IN-VITRO EVALUATION OF ANTIMICROBIAL POTENTIAL OF AYURVEDIC POLY-HERBAL FORMULATION: SUDARSHAN CHURNA

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ABSTRACT
Ayurvedic medicine plays a crucial role in healthcare and serves the health need of a vast majority of people in developing countries. Sudarshan Churna (SC) is very potent ayurvedic medicine; composed of 42 medicinal plants, which is used traditionally in treatment of malaria, viral fever, and bacterial infection. In this study the activity of this formulation was compared with the standard antibiotics like Amikacin and Norfloxacin. Ethanol, methanol and acetone extract of Sudarshan Churna demonstrated good antimicrobial activity and thus can form the basis for the development of a novel antibacterial formulation.

KEY WORDS: Sudarshan Churna, antimicrobial activity, ayurvedic medicine.

INTRODUCTION
In the last few decades, there has been an exponential growth in the field of ayurvedic medicine (Indian Traditional System of Medicine (1). Herbal medicines are being used increasingly as dietary supplements to fight or prevent common diseases (2). The search and use of drugs and dietary supplements of plant origin have accelerated in recent years. Ethnobotanists, pharmacologists, microbiologists and natural product chemists are combing the earth for phytochemicals which could be developed for treatment of infectious diseases.
Out of 25 to 50% of current pharmaceuticals derived from plants, very few are used as antimicrobials. Since time immemorial, the traditional physicians have been using plants for prevention or cure of infectious conditions. Western medicine is trying to duplicate their success (3). Today 80% of the world’s population in African, Asian, Latin American and Middle Eastern countries is using plants as traditional health remedies due to minimal side effects (4-6). In present scenario, pharmaceutical companies are investing significant amount of time and money for development of therapeutics based upon natural products obtained from plants (7-8). It is well known that use of most of the modern antimicrobials is fraught with adverse effects. The problem gets complicated since these antimicrobials are used for an extended period of time. Therefore, there is need to explore antibacterial activity of certain herbs and to create evidences for their efficacy. In case of Sudarshan Churna the key ingredient is *Swertia chirata* which constitutes 50% of its total composition (9). Ayurvedic Churnas are solid dosage form of medicaments meant for internal use. The dose is 1-2 tea spoonful which may be increased or decreased according to age and severity of disease. Churnas can be administered with water, milk, fruit juices or any other suitable liquid depending on the nature of disease. They may be given by mixing with honey in equal quantity, with sugar twice the quantity and with the milk four times the quantity as that of the drug (10). The *Sudarshan Churna* was procured from local market of Faridkot and subjected to study the *in-vitro* antimicrobial activity to justify the traditional claims of the Polyherbal formulation.

The dose of Sudarshan Churna is 2-4 gm.

**Therapeutic uses**

The *Sudarshan Churna* is used to treat Yakrt (Liver), Pliha vrddhi (Splenomegaly), Jvara (Fever), Visama jvara (Intermittent fever), Jirna jvara (Chronic fever), and Gulma (Abdominal Lump) (11).

**MATERIAL & METHODS**

**Drugs and chemicals**

The present study was conducted in the Department of Microbiology, Government Medical College, Faridkot. *Sudarshan Churna* was procured from the local market of Faridkot.

**Microbial strains**

The various different strains of microorganisms were used and were purchased from M.T.C.C. Institute of Microbial Technology (IMTECH) Chandigarh in lyophilized form with
complete detail of growth media required for converting these to the active culture. These are as follows *Pseudomonas aeruginosa* (MTCC 424), *Staphylococcus aureus* (MTCC 3160), *Klebisiella pneumonia* (MTCC 3384), *Bacillus subtilis* (MTCC *121*), *Escherichia coli* (MTCC 739).

**Extraction of drug material**
Method of Parekh *et al.* (2005) was used for the extraction of drug material (after some modifications). The aqueous extract was prepared by adding 20 g of herbal preparations in 200 ml distilled water, heated at 60°C for 2 h, filtered and the filtrate was evaporated on sand bath. The dry mass (3.6%w/w) was then stored at 4°C. The organic solvent extract was prepared by adding 20 g herbal preparation (powder) in 200 ml of organic solvent (acetone, ethanol and methanol) in screw-capped bottles and was put at 190-220 rpm on a rotary shaker. After 24 h of shaking, the extract was filtered, evaporated in vacuum and dried by rotary evaporator at 60°C (12-13). Dried extracts (2.9%, 3.1%, 3.7% w/w respectively) were stored in labeled sterile screw capped bottles at 4°C and later used for the *in vitro* study.

**Formulation of extract**
A known amount of powder was suspended in corresponding solvent to get desired concentration of suspension for the study of antimicrobial activity on the day of experimentation (14).

**Antimicrobial activity**
Antibacterial activity was determined by using Disc Diffusion Method. The impregnated filter paper discs were employed to determine the antibacterial activity of both aqueous and organic solvent extracts of herbal preparation (15). For antibacterial properties, 0.1 ml bacterial suspension of $10^5$CFU ml$^{-1}$ was swabbed on Nutrient Agar plate to form lawn culture. The aqueous, acetone, ethanol and methanol extracts were prepared in their respective solvents. The filter paper discs (6mm in diameter) were separately impregnated with different concentrations of extract and then placed on the agar plates which had previously been inoculated with the test microorganisms. Discs were soaked in various organic solvents, dried and were placed on lawns as negative control. After incubation of 24 h at 37°C, zone of inhibition of growth was measured in mm. The % inhibitory concentrations of the different extracts were measured and compared with the antibiotics (16-17) like Amikacin (30 µg) and Norfloxacin (10 µg) as standard.
RESULTS

From table it is evident that Ethanolic extracts of formulation showed more activity against *Pseudomonas aeruginosa* as compared to other extracts of the formulation, Acetone extract showed more significant activity against *Staphylococcus aureus* which is comparable with standard antibiotics, while ethanol extract also showed significant antimicrobial activity against *Staphylococcus aureus* which is comparable with norfloxacin (as shown in the table). *Klebsiella pneumoniae* showed sensitivity against ethanol and acetone extract which is comparable to norfloxacin while in case of *Bacillus subtilis* the methanol extract shows significant antimicrobial activity which is nearby the activity shown by standard antibiotics norfloxacin on the other hand *Escherichia coli* was found sensitive to methanol, ethanol and acetone extracts of formulation which is comparable with standard antibiotics.

ANTIMICROBIAL ACTIVITY

Table Antimicrobial activity of different extract of Sudarshan churna

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Microbial strains</th>
<th>Type of Extract</th>
<th>Zone of inhibition at different concentration (%) of extracts (mm)</th>
<th>Standard Antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Methanol</td>
<td>100 80 60 40 20 C</td>
<td>Amikacin (30 µg)</td>
</tr>
<tr>
<td>1.</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>15 13 12 8 - 7</td>
<td></td>
<td>Norfloxacin (10 µg)</td>
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<tr>
<td></td>
<td>(MTCC 424)</td>
<td>Ethanol</td>
<td>20 17 14 12 - 7</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Acetone</td>
<td>17 13 12 7 - 7</td>
<td>34</td>
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<td></td>
<td></td>
<td>Water</td>
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<tr>
<td>2.</td>
<td><em>Staphylococcus aureus</em></td>
<td>18 16 10 8 - 6</td>
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<tr>
<td></td>
<td>(MTCC 3160)</td>
<td>Methanol</td>
<td>18 16 10 8 - 6</td>
<td>23</td>
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<td></td>
<td></td>
<td>Ethanol</td>
<td>18 12 11 9 - 7</td>
<td>17</td>
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<td></td>
<td></td>
<td>Acetone</td>
<td>21 16 11 9 - 7</td>
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<td></td>
<td>Water</td>
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<td>3.</td>
<td><em>Klebsiella pneumonia</em></td>
<td>17 12 8 - - 6</td>
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<td></td>
<td>(MTCC 3384)</td>
<td>Methanol</td>
<td>17 12 8 - - 6</td>
<td>27</td>
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<td></td>
<td></td>
<td>Ethanol</td>
<td>20 13 12 9 - 7</td>
<td>19</td>
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<td></td>
<td></td>
<td>Acetone</td>
<td>20 18 16 12 - 9</td>
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<td>Water</td>
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<td>4.</td>
<td><em>Bacillus subtilis</em></td>
<td>18 13 11 8 - -</td>
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<td></td>
<td>(MTCC *121)</td>
<td>Methanol</td>
<td>21 17 14 10 - 8</td>
<td>34</td>
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<td></td>
<td></td>
<td>Ethanol</td>
<td>18 13 11 8 - -</td>
<td>25</td>
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<td></td>
<td>Acetone</td>
<td>18 15 10 7 - -</td>
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<td>Water</td>
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<td>5.</td>
<td><em>Escherichia coli</em></td>
<td>19 17 14 9 - 6</td>
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<td></td>
<td>(MTCC 739)</td>
<td>Methanol</td>
<td>19 17 14 9 - 6</td>
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<td></td>
<td></td>
<td>Ethanol</td>
<td>21 20 17 13 8 8</td>
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<td>Acetone</td>
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<td>Water</td>
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MTCC- Microbial Type Culture Collection C- Control
DISCUSSION

*Sudarshan Churna* contains 42 different constituents including 50% of *Swertia chirata* Buch Ham and the formulation is described in the ancient ayurvedic literature. A survey on the activities of the constituents revealed that *Swertia chirata*, *Ureria picta*, *Curcumma longa*, *Terminalia chebula*, *Asparagus racemosus*, *Acorus calamus*, *Zingiber officinale*, *Azadiracta indica*, *Glycerrhiza glabra* are reported to be effective as antimicrobial herbs (18-23). The Sudarshan Churna contains flavonoids and sterol, which may responsible for antimicrobial activity (17, 24-25).

The reported antimicrobial activity in the present study seems to be the outcome of antimicrobial action of its active components like flavonoids and sterols.

CONCLUSION

Our findings suggest that the Ayurvedic herbal preparation *Sudarshan Churna* extracts have antimicrobial properties and they can be used in the treatment of infectious diseases. On comparing the zone of inhibition of *Sudarshan Churna* extract to that of standard antibiotics (Amikacin and Norfloxacin) it is concluded that, the formulation in question shows activity against *E. coli*. whereas the standard antibiotics shows no activity i.e. the bacterial strain is resistant towards these two antibiotics. The most active extract can be further evaluated pharmacologically as well as for its chemically active components in the formulation.

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