ABSTRACT

Anti microbial activity of three medicinal plants (Murraya, Azadirachta, and Ocimum) on Urinary tract pathogens were investigated. The methanolic extracts of leaf of all three medicinal plants were the potent antimicrobial agent than ethanolic extract. Methanolic extract of all the three plants inhibited the growth of Klebsiella, Escherichia and Serratia while the ethanolic extract inhibited less. The highest antibacterial activity was found against Escherichia with methanolic extract of Leaf and Bark of Murraya and Leaf extract of Azadirachta. Bark extract of Azadirachta Azadirachta showed highest inhibitory activity against Serratia with methanolic extract and leaf with Escherichia. Ocimum leaf extract possess maximum antibacterial activity against Serratia with methanolic extract and leaf with Klebsiella. Escherichia was found to be most sensitive than Klebsiella and Serratia. The highest antibacterial activity was found against all the Urinary tract pathogens with methanolic extract of Leaf and Bark of Murraya.

Keywords: Medicinal Plants, Antibacterial activity, Methanolic extract, Ethanolic extract.

INTRODUCTION

Medicinal plants are part and parcel of human society to combat diseases, from the dawn of civilization. Medicinal plants are considerably useful and economically essential. They contain active constituents that are used in the treatment of many human diseases. The plant extracts have been developed and proposed for use as antimicrobial substances. Plants used in traditional medicine contain a vast array of substances that can be used to treat chronic and infectious diseases.

The use of plant extracts and photochemical, both with known antimicrobial properties can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency. Many plants have been used because of their antimicrobial traits, which are chiefly due to synthesized during secondary metabolism of the plant.

In modern days, the antioxidants and antimicrobial activities of plant extract have formed the basis of many applications in pharmaceuticals, alternative medicines and natural therapy. Recently extracts of plant have provoked interest as sources for their potential uses as alternative medicines for the treatment of many infectious diseases.

Bacterial resistance to antibiotics represents a serious problem for clinicians and the pharmaceutical industry and great efforts are being made to reverse this trend, and one of them is the widespread screening of medicinal plants from the traditional system of medicine hoping to get some newer, safer, and more effective agents that can be used to fight infectious diseases.

Azadirachta indica A. Juss (syn. Melia azadirachta) is well known in India and its neighbouring countries for more than 2000 years as one of the most versatile medicinal plants having a wide spectrum of biological activity. Every part of the tree has been used as traditional medicine for household remedy against various human ailments from antiquity.

Murraya koenigii L. (curry leaf) belonging to family Rutaceae is used as a spice for its characteristic flavour and aroma. It is reported to have anti-oxidant, anti-diabetic, anti-carcinogenic, anti-dysenteric, stimulant, hypoglycaemic and antimicrobial activities. Biologically active carbazole alkaloids are reported to have antimicrobial properties.

Ocimum sanctum commonly known as holy basil or Tulsi a herbaceous sacred plant found throughout India. Essential oils of tulsi have antibacterial with emphasis on anti tuberculosis, antifungal and antiviral properties.

MATERIALS AND METHODS

Plant material

The plants of Mkoenigii (MK), Azadirachta indica (AI) and Ocimum sanctum(OS) were collected from the Local Nursery of Jaipur. Different parts including Leaf, Bark and Roots were separated, washed thoroughly with distilled water, shade dried, powdered using blender and stored.

Solvent Extraction

After authentication the powdered parts were extracted with methanol, ethanol, petroleum ether and acetone using Soxhlet’s apparatus for 12-14 h. The extracts were concentrated, percentage yield calculated and then subjected to preliminary phytochemical analysis.

Antimicrobial Activity

The in vitro screening for antimicrobial study was carried out using selected urinary tract infection (UTI) causing pathogens which includes three gram negative bacteria (Escherichia coli, Klebsiella pneumoniae and Serratia marcescens).

These organisms were identified by following the standard microbiological methods. The antibacterial screening of the extracts were carried out by determining the zone of inhibition using well diffusion method. The strains of microorganisms obtained were inoculated in conical flask containing 100 ml of nutrient broth. These conical flasks were incubated at 37°C for 24 h and were referred to as seeded broth.

Different concentrations of the extracts were prepared by reconstituting with methanol and ethanol. The test microorganisms were seeded into respective medium by spread plate method 10 µl (10 cells/ml) with the 24h cultures of bacteria growth in nutrient broth. One ml of this was used in flooding over nutrient agar plates in the well diffusion method of the in vitro antimicrobial sensitivity test.

The plates were left for 5mins after which they were dried at 37°C for 1hour. Four wells, equally distant, were bored round the plate using a sterile cork borer. Various concentrations of the diluted extracts were put inside the wells. Solvents such as Methanol and ethanol were put inside the well in separate petriplates to serve as negative control while Chloramphenicol (1mg/ml) was used as positive control in the separate petriplates. The plates were left free for 1 hour after which there were incubated at 37°C for 24 hours and were examined for zones of inhibition.

Phytochemical Screening

The methanolic extracts of different plants were used as samples for qualitative phytochemical screening for tannins, alkaloids, glycosides, terpenoid, steroid and flavonoids following the standard procedures of Trease and Evans, 1989.
towards this goal is the development of new chemotherapeutic agents. The first step
Plants are important source of potentially useful structures for the Murraya
Urinary tract pathogens with methanolic extract of Leaf and Bark of
2).
Ocimum
Escherichia
extract of this plant showed highest inhibitory activity against against
Serratia.
Kleibsiiella
Phytoconstituents OS AI MK
Carbohydrate - - -
Tannin + + -
Alkaloid + - +
Flavonoids - + -
Steroid + - -
Glycoside + - -

(+): Present; (-): Absent

Table 1: The phytochemical screening of the Plant extracts.

Table 2: Antimicrobial activity of methanol and ethanol extracts of plants against UTI Pathogens

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Organic solvent</th>
<th>Leaf (mm)</th>
<th>Bark (mm)</th>
<th>Leaf (mm)</th>
<th>Bark (mm)</th>
<th>Leaf (mm)</th>
<th>Chloramphenicol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Methanol</td>
<td>27</td>
<td>24</td>
<td>26</td>
<td>25</td>
<td>21</td>
<td>29</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>Ethanol</td>
<td>19</td>
<td>15</td>
<td>13</td>
<td>14</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Escherichia</td>
<td>Methanol</td>
<td>29</td>
<td>29</td>
<td>18</td>
<td>12</td>
<td>20</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>23</td>
<td>20</td>
<td>21</td>
<td>10</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Serratia</td>
<td>Methanol</td>
<td>22</td>
<td>24</td>
<td>22</td>
<td>20</td>
<td>18</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>16</td>
<td>12</td>
<td>12</td>
<td>17</td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>

Values are statistically significant at (p<0.05)

RESULTS AND DISCUSSION
The phytochemical analysis of the leaf powder and various extracts gave the results as depicted in Table-1.

Results obtained from the susceptibility testing of the organisms revealed that the tested three medicinal plants extracts possess potential antibacterial activity against Klebsiella, Escherichia and Serratia. When tested by agar diffusion method the methanolic extract was the potent antimicrobial agent than ethanolic extract. Methanolic extract inhibited Klebsiella, E.coli and Serratia while the ethanolic extract inhibited less (Table-2).

The highest antibacterial activity was found against Escherichia (29mm) with methanolic extract of Leaf and Bark of Murraya (Table-2).

Azadirachta leaf extract possess maximum antibacterial activity against Klebsiella (26mm) with methanolic extract (Table 2). Bark extract of this plant showed highest inhibitory activity against Klebsiella (25mm) with methanolic extract and least with Escherichia (12mm) (Table 2).

Ocimum leaf extract possess maximum antibacterial activity against Klebsiella (22mm) with methanolic extract and least with Serratia (Table 2).

Klebsiella was found to be most sensitive than Escherichia and Serratia. The highest antibacterial activity was found against all the Urinary tract pathogens with methanolic extract of Leaf and Bark of Murraya.

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the in vitro antibacterial activity assay 15. Many reports are available on the antiviral, antibacterial, antifungal, antihelminthic, antimolluscal and anti-inflammatory properties of plants 17. Some of these observations have helped in identifying the active principle responsible for such activities and in the developing drugs for the therapeutic use in human beings.

However, not many reports are available on the exploitation of antifungal or antibacterial property of plants for developing commercial formulations for applications in crop protection. The phytochemical research based on ethno-pharmacological information is generally considered an effective approach in the discovery of new anti-infective agents from higher plants 19.

Knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of such economic materials as tannins, oils, gums, precursors for the synthesis of complex chemical substances.

In addition, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies 15. Chemically constituents may be therapeutically active or inactive. The ones which are active are called active constituents and the inactive ones are called inert chemical constituents 20.

REFERENCES
20. Duraipandian V, Ayyanar M and Ignacimuthu S: Antimicrobial Activity of Some Ethnomedical Plants Used by Paliyar Tribe from Tamil Nadu, India. BMC complementary and alternative medicine 2006; 635.