INHIBITORY EFFECT OF DIFFERENT PLANT EXTRACTS ON SALMONELLA TYPHIMURIUM GROWTH

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ABSTRACT

The present study aimed at evaluating the in vitro antimicrobial activity of hot and cold aqueous extracts of some medicinal plants against Salmonella typhimurium growth. The hot and cold aqueous extracts of Ocimum sanctum (Tulsi), Annona reticulate (Custard apple), Lawsonia sp. (Mehndi) and Mentha piperita (Pudina) were used for the study. The hot and cold aqueous extracts of Lawsonia sp. leaves presented the highest inhibitory activity having a mean MIC value of 0.001 mg/ml and 0.01 mg/ml, respectively on Salmonella typhimurium growth compared to other leaves extracts.

Key-words: Medicinal plants, Antimicrobial activity, Minimal inhibitory concentration.

INTRODUCTION

Since the discovery of penicillin in 1929 by Alexander Fleming, the search and manufacture of antibiotics continues to be on a steep rise. However, problems like the development of allergy, an increase in the number of antibiotic resistant strains and adverse side effects on the body leads to a serious search for alternate medicines. Medicinal plants are considerably useful and economically essential, as the active ingredients present in them are found to be effective in reducing microorganisms. Plant extracts, including their essential oils and essences, have been recognized that they possess antimicrobial properties against bacteria, moulds, and yeast (Smith Palmer et al., 1998; Ekwenye and Njoku, 2006; Ghosh et al., 2007).

The demand for more and more drugs from plant sources is continuously increasing. It is therefore essential to evaluate plants of medicinal value systematically for various ailments that are used in traditional medicine. Hence, there is a need to screen medicinal plants for their promising biological activity. The present study aimed at evaluating the in vitro antimicrobial activity of Ocimum sanctum (Tulsi), Annona reticulate (Custard apple), Lawsonia sp. (Mehndi) and Mentha piperita (Pudina) were used to test their inhibitory effect against the bacteria Salmonella typhimurium.

MATERIAL AND METHOD

Plant material: Leaves of medicinal plants Ocimum sanctum (Tulsi), Annona (Custard apple reticulate), Lawsonia sp. (Mehndi) and Mentha piperita (Pudina) growing in the premises of the college were used to prepare the aqueous extract.

Bacterial culture: Salmonella typhimurium culture was procured from the Institute of Microbial Tech (IMTECT) Chandigargh. Strain no. MTCC No. 98.

Preparation of plant extracts: Surface of leaves was wiped gently with a moist muslin cloth. After which the leaves of Ocimum sanctum (Tulsi), Annona reticulate (Custard apple), Lawsonia sp. (Mehndi), Mentha piperita (Pudina) were shade dried at room temperature (28-35 °C). The dried leaves were powdered and sieved through a sterile muslin cloth. The aqueous extracts of leaves from the four plants were prepared, by the method proposed by Tandle et al. (1986).

Cold aqueous extract: One gram of leaves powder of Ocimum sanctum, Annona reticulate, Lawsonia sp. and Mentha piperita was added to sterile 10 ml distilled water and left overnight at room temperature. It was then filtered.
Hot aqueous extract: One gram of leaves powder of *Ocimum sanctum*, *Annona reticulata*, *Lawsonia* sp. and *Mentha piperita* was added to sterile 10 ml distilled water and heated for 5 min. The extract was then cooled and filtered volume was made up to 10 ml with sterile distilled water.

Preparation of bacterial inoculum: A 24 hr. broth culture of *Salmonella* having a cell count of 10⁹ cells/ml was used to inoculate in the dilution tubes. The count was determined with the help of brown’s opacity tubes. The purity and cultural characteristics of *Salmonella typhimurium* were observed by isolating pure culture in differentiating and selective media i.e., Mac-conkey and BGA (Brilliant green agar). Growth from single colony was stained by the Gram’s Method. Motility was determined by hanging drop preparation.

Assessment of the minimum inhibitory concentration (MIC): The MIC of cold aqueous (Caq) and hot aqueous (Haq) extract of *Ocimum sanctum*, *Annona reticulata*, *Lawsonia* sp. and *Mentha piperita* were assessed by the standard tube dilution method Table 1 (Cruickshank et al., 1980). Serial dilutions of the extracts were prepared in peptone water. To all tubes of the series, a loop full of bacterial inoculum was added from the 24 hrs standardized broth culture. The tubes were incubated at 37°C for 24 hour and then examined for the turbidity indicated by bacterial growth. The highest dilution of the extract which showed no turbidity was considered as the MIC of that particular extract against the *Salmonella typhimurium*. The observation was confirmed by streaking a loop full from the tube on sterile nutrient agar slants, incubating at 37°C for 24 hr and observing for growth. Absence of growth confirmed complete inhibition of bacterium.

<table>
<thead>
<tr>
<th>Plants</th>
<th>Cold extract (mg/ml)</th>
<th>Hot extract (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ocimum sanctum</em> (Tulsi)</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td><em>Annona reticulata</em> (Custard apple)</td>
<td>10</td>
<td>0.1</td>
</tr>
<tr>
<td><em>Lawsonia</em> sp. (Mehndi)</td>
<td>0.001</td>
<td>0.01</td>
</tr>
<tr>
<td><em>Mentha piperita</em> (Pudina)</td>
<td>1</td>
<td>0.1</td>
</tr>
</tbody>
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RESULTS AND DISCUSSION
The cold and hot aqueous extracts of *Lawsonia sp.* were the most effective inhibitor having a mean MIC value of 0.001mg/ml and 0.01mg/ml respectively on *Salmonella typhimurium* growth compared to other leaves extracts. Among cold aqueous extracts, *Lawsonia sp.* leaves extracts had the least MIC value of 0.001mg/ml followed in a decreasing order of effectiveness by *Mentha piperita* (1mg/ml). The extracts of *Ocimum sanctum* and *Annona reticulata* leaves were the least effective with an MIC of 10mg/ml. Among hot aqueous extracts, *Lawsonia sp.* leaves extracts had the most effective inhibitors followed by *Annona reticulata* and *Mentha piperita* leaves extracts having the same MIC value of 0.1mg/ml. *Ocimum sanctum leaves* were the least effective inhibitor. In case of *Lawsonia sp.* the Caq was a better inhibitor compared to its Haq. While the Haq of Custard apple leaves and *Mentha piperita* were better inhibitors compared to their Caq. In case of *Ocimum sanctum both* the hot and cold aqueous extract had the same MIC value of 10mg/ml. Results of the present study were in line with the scientific investigations of Ekwenge and Njoku (2006). They tested the entire plant extracts of *Phyllanthus niruri* for its antibacterial effect on *E. coli*, *S. aureus* and *Salmonella typhi*. The aqueous and ethanol extracts of *P. niruri* showed high inhibition against *S. aureus*, *E. coli* and *S. typhi*. Both aqueous and ethanol extracts were inhibitory to the above said organisms. Ghosh et al. (2007) studied antibacterial activity of *Terminalia bellerica*,
**Terminalia chebula, Emblica officinalis, Punica granatum and Lawsonia inermis.** Shan *et al.*, (2007) also found the inhibitory effect of clove extracts against *S. anatum*. This result suggests that hot and cold aqueous extract of *Lawsonia sp. (Mehndi)* is useful as antimicrobial agent to reduce the microbial level of *Salmonella typhimurium*.

**REFERENCES**


