ANTIMICROBIAL ACTIVITY OF METHANOLIC AND ACETONIC EXTRACTS OF
AZADIRACHTA INDICA, SARACA ASOCA AND CURCUMA LONGA

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ABSTRACT

In the present work an attempt has been made to analyse the antibacterial and antifungal potential of methanolic and acetonic extracts of Azadirachta indica, Saraca asoca and Curcuma longa against E.coli and B. subtilis among bacterial strains and A. niger and A. fumigatus among fungal strains by disc diffusion method and the antibacterial and antifungal activities were determined by measuring the diameter of zone of inhibition. The methanolic extracts of Curcuma longa possessed the highest antibacterial with a zone of inhibition of 20 mm for E.coli and highest antifungal activity with a zone of inhibition of 22 mm for A. fumigatus. Acteonic extracts, exhibited lesser antibacterial activity with respect to B. subtilis 8 mm and antifungal activities with respect to A. niger being 8 mm when compared to the methanolic extracts.

KEYWORDS: Antibacterial, Antifungal, Methanolic Extracts, Acteonic Extracts

INTRODUCTION

According to World Health Organization medicinal plants serve as the best source for a variety of drugs. Therefore, such plants are being investigated for better understanding of their medicinal properties (Alo, et al., 2012). Plants have been used since ancient times to treat common infectious diseases whether locally within the dermis or a blood infection (Ozcan et al., 2009). Resistance towards drugs developed by pathogenic microorganisms due to their indiscriminate use and side effects due to synthetic drugs, has recently drawn much attention towards plant extracts and biologically active compounds isolated from plant species used in herbal medicine. Medicinal plants represent an alternate to synthetic drugs for the treatment of several non severe infectious diseases and can also be a possible source for new potent antibiotics to which pathogen strains are not resistant. (Mathur et al., 2011). In the present study Antibacterial and antifungal activity of methanolic and acetonic extracts of Azadirachta indica, Saraca asoca and Curcuma longa has been studied. Antibacterial and antifungal properties of Azadirachta indica, Saraca asoca and Curcuma longa have previously been reported by several authors (Pradhan et al., 2009, Maragathavalli et. al., 2012 Mahesh and satish, 2008 and Pundir and Jain 2010). Leaves of Azadirachta indica possess antibacterial, antifungal, antiviral, and even antiparasitic property and therefore exhibit wide range of pharmacological activities of neem leaf which may include immunodulatory, anti-inflammatory, antihyperglycaemic, antiallergic, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic and anticarcinogenic properties. (Subapriya et al., 2005). The leaf extracts of the plant as well as exhibit antidermatophytic activity against dermatophytes such as Trichophyton ruberum, Trichophyton , Mentagrophytes, Tricophyton violaceum, Microsporum nanum and Epidermophyton floccosum.(Heinrich et al., 2005). Saraca asoca exhibits antibacterial and antifungal activities against several bacterial Bacillus subtilis, E. coli, Salmonella typhosa and staphylococcus aureus and fungal Alternaria cajani, Helminthosporium sp., Curvularia lunata and Fusarium sp., species.Besides this, Saraca asoca have also been reported to have Anti-cancer activity, Anti-menorrhagic activity, Anti-oxytoxic activity (Pradhan et al., 2009). Phytochemicals present in turmeric have been reported to have potential effects
on diseases, such as cancer, alzheimer’s disease, arthritis and diabetes (Mishra and Palanivelu, 2008 and Mahady et al., 2002). The present investigation was undertaken considering the vast potentiality of plants as sources of antibacterial and antifungal agents.

Therefore, in the present work an attempt has been made to analyse the antibacterial and antifungal potential of methanolic and acetonic extracts of *Azadirachta indica*, *Saraca asoca* and *Curcuma longa* against selected pathogenic bacterial and fungal strains.

**MATERIALS AND METHODS**

**Collection Processing of Plant Materials**

The fresh leaves of *Azadirachta indica*, *Saraca asoca* and *Curcuma longa* were collected from the Nursery of School of Forestry and Environment, SHIATS, Allahabad. The fresh leaves were surface sterilized simply by washing under tap water and distilled water and were shade dried.

**Preparation of Methanolic and Acetonic Extracts**

The completely shade dried leaves were grounded to fine powder and allowed soxhlet for further acetonic and methanolic extract preparation. The obtained extracts were then subjected to rotary evaporator and evaporated to dryness and stored in air tight bottles at 4°C for further use.

**Preparation of Methanolic and Acetonic Extracts**

5gm dried leaves of *Azadirachta indica*, *Saraca asoca* and *Curcuma longa* were taken in separate Erlenmeyer flasks to which 50ml of required solvent i.e., methanol and acetone were added and kept in an orbital shaker for 24 h. After 24 h, it was filtered and filtrate was collected and kept in incubator at 37°C till all solvents completely evaporated from mixtures. The remaining powdered extract was weighed and dissolved in double amount of DMSO. It was further stored at 4°C till use.

**Microorganisms**

Two different bacterial strains viz., *E.coli* and *B. subtilis* and two different fungal strains viz., *Aspegillus niger* and *Aspergillus fumigatus* were used for testing antibacterial and antifungal activities. The test organisms used in this study were obtained from Microbiology department of SHIATS. The bacterial strains were cultured and maintained on nutrient agar media slants and the fungal strains were cultured and maintained on potato dextrose agar slants. The cultures were maintained by subculturing periodically and preserved at 4°C.

**Antibacterial Activity**

The antibacterial activity was done using methanolic and acetonic extracts of *Azadirachta indica*, *Saraca asoca* and *Curcuma longa* by disc diffusion method (Sardari et al., 1998). Filter paper disc (6mm in diameter) impregnated with acetonic and methanolic extracts were placed on test organism seeded plates test organism used were *E.coli* and *B. subtilis*. Autoclaved distilled water was used as control. The plates were incubated at 37°C for 24 h, after which the results were observed in terms of zone of inhibition. The diameters of the zones were measured in mm.

**Antifungal Activity**

The antifungal activity was done by disc diffusion method (Mahesh and Satish, 2008) using methanolic and acetonic extracts of *Azadirachta indica*, *Saraca asoca* and *Curcuma longa*. Potato dextrose agar plates were inoculated with each fungal culture viz. *Aspergillus niger* and *Aspergillus fumigatus* separately. Filter paper disc (6mm in diameter)
impregnated with acetonic and methanolic extracts were placed on test organism seeded plates test organism used were Aspergillus niger and Aspergillus fumigatus. Autoclaved distilled water was used as control. The plates were incubated at 28°C for 24 h, after which the results were observed in terms of zone of inhibition. The diameters of the zones were measured in mm.

RESULTS

The antibacterial and antifungal activities were estimated using methanolic and acetonic extracts of Azadirachta indica, Saraca asoca and Curcuma longa against E.coli and B. subtilis and A. niger and A. fumigatus respectively by disc diffusion method and the antibacterial and antifungal activities were determined by measuring the diameter of zone of inhibition.

Table 1: Antibacterial Activity of Azadirachta indica against E. coli and B. subtilis

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Acetonic Extract (Zone of Inhibition in mm.)</th>
<th>Methanolic Extract (Zone of Inhibition in mm.)</th>
<th>Distilled Water (Control) (Zone of Inhibition in mm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>10</td>
<td>16</td>
<td>00</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>12</td>
<td>14</td>
<td>00</td>
</tr>
</tbody>
</table>

Figure 1: Antibacterial Activity of Azadirachta indica against E.coli and B.subtilis

The results of this study reveal that Azadirachta indica, Saraca asoca and Curcuma longa possesses potential antibacterial activity against E. coli and B. subtilis. With the highest antibacterial activity against E. coli 20 mm for methanolic extract of Curcuma longa (Table 3 and Figure 3) and a minimum antibacterial activity in E. coli with a zone of inhibition of 8 mm was observed for acetonic extract of Saraca asoca (Table 2 and Figure 2). Distilled water (control) showed no zone of inhibition. Similar results were seen with ethanolic and methanolic extracts of Azadirachta indica by Maragathavalli et al. (2012) and ethanolic extracts of Saraca asoca showed similar results when tested against E. coli (Singh et al., 2009; Pradhan et al., 2009).

Table 2: Antibacterial Activity of Saraca asoca against E. coli and B. subtilis

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Acetonic Extract (Zone of Inhibition in mm.)</th>
<th>Methanolic Extract (Zone of Inhibition in mm.)</th>
<th>Distilled Water (Control) (Zone of Inhibition in mm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>10</td>
<td>12</td>
<td>00</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>8</td>
<td>10</td>
<td>00</td>
</tr>
</tbody>
</table>
Figure 2: Antibacterial Activity of *Saraca asoca* against *E. coli* and *B. subtilis*

Table 3: Antibacterial Activity of *Curcuma longa* against *E. coli* and *B. subtilis*

<table>
<thead>
<tr>
<th>Solvent Bacterial Species</th>
<th>Acetonic Extract (Zone of Inhibition in mm)</th>
<th>Methanolic Extract (Zone of Inhibition in mm.)</th>
<th>Distilled Water (Control) (Zone of Inhibition in mm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>18</td>
<td>20</td>
<td>00</td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td>16</td>
<td>18</td>
<td>00</td>
</tr>
</tbody>
</table>

Figure 3: Antibacterial Activity of *Curcuma longa* against *E. coli* and *B. subtilis*

Similarly, the methanolic and acetonc extracts of the three medicinal plants posseses antifungal activity against *A. niger* and *A. fumigatus* with the maximum zone of inhibition against *A. niger* 22 mm for methanolic extract of *Curcuma longa* (Table 6 and Figure 6) and the minimum inhibition was shown by acetonic extract of *Azadirachta indica* against *A. niger* 8 mm. Distilled water (control) showed no zone of inhibition.

Table 4: Antifungal Activity of *Azadirachta indica* against *A. niger* and *A. fumigatus*

<table>
<thead>
<tr>
<th>Fungal Species</th>
<th>Solvent</th>
<th>Acetonic Extract (Zone of Inhibition in mm.)</th>
<th>Methanolic Extract (Zone of Inhibition in mm.)</th>
<th>Distilled Water (Control) (Zone of Inhibition in mm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. niger</em></td>
<td></td>
<td>8</td>
<td>20</td>
<td>00</td>
</tr>
<tr>
<td><em>A. fumigatus</em></td>
<td></td>
<td>10</td>
<td>15</td>
<td>00</td>
</tr>
</tbody>
</table>
Antimicrobial Activity of Methanolic and Acetonic Extracts of *Azadirachta indica*, *Saraca asoca* and *Curcuma longa*

**Figure 4:** Antifungal Activity of *Azadirachta indica* against *A. niger* and *A. fumigatus*

**Table 5:** Antifungal Activity of *Saraca asoca* against *A. niger* and *A. fumigatus*

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Fungal Species</th>
<th>Acetonic Extract (Zone of Inhibition in mm.)</th>
<th>Methanolic Extract (Zone of Inhibition in mm.)</th>
<th>Distilled Water (Control) (Zone of Inhibition in mm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>A. niger</em></td>
<td>15</td>
<td>16</td>
<td>00</td>
</tr>
<tr>
<td></td>
<td><em>A. fumigatus</em></td>
<td>16</td>
<td>20</td>
<td>00</td>
</tr>
</tbody>
</table>

**Figure 5:** Antifungal Activity of *Saraca asoca* against *A. niger* and *A. fumigatus*

**Table 6:** Antifungal Activity of *Curcuma longa* against *A. niger* and *A. fumigatus*

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Fungal Species</th>
<th>Acetonic Extract (Zone of Inhibition in mm.)</th>
<th>Methanolic Extract (Zone of Inhibition in mm.)</th>
<th>Distilled Water (Control) (Zone of Inhibition in mm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>A. niger</em></td>
<td>14</td>
<td>20</td>
<td>00</td>
</tr>
<tr>
<td></td>
<td><em>A. fumigatus</em></td>
<td>12</td>
<td>22</td>
<td>00</td>
</tr>
</tbody>
</table>

**Figure 6:** Antifungal Activity of *Curcuma longa* against *A. niger* and *A. fumigatus*
DISCUSSIONS

Plants are becoming an important source of potentially useful structures for the developments of new chemotherapeutic agents (Mahesh and Satish, 2008). Medicinal plants have attracted the attention of several biological communities (Penecillia and Magno, 2011). From the present investigation it was observed that the leaves of three medicinal plants viz. Azadirachta indica, Saraca asoca and Curcuma longa possess potential antibacterial activity against E. coli and B. subtilis and antifungal activity against A. niger and A. fumigatus. It has been speculated that the methanolic extracts possessed the highest antibacterial and antifungal properties against the selected bacterial and fungal cultures with Curcuma longa as the best antibacterial and antifungal agent. Acetonic extracts was capable of inhibiting the pathogenic bacterial and fungal species however, lesser antibacterial and antifungal activities were observed when compared to the methanolic extracts, with Azadirachta indica being the least effective against fungal pathogens and Saraca asoca least effective against bacterial pathogens. Similar results were obtained with methanolic and acetonic extracts of Acacia nilotica, Sida cordifolia, Tinospora cordifolia, Withania somnifera and Ziziphus mauritiana when tested against several bacterial and fungal species (Mahesh and Satish, 2008). In another study, methanolic extracts of Eucalyptus camaldulensis inhibited the growth of Bacillus subtilis and Staphylococcus aureus (Babayi et al., 2004). Plants generally produce secondary metabolites which constitute an important source of antimicrobial agent and many pharmaceutical drugs (Varaprasad et al., 2009). The inhibitory effects of plants on microorganisms may be therefore, due to the presence of certain phytochemicals such as flavanoids, alkaloids, phenols and saponins (Babayi et al., 2004).

Therefore, from the present investigation it is observed that leaves of Azadirachta indica, Saraca asoca and Curcuma longa have highly potent medicinal values and can therefore be utilized as the source of antimicrobial compounds.

CONCLUSIONS

It can be concluded that methanolic plant extracts give the maximum inhibition against several pathogenic bacterial and fungal species, the acetonic extracts were also reported to be having antibacterial as well as antifungal properties but, to a lesser extent as compared to those of the methanolic extracts. Thus, it can be said that common plants have great potential as antimicrobial compounds; hence, drug development plant based compounds could be useful in meeting the demand of lesser side effects of synthetic drugs. They can therefore be used for the treatment of several harmful infectious diseases caused by resistant microorganisms.

REFERENCES


