents, Medicinal plant extracts, Methanol extracts, Gram negative bacteria

INTRODUCTION
Novel drug discovery from medicinal plants is an unlimited and vast field of scientific exploration. It should follow a reverse pharmacological approach to reduce cost and duration of development. Traditional medicinal literatures serve as a powerful search engine in the context of providing the lead in discovery and research of new drugs. The universal role of plants in the treatment of disease is exemplified by their employment in all the major systems of medicines. Ayurvedic (Hindu) or Unani (Islamic) systems centred in Western Asia and the Indian subcontinents. Looking to the present scenario, there is an urgent need to discover new antimicrobial agents for human and veterinary therapeutic use as resistance to the current allopathic drugs increases in severity and extent. This phenomenon has occurred with some important pathogenic bacteria and as a result of which more than two million people worldwide are at a high risk of bacterial diseases. The current study is undertaken to identify new and structurally novel natural products showing antimicrobial activity with new modes of action. Secondary plant metabolites with unknown pharmacological activities have been investigated as a source of medicinal agents. It was anticipated that phytochemical with adequate antibacterial efficacy can be used for the treatment of bacterial infections. Since time immemorial man has used various parts of plants in the treatment and prevention of various ailments.

AIMS AND OBJECTIVES
To evaluate the activity of medicinal plant extracts against gram positive and gram negative bacteria, yeast and fungi in vitro and to carry out preliminary phytochemical screen...
-ing of these plants to reveal the presence of active principals in the plants.

**MATERIAL AND METHODS**

**Microbial cultures**

Antimicrobial activity is tested against bacteria, fungi and yeasts. Microorganisms used are listed in the Table 1. They are procured from National Collection of Industrial Microorganisms (NCIM), Pune, India. The cultures were grown on their respective selective media to check the purity and ensure their optimum growth before testing.

**Plant material**

Fresh plants were procured from the botanical garden of Navsari Agriculture University, Navsari, Gujarat, India and then the plant extracts were prepared according to the following procedure.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Name of the microbe</th>
<th>NCIM, Pune, India</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Escherichia coli</em></td>
<td>5051</td>
</tr>
<tr>
<td>2</td>
<td><em>Salmonella typhi</em></td>
<td>2501</td>
</tr>
<tr>
<td>3</td>
<td><em>Proteus vulgaris</em></td>
<td>2027</td>
</tr>
<tr>
<td>4</td>
<td><em>Bacillus subtilis</em></td>
<td>2063</td>
</tr>
<tr>
<td>5</td>
<td><em>Staphylococcus aureus</em></td>
<td>2127</td>
</tr>
</tbody>
</table>

The plant material after identification and authentication were washed with first luke warm water followed with by boiling water to remove the microbes present on its surface. Oven drying was carried out at 45°C for 14 days. Air dried plant materials were finely ground before being infused in water, acetone and methanol. Water, acetone and methanol extractions were carried out using soxhlet extractor. Powdered dried leaves (100 grams) were extracted with 200 ml of the solvent. The extracts were filtered using whatman filter paper no. 42 (125 mm). The filtered extracts were stored in air tight dark bottles at room temperature for antimicrobial activity. The extract was dried by keeping it at room temperature in a steady air current. They were stored at 4°C until required for testing. The extracts were dissolved in 50% Di methyl sulfoxide (DMSO) before use.

The plants used in the study are *Tinospora cordifolia*, commonly known as Guduchi, *Withania sominifera*, commonly known as Ashwagandha, *Asparagus racemosus*, commonly known as Satavari and *Ocimum sanctum*, commonly known as Tulsi. (Fig.1)

![Image of plant materials](image-url)
Assay medium
Mueller-hinton agar medium (Hi media no.M173-500G) was used to check the potency of the drug against different microorganisms under study. Kirby-bauer technique was adopted to check the antimicrobial action of medicinal plant extracts.\textsuperscript{10}

Antimicrobial activity
The antimicrobial activity was performed by agar well diffusion method. 20 ml of sterile mueller-Hinton agar was poured in sterile petridishes. The plates were allowed to solidify before use. The organisms (0.2 ml) were spread on the agar plate uniformly using a spreader. 8 mm bores were made all equidistance from one another on the medium with a sterile cup borer. 20 µl of different combinations of plant extracts were added to respective bores. The plates were incubated at 37°C for 24 hours and the zone of inhibition was measured.\textsuperscript{11} (Fig. 2)

Fig. 2 : Antimicrobial activities of \textit{B. subtilis} and \textit{E.coli} by agar well diffusion method

Phytochemical screening
Different qualitative chemical tests were carried out using standard procedures on the aqueous extract in order to identify the constituents as described by Sofowora\textsuperscript{12}, Trease and Evans\textsuperscript{13}, Harborne\textsuperscript{14} and Edeoga.\textsuperscript{15}

Qualitative analysis on phytochemical constituents

Alkaloids
1ml of the filtrate with 2ml of Drangenddroff’s reagent shows turbid orange colour.

Tannins
1ml of the filtrate with 2ml of ferric chloride gives dark green colour.

Saponins
1 ml of the filtrate with 2 ml of distilled water, shaken vigorously and then allowed to stand for 10 minutes. Development of foam on the surface of the mixture, lasting for 9 to 11 minutes indicates the presence of saponins.

Anthraquinones
1ml of the filtrate with 10ml of benzene is filtered. 5ml of 10% ammonia (v/v) is then added to the filtrate and shaken well. Presence of anthraquinones is indicated by the development of pinkish colour in the solution.

Anthocyanides
1 ml of the filtrate with 5 ml of dilute HCl shows the development of pale pink colour.

Phenolic flavonoids
1 ml of the filtrate with 2 ml of 10% lead acetate gives brown precipitate.

Flavonoids
1 ml of the filtrate with 2 ml of dilute NaOH shows the development of golden yellow colour.\textsuperscript{16-19}

Carbohydrates
Take 1ml of the filtrate with 5ml of Benedicts reagent and boil it for 5 minutes. Bluish green colour indicates the presence of carbohydrates. Addition of few drops of Molischs reagent and few drops of concentrated H$_2$SO$_4$ to 1 ml of the filtrate, gives A purple colour. Addition of few drops of Fehlings a reagent to 1ml of the filtrate leads to the development of green colour. Addition of few drops of Fehlings B reagent to 1ml of the filtrate leads to the development of brown colour.
Proteins
Addition of 5 to 6 drops of millions reagent to 1 ml of the filtrate leads to the development of white precipitates turning red on heating.

Steroids
Addition of 10 ml of chloroform and 10 ml of H₂SO₄ slowly by the sides of the test tube containing 1 ml of the filtrate leads to the upper layer turning red and the sulphuric acid layer exhibiting a greenish yellow fluorescence.

Terpenoids
On addition of 2 ml chloroform and a few drops of concentrated sulphuric acid carefully to 1 ml of the filtrate leads to the formation of reddish brown colour at the interface.

Cardiac glycosides
Addition of 1 ml of FeCl₃ reagent (consisting of 1 volume of 5% FeCl₃ solution and 99 volume of glacial acetic acid) and a few drops of concentrated sulphuric acid to 1 ml of the filtrate shows the appearance of greenish blue colour within a few minutes.

Phlobatannins
Addition of few drops of 1% HCl to 1 ml of the filtrate leads to the formation of red precipitates.

RESULTS AND DISCUSSION
Fig. 3 helps to conclude that the different extracts of medicinal plants showed antimicrobial activity against different test organisms. Growth of Proteus vulgaris was greatly suppressed by the different plant extracts where as Salmonella typhi showed the least suppression of growth. The plant extracts obtained in alcohol and methanol showed better antimicrobial effect than the extract obtained in water.

![Graphical representation of antimicrobial activities against different test organisms](image-url)
Comparative studies showed that *Asparagus racemosus* was not effective against all different microorganisms in comparison to *Ocimum sanctum* and *Withania sominifera*. The least effective was *Tinospora cordifolia*. The phytochemicals in these plant extracts have protective and disease preventive properties (Table 2). It indicates the results of qualitative analysis of the various phyto constituents in alcohol and methanol extracts of medicinal plants under study.

**Table 2**: Qualitative analysis of the various phyto constituents in alcohol and methanol extracts of medicinal plants

<table>
<thead>
<tr>
<th>Name of the test</th>
<th>Oscimum sanctum</th>
<th>Tinospora cordifolia</th>
<th>Asparagus racemosus</th>
<th>Withania sominifera</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test for alkaloids</td>
<td>E. E.</td>
<td>E. E.</td>
<td>E. E.</td>
<td>E. E.</td>
</tr>
<tr>
<td>Dragendorff’s test</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Test for tannins</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Test for saponins</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Test for anthraquinone</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Test for anthrocyanosides</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Test for phenolic flavonoids</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Test for flavonoids</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Test for carbohydrates</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Benedicts test</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Test for proteins</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Test for steroids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Test for terpenoids</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Test for cardiac glycosides</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Test for phlobatannins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Signages : Present (++), Moderately present (+), Absent (-), Ethanol extract : E. E., Methanol extract : M. E.

Preliminary phytochemical screening revealed the presence of alkaloids, tannins, saponins, flavonoids, carbohydrates, proteins, steroids, terpenoids and cardioglycosides in most of the plant extracts. Presence of phenolic flavonoids and anthrocyanosides were observed in very few samples where as anthraquinones and phlobatannins were totally absent. Some of these phytochemicals are assumed to be associated with antibacterial activity.

**CONCLUSION**

From the above studies, it is concluded that the plant species under study have many beneficial effects due to the presence of the above secondary metabolites which are likely to combat many diseases and also boost the immune system. The phytochemical characterization of the extracts, identification of responsible bioactive compounds and quality standards is a subject for further study in future.

**REFERENCES**


