Phytochemical analyses, antibacterial and antifungal activity of leaves from Abutilon indicum (L.) Sweet

D. Saranya, J. Sekar* and G. Adaikala Raj

Department of Botany, Annamalai University, Annamalainagar, Tamil Nadu, India

ABSTRACT
Phytochemical analyses of different extracts of Abutilon indicum (L.) Sweet was analysed. Leaves were extracted successively with different solvents viz., Petroleum ether, chloroform, ethyl acetate and methanol. The antimicrobial activity of different extracts was carried out against Staphylococcus aureus, Streptococcus pyogenes, Enterococcus faecalis, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, Vibrio cholerae, Candida albicans, Candida parapsilosis and Candida tropicalis by using the extent of the inhibitory zone, Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) were also determined. The ethyl acetate extract of Abutilon indicum showed the highest antimicrobial activity against all the bacterial and fungal strains tested than the other solvent extracts. The mean zones of inhibition produced by the extracts in agar diffusion assays against the tested bacterial strains ranged from 7.0 to 23.1 mm. The lowest MIC (62.5 and 125 µg/ml), MBC (125 and 1000 µg/ml) and MFC values (500 and 1000 µg/ml) were recorded. The ethyl acetate extract of A. indicum leaves showed the presence of strong phytochemicals viz., flavonoids, phenolic compounds, tannins, steroids, glycosides, saponins, terpenoids and alkaloids than other extracts. The highest mean of zone inhibition (23.1 mm) was observed in the ethyl acetate extract of A. indicum against Staphylococcus aureus. These finding suggest that the ethyl acetate extract of A. indicum can be used as an antimicrobial substance for the treatment of bacterial and fungal infections.

Key words: Antimicrobial activity, Abutilon indicum, MIC, MBC, MFC.

INTRODUCTION
A large number of secondary metabolites such as tannins, alkaloids, phenolics and terpenes are responsible for the valuable pharmacokinetic properties of medicinal plants and nucleic acids. Medicinal plants are indeed the most important source of life saving drugs for the majority of the world’s population. For this study it is important to select suitable biotechnological tools that would be helpful to multiply and conserve the critical genotypes of medicinal plants. Medicinal plants, since times immemorial, has been used virtually in all cultures as a source of medicine. Medicinal plants play a key role in world health care systems [1].

Infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health, despite the tremendous progress in human medicine. Their impact is particularly large in developing countries due to the relative unavailability of medicines and the emergence of widespread drug resistance [2]. one of the more alarming recent trends in Infectious diseases has been the increasing frequency of antimicrobial resistance among microbial pathogens causing nosocomial and community-acquired infections. Numerous classes of antimicrobial agents have now become less effective as a result of the effective pressure of antimicrobial usage [3].
Bacteria are the leading cause of nosocomial diseases, and viruses are a distant second. Occasionally, fungi cause disease but rarely protozoa are involved. Mostly nosocomial diseases are caused by Gram-negative bacilli like Escherichia coli. The use of therapeutic and diagnostic equipment (such as intravenous and urinary catheters), surgical procedure and transplantation has increased the risk of nosocomial diseases [4]. Bacteria have evolved numerous defenses against antimicrobial agents and drug-resistant pathogens. In the recent years, incidence of multidrug resistance in pathogenic and opportunistic bacteria has been increasingly documented [5]. Bacterial infection causes a high rate of mortality in the human population and aquaculture organisms [6]. For example, Enterococcus faecalis is the causative agent of inflammatory bowel disease [7], E. coli, Staphylococcus aureus and Pseudomonas aeruginosa cause diseases such as mastitis, abortion, and upper respiratory complications, while Salmonella sp. causes diarrhea and typhoid fever (8). P. aeruginosa is an important and prevalent pathogen among burned patients capable of causing life-threatening illness [7].

The fungi cause opportunistic infections in immunocompromised patients like those suffering from AIDS, cancer, diabetes, or undergoing treatment with immunosuppressive agents after transplant surgeries. Among these, Candidiasis has become a major public health problem as it is one of the leading causes of fungal infection in immune-suppressed population, particularly in AIDS patients leading to oropharyngeal and oesophageal Candidiasis [9]. Fungal related diseases may not be as common as other microbial infections but, when present, they are difficult to treat especially in immunosuppressed persons [10]. Candida albicans is the most common species associated with Candidiasis and is the most frequently recovered species from hospitalized patients. Candidiasis encompasses infections that range from superficial, such as oral thrush [11]. The increase of C. albicans infections parallels medical advancements such as invasive procedures, immunosuppressive treatments for organ transplants and widespread use of broad-spectrum antibiotics [12]. Candida and Aspergillus species have been found to be the most common etiological agents in nosocomial blood stream fungal infections (BSI). The most common species accounting for more than 90 % of all Candida-associated BSIs are C. albicans, C. glabrata, C. parapsilosis, C. tropicalis, and C. Krusei, while Aspergillus fumigatus, A. flavus, A. niger and A. terreus are the most common isolated species in Aspergillus-associated BSIs [13].

A major problem in antimicrobial chemotherapy is the increasing occurrence of resistance to antibiotics, which leads to the insufficiency of antimicrobial treatment. The overuse of antibiotics and consequent antibiotic selection pressure is thought to be the most important factor contributing to the appearance of different kinds of resistant microbes [14]. Antibiotics provide the main basis for the therapy of microbial infections. Since, the discovery of these antibiotics and their uses as chemotherapeutic agents, there was a belief in the medical fraternity that this would lead to the eventual eradication of infection diseases [15].

Abutilon indicum belongs to the family Malvaceae and distributed in all parts of tropical and subtropical region of India. All parts of the plant have been recognized to have medicinal properties. The plant is commonly called as Thotti in Tamil. The traditionally, the plant used as anthelmintic, anti-inflammatory and is useful in urinary and uterine discharges, piles and lumbago [16], jaundice, ulcer and leprosy. A. indicum leaves are used in the treatment of toothache, lumbago, piles, anti-fertility and liver disorders [17]. Root and bark are used as aphrodisiac, antidiabetic [18], nerve tonics and diuretic. The plant extracts and their products for antimicrobial activity have shown that a potential source of novel antibiotic prototypes of higher plants [19].

Hence, present study was carried out to evaluate the phytochemical and antimicrobial activity of petroleum ether, chloroform, ethyl acetate and methanol extracts of leaves from Abutilon indicum against bacterial and fungal strains.

**EXPERIMENTAL SECTION**

**Collection of Plant material and Extraction**

The leaves of Abutilon indicum (Malvaceae) was collected from kadavachery village (Lat, 11.24 ºN; Long, 79.44 ºE), Cuddalore, District, Tamil Nadu, India. During the months of August to September 2014, Herbarium was deposited (AUBOT#432), Department of Botany, Annamalai University, Annamalainagar. Collected leaves were washed with water, then surface sterilized with 10% sodium hypochlorite solution rinsed with sterile distilled water and shade dried under room temperature. The samples were ground in to a fine powder. One hundred grams of fine powder was used for extraction with different organic solvents like non-polar to polar viz., petroleum ether, chloroform, ethyl acetate and methanol for 8 hours using Soxhlet apparatus. The solvents were evaporated under vacuum in a rotary evaporator (Heidolph, Germany) and the dried extracts were stored at 4°C until further use.
Phytochemical analysis
The Petroleum ether, chloroform, ethyl acetate and methanol extracts of leaves of *Abutilon indicum* was used for qualitative phytochemical analyses. Phytochemicals such as, flavonoids, tannins, steroids, glycosides, saponins, phenolic compounds, terpenoids and alkaloids were analyzed according to described [20, 21].

Microorganisms
Seven clinical bacterial strains isolates Gram–positive bacteria: *Staphylococcus aureus, Streptococcus pyogenes, Enterococcus faecalis* and Gram–negative bacteria: *Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, Vibrio cholerae* and three fungal species: *Candida albicans, Candida parapsilosis* and *Candida tropicalis* were used in the present study. The stock cultures were maintained on Muller Hinton Agar medium and Sabouraud Dextrose Agar at 4 °C for bacterial and fungal respectively. The isolates obtained from Raja Muthiah Medical College Hospital, Annamalai University, Tamilnadu. *In vitro* antibacterial activity was determined by using Muller Hinton Agar (MHA) and Muller Hinton Broth (MHB). *In vitro* antifungal activities were determined by using Sabouraud Dextrose Agar (SDA) and Sabouraud Dextrose Broth (SDB) (fungi) were obtained from Himedia, Mumbai.

Antibacterial and Antifungal assays

Disc diffusion method
The agar diffusion method [22] was employed for the initial assessment of antibacterial potential of the extracts. Petri plates were prepared by pouring 20 ml of MHA and SDA allowed solidifying for the use in susceptibility test against bacteria and fungi. The standard inoculums using bacterial suspension containing $10^8$ CFU per ml, *Candida* suspension containing $10^6$ CFU per ml were swabbed on the top of the solidified sputure media. Plates were dried and uniformly spread. The excess inoculums were drained and the plates were allowed to dry for 5 min. After drying, the disc with extracts were placed on the surface of the plate with sterile forceps and gently pressed to ensure the contact with the incubated agar surface. Ciprofloxacin (10µg/disc) for bacteria and Amphotercin - B (100units/disc) for yeast was used as positive control. 5 per cent (Dimethyl sulphoxide DMSO) was used as blind control in these assays. Finally, the inoculated plates were incubated at 37 °C for 24 h. The zone of inhibition was observed and measured in millimeters. Each assay in this experiment was repeated three times.

Minimum inhibitory concentration (MIC)
The minimum inhibitory concentration was determined in MHB for bacteria and SDA for fungi described by broth macro dilution method [23]. The plant extracts were dissolved in 10 per cent DMSO to obtained 2 mg/ml. 0.5 ml of stock solution was incorporated into 0.5 ml of MHB to get a concentration of 1000, 500, 250 to standardized suspension of the test organism was transferred into each tube. The control tube contained only organism and devoid of plant extracts. The culture tubes were incubated at 37°C for 24 h. The lowest concentrations, which did not show any growth of tested organism after macroscopic evaluation were determined as MIC.

Minimum bactericidal concentration (MBC) and Minimum fungicidal concentration (MFC)
The MBC and MFC of the extracts were determined (24] by plating 100 µL of sample from each MIC assay tube with growth inhibition into freshly prepared MHB and SDA and the plates were incubated at 37°C for 24 h. The MBC and MFC values were recorded at the lowest concentration of the extracts that did not permit any visible bacterial/fungal colony growth on the agar plate during the period of incubation.

RESULTS
The petroleum ether, chloroform, ethyl acetate and methanol extracts of the leaf of *Abutilon indicum* revealed the presence of phytochemicals such as alkaloids, flavonoids, Cardiac glycosides, phenolic compounds, saponins, steroids, tannins and terpenoids. The ethyl acetate extracts of *A. indicum* revealed the presence of strong phytochemicals, alkaloids, terpenoids, steroids, flavonoids and phenolic compounds, than root and bark. The methanol and chloroform extracts were present in all phytochemicals except cardiac glycosides and alkaloids. The petroleum ether extracts were absent in all the phytochemical except phenolic compounds, steroids, terpenoids and tannins the results are presented in Table 1.
The different solvent viz., petroleum ether, chloroform, ethyl acetate and methanol extracts of A. indicum leaves showed the varied level of activities against the bacterial and fungal strains tested. All the extracts of A. indicum possessed significant antibacterial and antifungal activity against all the bacterial and fungal strains tested. When compared to the available antibiotics tested. The mean values are presented in Table 2. When the different extracts were tested against the test bacterial and fungal strains by using disc diffusion method MIC, MBC and MFC were also determined. The mean zones of inhibition obtained were between 7.0 to 23.1mm. The Ciprofloxacin (10µg/disc) antibacterial positive control produced the mean zone of inhibition were from 27.3 to 30.1mm and Amphotercin-B (100µg/disc) antifungal positive control produced the mean zone of inhibition were between 13.5 and 16.1. The 10% DMSO did not produced the any zone of inhibition. The MIC values of different extracts of A. indicum ranged between 62.5 and 1000 µg/ml, while the MBC values were between 125 and 1000µg/ml and MFC values were between 500 and 1000µg/ml. The highest mean of zone of inhibition (21.3) and the lowest MIC (62.5 µg/ml) values were obtained the ethyl acetate extract of A. indicum against Staphylococcus aureus. The antifungal activity of highest mean zone of inhibition (14.6 mm) and the lowest MIC (250 µg/ml) and MBC (500 µg/ml) values were observed in ethyl acetate extract of A. indicum against Candida parasapsilosis.

Table 1. A preliminary phytochemical analyses of different extracts of leaves of Abutilon indicum

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Phytoconstituents</th>
<th>Petroleum ether</th>
<th>Chloroform</th>
<th>Ethyl acetate</th>
<th>Methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Cardiac glycosides</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Phenolic compounds</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Saponins</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Steroids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

(++) = Strong; (+) = Positive (present); (-) =Negative (absent)
<table>
<thead>
<tr>
<th>S. No.</th>
<th>Microbial strains</th>
<th>Mean zone of inhibition* (mm)</th>
<th>Concentration of the extracts (µg/disc)</th>
<th>MIC (µg/ml)</th>
<th>MBC/ MFC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1000</td>
<td>500</td>
<td>250</td>
</tr>
<tr>
<td>6</td>
<td>Pseudomonas aeruginosa</td>
<td></td>
<td>Petroleum ether</td>
<td>10.0±0.30</td>
<td>9.3±0.57</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Chloroform</td>
<td>11.1±0.28</td>
<td>10.0±0.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ethyl acetate</td>
<td>12.1±0.28</td>
<td>11.0±0.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Methanol</td>
<td>11.6±0.78</td>
<td>10.6±0.16</td>
</tr>
<tr>
<td>7</td>
<td>Vibrio cholerae</td>
<td></td>
<td>Petroleum ether</td>
<td>11.0±0.50</td>
<td>10.0±0.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Chloroform</td>
<td>11.6±0.76</td>
<td>9.8±0.86</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ethyl acetate</td>
<td>13.5±0.50</td>
<td>11.1±0.28</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Methanol</td>
<td>13.1±0.28</td>
<td>10.6±0.76</td>
</tr>
<tr>
<td>8</td>
<td>Candida albicans</td>
<td></td>
<td>Petroleum ether</td>
<td>10.6±0.76</td>
<td>9.1±0.78</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Chloroform</td>
<td>12.1±0.50</td>
<td>10.1±0.28</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ethyl acetate</td>
<td>14.0±0.50</td>
<td>11.0±0.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Methanol</td>
<td>13.1±0.28</td>
<td>10.6±0.16</td>
</tr>
<tr>
<td>9</td>
<td>Candida parapsilosis</td>
<td></td>
<td>Petroleum ether</td>
<td>11.3±0.37</td>
<td>10.1±0.28</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Chloroform</td>
<td>13.1±0.28</td>
<td>10.0±0.57</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ethyl acetate</td>
<td>14.6±0.76</td>
<td>11.3±0.57</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Methanol</td>
<td>13.8±0.86</td>
<td>10.8±0.76</td>
</tr>
<tr>
<td>10</td>
<td>Candida tropicalis</td>
<td></td>
<td>Petroleum ether</td>
<td>9.0±0.50</td>
<td>8.1±0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Chloroform</td>
<td>9.8±0.6</td>
<td>8.5±0.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ethyl acetate</td>
<td>11.0±0.50</td>
<td>9.6±0.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Methanol</td>
<td>10.1±0.28</td>
<td>9.0±0.50</td>
</tr>
</tbody>
</table>

* Diameter of zone of inhibition (mm) including the disc diameter of 6 mm

**Mean of three assays; ± - Standard deviation; * Significant at P<0.05

**DISCUSSION**

The present study petroleum ether, chloroform, ethyl acetate and methanol extracts of *Abutilon indicum* leaves were used to analyse the phytochemicals such as alkaloids, flavonoids, phenolic compounds, saponins, tannins, glycosids, steroids and terpenoids. The ethyl acetate and methanol extracts of *A. indicum* leaves showed the presence of phytochemicals namely flavonoids, phenolic compounds, saponins, tannin, glycosids steroids and terpenoids strongly than the other extract. It has been unveiled that *Abutilon indicum* contains many biologically active compounds such as phenols, tannins, alkaloids, flavanoids glycosides, proteins, amino acids, sesquiterpenes, steroids, sterols, terpenoids, terpenes, carbohydrate, β-sitosterol, gallic acid, p-coumaric acid, quercetin-3-O-beta glycopyranoside etc [25]. The antibacterial and antifungal activities of *A. indicum* plant may be due to the presence of phytochemicals. There are several evidences on the presence of antimicrobial metabolites like tannins, flavonoid, glycosides, essential oils, furostanol, spirostanol, saponins, phytosterols,amides, alkaloids, etc in the studied plant species [26]. Secondary metabolites such as polyphenols are not required for plant development and growth, but are involved in plant communication and defense [27]. Tannins and saponins are plant metabolites well known for their antimicrobial properties [28]. Flavonoids have both antifungal and antibacterial activities. They possessed anti-inflammatory properties also [29]. Saponins, flavonoids, terpenes and steroids are known to have antimicrobial and curative properties against several pathogens [30].

The antimicrobial effects of plant materials are believed due to secondary products present in the plant, although it is usually not attributed to a single compound, but to a combination of metabolites [31]. The exact mechanism of bacterial growth inhibition of the plant materials is yet to know. One of the most important mechanisms is the hydrophobic activity of the bioactive compounds which enables them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable. Extensive leakage from the bacterial cells or the exit of critical molecules and ions will consequences death of the bacteria [32].

In present results indicated that the different solvents viz., petroleum either, chloroform, ethyl acetate and methanol extracts of *Abutilon indicum* significant antibacterial activity against all the bacterial and fungal strains tested. The ethyl acetate extract of *Abutilon indicum* showed the highest antibacterial activity than other extracts against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Candida albicans*, *Candida parapsilosis* and *Candida tropicalis*.
Vibrio cholerae, Candida albicans, Candida parapsilosis and Candida tropicalis. The highest mean of zone of inhibition (23.1) and the lowest MIC (62.5 µg/ml), MBC (125 µg/ml) and MBC (250 µg/ml) values were obtained for ethyl acetate extract of A. indicum against Staphylococcus aureus. The antibacterial activity was recorded for the ethyl acetate extracts of Stoechospermum marginatum and C. chemnitzia against Bacillus subtilis, Streptococcus pyogenes, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Vibrio cholerae, Shigella flexneri, Proteus mirabilis and P. vulgaris. [33, 34]. Screened petroleum ether, chloroform, ethyl acetate, methanol and water extracts of Cassia fistula flowers against Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis, Enterococcus faecalis and one “Gram-negative” bacterium Pseudomonas aeruginosa. The results revealed that the highest zones of inhibition were recorded with ethyl acetate extract against S. epidermidis (23 mm), S. aureus (19 mm), B. subtilis (15 mm) and E. faecalis (13 mm). [35]

In the present study different solvent extracts of Abutilon indicum tested for antibacterial and antifungal activities. The ethanol, chloroform, ethyl acetate and aqueous extracts of Abutilon indicum showed inhibitory effect against E. coli, Staphylococcus aureus, Pseudomonas aeruginosa, Proteus vulgaris, Salmonella paratyphi, Shigella sonnei, Salmonella typhimurium and Klebsiella pneumonia. The antimicrobial activity of ethanol extracts of Abutilon indicum was significant against Gram positive bacteria Staphylococcus aureus, Gram negative bacteria E. coli and fungal Aspergillus niger and Candida parapsilosis. [37].

In the present study, Gram-positive bacteria were found to be more susceptible than the Gram-negative bacteria. The reason for different sensitivity between Gram-positive and Gram-negative bacteria could be ascribed to the morphological differences between these microorganisms [38]. The Gram-positive bacteria should be more susceptible since they have only an outer peptidoglycan layer, which is not an effective permeability barrier [39].

CONCLUSION

Since ethyl acetate extract of A. indicum showed the potential antimicrobial activity against all the bacterial fungal strains tested. Moreover, the present investigation indicated the potential source of antimicrobial activity of leaf extract of Abutilon indicum against variety of biologically active organisms and it is hope that the present results will provide a starting point for investigations aimed at exploiting new natural antibacterial substances present in the Abutilon indicum.

Acknowledgement

The authors are thankful to Dr. K. Arumugam, Professor and Head, Department of Botany, Annamalai University for providing necessary laboratory facilities.

REFERENCES


