Phytochemical Screening and Antimicrobial Assessment of Leaves of Adhatoda vasica, Azadirachta indica and Datura stramonium

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Abstract
The present study was aimed to investigate the phytochemical screening and antimicrobial assessment of Adhatoda vasica, Azadirachta indica and Datura stramonium leaf extract. These plants were collected; aqueous and alcoholic extracts were prepared by soxhlet extraction process. The phytochemical analysis of extracts were performed, and antimicrobial activity of aqueous and alcoholic extracts of all plant material at dose of 100 mg/ml and 200 mg/ml concentrations against various strains were done. These extract were studied through agar diffusion method against E. coli, S. aureus, S. Bacillus bacteria and Rhizopus fungi and their comparison with standard ofloxacin. The phytochemical study inferred the presence of secondary metabolites which assist their herbal properties. Azadirachta indica was effective against all of these micro-organisms. While Adhatoda vasica and Datura stramonium were less effective as compared to Azadirachta indica and ineffective against Rhizopus fungi. Azadirachta indica had shown almost equal anti-microbial activity, against all species of microbes which was taken in study as compared to standard ofloxacin. Thus in future, extract of these plants may be beneficial for another several species of microbes and mixture of these plants may be more effective as compared to standard drugs.

Keywords: Adhatoda vasica, Azadirachta indica, Datura stramonium, Antimicrobial assessment

1 Introduction
According to World Health Organization, medicinal plants are the best source to obtain a variety of newer herbal drugs. The status of herbal medicine has been fast growing all over the world during the last few decades. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. During the twentieth century, when exploring the natural environment, man has made great discoveries that have enabled him to use a considerable number of natural resources.

The ancient man discovered the therapeutic value of some herbs by trial and error. Plants are rich in a variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, phenols, steroids, glycosides and volatile oils. Bacterial resistance to antibiotics is increasingly becoming a concern to public health. Currently used antibiotic agents are failing to bring an end to many bacterial infections due to super resistant strains. Plants have a great potential for producing new drugs of great benefit to mankind. Medicinal plants represent a rich source from which antimicrobial agents may be obtained.

Adhatoda vasica, an important Indian medicinal plant has long been used in ayurvedic system of medicine. The plant has been found to have a diverse number of pharmacological activities include Respiratory tract infection, cough formulation, expectorant, anti-spasmodic and bleeding pills. Recently various researchers have found greater interest in anti-microbial activity against several species in different studies.

Azadirachta indica is a tree from the Meliaceae family originated from India. It is also widely distributed in Asia, Africa and other tropical parts of the world. India encouraged scientific investigations of Azadirachta indica tree as the part of his program to give a new lease of life for Indian tradition and also increase commercial interest on Azadirachta indica. Currently some authors believe that no other plant or tree in the world has been so extensively researched or used, in all possible capacities.
It has been used in Ayurvedic medicine for more than 4000 years due to presence of its medicinal value. Neem contain more than 300 chemical constituent such as azadirachtin, meliacin, gedunin, salnin, nimbin, valassin and many other derivatives of these principles. The main active component azadirachtin is commonly used as the biological marker for this plant Neem is called ‘arista’ in Sanskrit a word that means perfect, complete and imperishable.

It is known for its pesticide activity against more than 400 insect pests and pharmacological activities, such as anti-inflammatory, anti-malarial, anti-fertility, antimicrobial, hepatoprotective, wound healing and various other activities.

*Datura stramonium* more commonly known as jimson weed or thorn apple is a wild-growing flowering plant belonging to the family Solanaceae and is a medicinal value with anti-inflammatory, antioxidant, analgesic, neurotoxic, hypolipidemic, hypoglycaemic and various other activities.

*Datura stramonium* was also found effective against few species of micro-organism in several studies. Therefore, our aim was to evaluate the phytochemical study and anti-microbial activity of the aqueous and alcoholic extracts of *Datura stramonium*, *Adhatoda vasica*, *Azadirachta indica* leaf against few species of bacteria and rhizopus fungus.

### 2 Materials and Methods

#### 2.1 Plant collection and identification

The plants used in this study were *Azadirachta indica*, *Adhatoda vasica* and *Datura stramonium* collected locally from oriental college of pharmacy, Bhopal (M.P). The collected material was authenticated by Dr. V.K. Rawat, botanist, department of biotechnology, Barkatullah University, Bhopal (M.P).

#### 2.2 Preparation of the crude extracts

The leaves of *Azadirachta indica*, *Adhatoda vasica* and *Datura stramonium* were air-dried, coarsely powdered and were then extracted. Extraction process was performed in hot continuous process through soxhlet apparatus for 48 hours. Solvent used in extraction process were alcohol and water, thus alcoholic and aqueous extract were used in anti-microbial assessment. After the 48 hour content of round bottom flask were filtered through whatman filter paper and extracts were allow to evaporate in warm water and dried in room temperature. Condensed extracts were weighed and stored in air tight container for 4 °C till further investigation.

#### 2.3 Preliminary Phytochemical analysis

Preliminary phytochemical screening was performed to identify secondary metabolites (phytoconstituent) in alcoholic and aqueous extract.

#### 2.4 Preparation of the tested organisms

##### 2.4.1 Preparation of standard bacterial suspensions

The average number of viable, *E. coli*, *S. Aureus* and *S. bacillus* organisms per ml of the stock suspensions was determined by means of the surface viable counting technique. About (10^6-10^9) colony-forming units per ml was used. Each time, a fresh stock suspension was prepared; the experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

##### 2.4.2 Preparation of standard fungal suspensions

The fungal cultures *Rhizopus* fungi were maintained on Saboraud Dextrose Agar incubated at 25°C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspended in (100 ml) of sterile normal saline and the suspension was maintained for further use.

#### 2.5 Antimicrobial activity

##### 2.5.1 Testing for antibacterial activity

The cup-plate agar diffusion method was used to assess the antibacterial activity of the prepared extracts. 0.6 ml of standardized bacterial stock suspensions of *10^8-10^9* colony-forming units per ml was thoroughly mixed with 60 ml of sterile nutrient agar. 20 ml of the inoculated nutrient agar were distributed into sterile Petri dishes. The agar was left to set and in each of these plates, 4 cups, 10 mm in diameter, were cut using a sterile cork borer No. 4 and the agar discs were removed. Alternate cups were filled with 0.1 ml of each extracts using micropipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C for 18 hours. Three replicates were carried out for each extract against each of the test organism. Simultaneously addition of the respective solvents instead of extracts was carried out as controls.

After incubation the diameters of the growth inhibition zones were measured, averaged and the mean values were tabulated.

##### 2.5.2 Testing for anti-fungal activity

The same method as for fungal was followed. Instead of nutrient agar media, yeast and mould extract agar was used. The inoculated medium was incubated at 25°C for two days for the *Rhizopus* fungi.

### 3 Results

#### 3.1 Phytochemical study

Presence of classes of secondary metabolite may be a useful indicator of both efficacy and potential toxicity; hence test for the presence of phytochemical classes with known bioactivity was done. The phytochemical investigation of aqueous extracts of *Adhatoda vasica*, *Datura stramonium* and *Azadirachta indica* are displayed in...
The result indicates that maximum number of phytoconstituents were present in aqueous and alcoholic extracts of *Datura stramonium* compared to other extracts of *Adhatoda vasica* and *Azadirachta indica*. The aqueous extracts of *Adhatoda vasica* contain least number of phytoconstituents.

### 3.2 Antimicrobial activity

**Table 1: Phytochemicals present in various extracts**

<table>
<thead>
<tr>
<th>Phytoconstituent</th>
<th>Aqueous extract</th>
<th>Alcoholic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Adhatoda vasica</em></td>
<td><em>Azadirachta indica</em></td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = Detected, ++ = Strongly detected, - = Not detected

**Table 2: Zone of inhibition (diameter) of microorganism in agar diffused plate by aqueous and alcoholic extract of *Azadirachta indica***

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Aqueous extract 100mg/ml</th>
<th>Aqueous extract 200mg/ml</th>
<th>Ethanolic extract 100mg/ml</th>
<th>Ethanolic extract 200mg/ml</th>
<th>Standard ofloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>0.8cm</td>
<td>1.2cm</td>
<td>1.2cm</td>
<td>1.8cm</td>
<td>1.9cm</td>
</tr>
<tr>
<td><em>S. Aureus</em></td>
<td>0.8cm</td>
<td>1.3cm</td>
<td>1.0cm</td>
<td>1.6cm</td>
<td>2.2cm</td>
</tr>
<tr>
<td><em>S. bacillus</em></td>
<td>1.2cm</td>
<td>1.6cm</td>
<td>0.9cm</td>
<td>1.5cm</td>
<td>1.7cm</td>
</tr>
<tr>
<td><em>Rhizopus</em></td>
<td>1.8cm</td>
<td>2.0cm</td>
<td>1.6cm</td>
<td>1.9cm</td>
<td>NA</td>
</tr>
</tbody>
</table>
Table 3: Zone of inhibition (diameter) of microorganism in agar diffused plate by aqueous and alcoholic extract of *Adhatoda vasica*

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Aqueous extract 100mg/ml</th>
<th>Aqueous extract 200mg/ml</th>
<th>Ethanolic extract 100mg/ml</th>
<th>Ethanolic extract 200mg/ml</th>
<th>Standard ofloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>0.6cm</td>
<td>1.2cm</td>
<td>0.9cm</td>
<td>1.2cm</td>
<td>1.9cm</td>
</tr>
<tr>
<td><em>S. Aureus</em></td>
<td>0.3cm</td>
<td>0.7cm</td>
<td>1.2cm</td>
<td>1.5cm</td>
<td>2.2cm</td>
</tr>
<tr>
<td><em>S. bacillus</em></td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>0.9cm</td>
<td>1.7cm</td>
</tr>
<tr>
<td><em>Rhizopus</em></td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Table 4: Zone of inhibition (diameter) of microorganism in agar diffused plate by aqueous and alcoholic extract of *Datura stramonium*

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Aqueous extract 100mg/ml</th>
<th>Aqueous extract 200mg/ml</th>
<th>Ethanolic extract 100mg/ml</th>
<th>Ethanolic extract 200mg/ml</th>
<th>Standard ofloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>1.3cm</td>
<td>1.8cm</td>
<td>NA</td>
<td>0.9cm</td>
<td>1.9cm</td>
</tr>
<tr>
<td><em>S. Aureus</em></td>
<td>1.0cm</td>
<td>1.2cm</td>
<td>1.2cm</td>
<td>1.3cm</td>
<td>2.2cm</td>
</tr>
<tr>
<td><em>S. bacillus</em></td>
<td>1.2cm</td>
<td>1.4cm</td>
<td>0.8cm</td>
<td>0.9cm</td>
<td>1.7cm</td>
</tr>
<tr>
<td><em>Rhizopus</em></td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

**4 Discussions**

Prevalence of antibiotic resistance in infectious bacteria has been increasing since last few decades, ultimately increasing occurrence of infectious diseases in developed as well as developing countries, has raised the demand for the scientific community to search for new anti-bacterial components. Treatment of infections is compromised worldwide by the emergence of bacteria that are resistant to multiple antibiotics. There are several reasons for development of multidrug resistance, include chromosomal mutations, resistance is most commonly associated with extra chromosomal elements acquired from other bacteria in the environment. These include different types of mobile DNA segments, such as plasmids, transposons, and integrons. However, intrinsic mechanisms not commonly specified by mobile elements such as efflux pumps that expel multiple kinds of antibiotics are now recognized as major contributors to multidrug resistance in bacteria. Once established, multidrug-resistant organisms persist and spread worldwide, causing clinical failures in the treatment of infections and public health crises.

Due to these entire problems with synthetic molecules, several researchers are focusing on plant source because natural sources may be the best to find new and noble anti-bacterial substances that can help to resolve this problem to some extent. In India, as well as various other parts of the world, large plants have been used as effective anti-microbial agents. There are several drugs obtained from plant source like Quinine as anti-malarial, Vinristine, Vinblastine, Topotecon as anti-cancer etc.

In the present investigation *Adhatoda vasica*, *Azadirachta indica* and *Datura stramonium* leaf extract were found to be effective against *E. coli*, *S. aureus*, *S. bacillus* and *Rhizopus* fungi and might be due to the presence of secondary metabolites i.e. alkaloids, saponins, flavonoids, tannins, terpenoids etc. In this study, we used aqueous and alcoholic extract of all these plant against *E. coli*, *S. aureus*, *S. bacillus* and *Rhizopus* fungi and...
compared with each other as well as standard ofloxacin. *Azadirachta indica* was found to be maximum efficacy against all species of microbes which was taken in study as compared to *Adhatoda vasica* and *Datura stramonium* and almost equally effective as standard ofloxacin. *Adhatoda vasica* and *Datura stramonium* was found to be less effective as compared to both *Azadirachta indica* and ofloxacin.

The major finding of this article is to be evaluation of effectiveness of *Adhatoda vasica* and *Datura stramonium* and its comparison with *Azadirachta indica* and standard ofloxacin. Thus, in future mixture of these plants might be more effective as compared to standard drug against several species of microbes.

5 Conclusions

In the present investigation, aqueous and alcoholic leaf extract of *Adhatoda vasica, Datura stramonium* and *Azadirachta indica* were evaluated for anti-microbial activity through agar diffusion method against *E. coli, S. aureus, S. bacillus* and *Rhizopus* fungi. *Azadirachta indica* was effective against all of these microorganisms. While *Adhatoda vasica* and *Datura stramonium* were less effective as compared to *Azadirachta indica* and ineffective against *Rhizopus* fungi. *Azadirachta indica* had shown almost equal anti-microbial activity, against all species of microbes which was taken in study as compared to standard ofloxacin. These results indicate that the anti-bacterial and anti-fungal activity of these extracts might be due to the presence of phytochemicals i.e. alkaloids, saponins, flavonoids, tannins, terpenoids, amino acids etc.

6 References

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