ANTIMICROBIAL ACTIVITY OF PLANT EXTRACT OF SPHARANTHUS INDICUS L. AGAINST UTI CAUSING MICROORGANISM.

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ABSTRACT
The aqueous leaf extract and methanolic leaf extract of Spharanthus indicus L. showed antibacterial activity against extended spectrum beta lactamase (ESBL) producing bacteria E.coli, Pseudomonas, Klebsiella, MRSA (methicillin- resistant staphylococcus aureus), Salmonella, and Proteus. Methanolic leaf extract of Spharanthus indicus L. showed highest antibacterial activity against Klebsiella, E.coli (15mm, 14mm) and significant against other. The main purpose of the study is to eradicate the urinary tract infection problem across the world by using medicinal plants.

KEYWORDS: Antimicrobial, ESBL, Medicinal Plant, Aqueous extract.

INTRODUCTION
Plants are major source of herbal medicine and the presence of secondary metabolites in plants implicated them for many therapeutical activities. Also, the plants had provided a source of inspiration for novel drug compounds, as plants derived medicines have made large contributions to human health and well being. A major part of total population in developing countries still uses traditional folk medicine obtained from plant resources. The people of Indian villages used crude plants as medicine since Vedic period and still continued.

Worldwide, the infectious diseases caused the death of approximately one-half of all deaths in tropical countries. Perhaps the infectious diseases statistics should be very high and frequent in developing nations, but it might be remarkable that infectious diseases mortality rates were actually increasing in developed countries.
Nowadays, the herbal drugs popularity increased and used widespread. The research is still lagging behind to get the efficiency of plant derived medicines on microorganisms which induced pathogenesis in human beings and other animals. There were reports of various medicinal plants that had been used for years in daily life to treat disease all over the world. There were several reports on the antimicrobial activity of different herbal extracts in different regions of the world. Phytomedicines derived from plants have shown great promise in the treatment of intractable infectious diseases. The most important bioactive constituents of these plants are alkaloids, tannins, flavonoids and phenolic compounds. Stiffness and Douros reported that more than 50% of all modern clinical drugs were origin from natural products. The natural products might be played an important role in drug development programs in the pharmaceutical industry. It has been reported that aqueous and methanolic extracts from plants used in allopathic medicines were potential sources of antiviral, antitumoral and antimicrobial agents. The higher plants, as a source of medicinal compounds, had continued to play a dominant role in the maintenance of human health since ancient times.

In developing countries, infectious diseases remain the main cause of the high mortality rates recorded; the majority of rural people has limited access to formal and adequate health services and thus heavily resources to traditional healers (WHO, 1996). Indigenous herbal remedies are widely used against many infectious diseases, but only few of them have been scientifically investigated with their active component isolated and characterized. In modern medical practice, the alarming worldwide evidences of antibiotic resistance cause an increasing need for new compounds that can act either by inhibiting resistance mechanisms of microorganisms of medicinal importance. Medicinal plants represent a valuable source for this kind of compounds.

Urinary tract infections are serious health problem affecting millions of people each year. Urinary tract infection is defined as the proliferation of active microorganisms inside the urinary channel which are harmful to their environment. Bacteriuria is the isolation of bacteria in the urine specimen. Presence of $1 \times 10^5$ or more colony forming units (CFUs) of the same active microorganism per milliliter in two consecutive urine specimens has been a historically threshold value for the diagnosis of bacteriuria (Stauffer et al., 2004). The incidence of UTI is higher among females, in whom it commonly occurs in an anatomically normal urinary tract. Conversely, in males and children, UTI generally reveals a urinary tract
lesion that must be identified by imaging and must be present today are greater than they were in the past. The first bacteria that were detected to be resistant to several antibiotics were reported in Japan during 1950s (Schlegel & Schmidt, 1985). Main purpose of the study is to make suitable drug against UTI with the help of medicinal plant as number of antibiotics has become resistant to ESBL producing bacteria cause urinary tract infection.

**MATERIALS AND METHODS**

**Collection of ESBL producing bacterial strain sample**

Bacterial sample of *MRSA, E coli, Pseudomonas, Klebsiella, Salmonella and Proteus* were Collected from peoples general hospital bhopal were Identified by gram staning and biochemical characterization.

**Collection of plant**

*Spharanthus indicus L.*Was collected from Bhopal region and identified by Dr.Padma Shrivastava HOD, Department of Biotechnology, Govt. College, BHEL, Bhopal (M.P). The plant material was thoroughly washed with water and was kept for drying in shade at room temperature for 24 days. The thoroughly air dried plant material was grinded to powder to about 40-50 mesh size weighted and stored in large plastic bottles.

**Antimicrobial activity of medicinal plant**

<table>
<thead>
<tr>
<th>Sample No</th>
<th><em>Spharanthus indicus L.</em> (aqueous)</th>
<th><em>Spharanthus indicus L.</em> (Methanolic)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteus</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>E Coli</td>
<td>9</td>
<td>14</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td>MRSA</td>
<td>13</td>
<td>16</td>
</tr>
<tr>
<td>Salmonella</td>
<td>7</td>
<td>14</td>
</tr>
</tbody>
</table>
DISCUSSION

Out of these three the maximum zone of inhibition was observed in the methanolic extract of *Spharanthus indicus* L. against *Klebsiella*, *Proteus* & *E.coli*, *MRSA* and *Pseudomonas* respectively. While work done by Anjana Sharma (2009) using aqueous extract of plant *Terminalia chebula* and *Zinziiber officinale* showed the zone of inhibition against *Proteus* (1mm, 0mm), *E.coli* (9mm, 0mm), *Pseudomonas aeruginosa* (5mm, 4mm) and *Klebsiella pneumoniae* (5mm, 6mm) respectively, thus our studied plant is showing more effective results and can be used to prepare drug against disease caused by ESBL producing bacteria.

On the other hand work done by Aliyu et al (2008) by using aqueous extract of plants *Vernonia blumeoides* and *Phyllanthus amarus* against *MRSA* observed the maximum zone of inhibition is 13mm and 15mm respectively which is also less effective than our studied plants.

Urinary tract infection (UTI) is among the most common infections ranking next to upper respiratory infection with an increasing resistance to antimicrobial agents. These ailments affect patients in all age of groups and sexes. Majority of UTIs are not life threatening and do not cause any irreversible damage. Multiple antimicrobial resistances among gram negative organisms have been a long term and well recognized problem with urinary tract infection. Resistance has been observed in multiple genera including *E.coli, Enterobactor, Klebsiella, Proteus, Salmonella, Serrata, and Psedomonas* *Escherichia coli* has been documented as the most important pathogen associated with urinary tract infection in many countries. These bacteria normally live in the intestine, but they sometime get into the urinary tract. The incidence of UTI is greater in women than men who may be either due to anatomical predisposition or urothelial mucosal adherence to the mucopolysaccharide lining or other host factor. The ESBL (Extended Spectrum of Beta-Lactamase) producing bacteria has been

Figure 1: Zone of inhibition showed by aqueous and methanolic leaf extracts of *Spharanthus indicus* L. against ESBL producing bacteria.
identified in members of Enterobacteriaceae, are increasingly causing UTI. Some of the major treated to suppress the cause of infection and prevent recurrence. UTI can be restricted to the bladder (essentially in females) with only superficial mucosal involvement, or it can involve a solid organ (the kidneys in both genders, the prostate in males). Multiple drug resistance has significantly increased in recent years. The existence of enzymes of Extended-Spectrum β- Lactamase (ESBLs) producing organism that are resistant to virtually all β-lactam antibiotics have been reported (Philippon et al., 1989). Escherichia coli, Klebsiella, Streptococcus pyogenes, S. faecalis, Pseudomonas vulgaris, P. faecalis Proteus vulgaris MRSA are the bacteria responsible for Urinary tract infection.

**Preparation of extract**

For this purpose, dried powdered of leaves were used for extraction with aqueous solvent by sonicator and methnolic solvent by Soxhlet apparatus. Then water bath the solvent at 70-800 C in china dish to get semisolid crude extract.

**Determination of the antimicrobial activity of medicinal plant**

From the crude extract, the 500mg/ml dilution of plant paste was prepared for antimicrobial assay. The modified agar well diffusion method was employed to determine the antimicrobial activity of plant extract, 200μl of the extract (500mg/ml) were poured in to the well. All the plates were incubated at 370C for 24 hrs and zone of inhibition were observed in the form of mm (mili meter).

**RESULTS**

*Spharanthus indicus* L. showed significant antimicrobial activity against ESBL producing bacteria contributing factor for high level of urinary tract infection (UTIs) in predominantly areas is poor sanitary conditions and lack of proper hygiene. One of the most common UTIs is leucorrhea among women, characterized by whitish discharge from female genitalia.

**CONCLUSION**

From our study we concluded that the prevalence of ESBL producing bacteria in UTI patients are increasing day by day and antibiotics are failed to cure. So, the medicinal plants are the best alternate for treating such ESBL producing bacteria because antibiotics are expensive to use, in our study out of three plants methanolic extract of *Spharanthus indicus* L. showed significant antimicrobial activity against all six tested bacteria (E.coli, Klebsiella, Proteus, Salmonella, MRSA and Pseudomonas). So we can use the medicinal plant extracts
individually as well as in combination for better and cheaper treatment, because of high medicinal value of the plant.

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