**IN-VITRO ANTIMICROBIAL ACTIVITY OF OCIMUM BASILICUM LINN LEAF EXTRACTS**

Balamurugan, S

Botany wing (DDE), Division of Plant Pathology, Annamalai University, Annamalainagar – 608002(Tamil Nadu), India

**ARTICLE INFO**

**Article History:**
Received 10th December, 2012
Received in revised form 10th, December, 2012
Accepted 5th January, 2013
Published online 17th January, 2013

**Key words:**
Antimicrobial, antifungal, antibacterial, Ocimum basilicum, Labiatae

**ABSTRACT**

In-vitro antimicrobial activity of leaf extracts of Ocimum basilicum Linn. was assessed by disc diffusion methods against three bacterial strains (Bacillus subtilis, Escherichia coli and Salmonella typhi) and two fungal strains (Aspergillus niger and Candida albicans). Minimum inhibitory concentration was also determined. The most susceptible bacterial and fungal strains were E.coli and Calbicans, respectively. The leaf extracts in organic solvents (hexane, methanol and chloroform) showed good antimicrobial activity. Results of the present investigation indicate that leaf of Ocimum basilicum possess antimicrobial properties and hence can be exploited for future natural plant based antimicrobial agents.

**INTRODUCTION**

Biological screening of plant extracts is carried out throughout the world for the determination of their antimicrobial & antibacterial activity. Synthetic chemicals used to control plant diseases not only pollute the environment, but are also harmful to human health. Because of environmental and economic considerations, plant scientists are involved to find the cheaper and more environmental friendly bio-compounds for the control of plant diseases using touches from different plants. Many studies have shown that aromatic and medicinal plants are sources of diverse nutrient and non nutrient molecules, many of which showed antioxidant and antimicrobial properties which can protect the human body against both cellular oxidation reactions and pathogens.

Ocimum basilicum Linn. a member of the family Labiatae, is an evergreen erect aromatic plant found throughout India. Traditionally, it is used as an analgesic, aromatic, thermogenic, carminative and for the treatment of dysentery and skin eruptions due to the aromatic nature of leaves. Carbazole alkaloids, the major constituents of the plants are known to possess cytotoxic, antioxidative, antimutagenic and anti-inflammatory activities. The leaves are rich in monoterpenoids and sesquiterpenoids which exhibited antifungal activities. In the present investigation, an attempt has been made to study the antimicrobial screening of leaf extracts of Ocimum basilicum Linn. against pathogenic microorganisms.

**MATERIALS AND METHODS**

**Collection of plant materials**

The plants of Ocimum basilicum Linn. were collected from the Experimental Orchards, Department of Agriculture, Annamalai University, Annamalainagar, Tamil nadu, India. The plant was identified and voucher specimen was deposited to the Herbarium, Department of Botany, Annamalai University. Leaves were separated, washed thoroughly with distilled water, shade dried, powder using blender and stored in air tight container until for further use.

**Preparation of leaf extracts**

The powdered leaves (500g) were extracted with hexane, methanol and chloroform using Soxhlet’s apparatus for 12-14 h. The organic extracts were separately filtered with Whatman No.1 paper and evaporated to dryness on water bath to obtain semi-solid mass. However, aqueous extraction of leaves is performed by using hot water maceration. The dried extracts were stored at 5°C in the refrigerator until for further study.

**ANTIMICROBIAL SCREENING**

**Test microorganisms**

In-vitro antimicrobial activity was evaluated against five pathogenic microorganisms, Gram positive Bacillus subtilis, (ATCC 6633), Gram negative Escherichia coli (ATCC 25922), Salmonella typhi (ATCC 3492) and fungal strains Aspergillus niger (ATCC 322) and Candida albicans (ATCC 4718). All these microorganisms were obtained from Rajah Muthiah Medical College Hospital (RMMCH), Annamalai University. The bacterial culture were grown and maintained on Nutrient Broth medium at 37°C for 24 h while the fungal cultures were maintained on Potato Dextrose Agar slants and incubated at 27°C for 48 hours.

**Antimicrobial assay**

Antimicrobial assay of crude extracts was performed against five test pathogenic strains by disc diffusion method. The nutrient agar and potato dextrose agar plates were seeded with suspension (10^6cfu/ml) of the bacterial and fungal strains, respectively. The sterilized Whatman no.1 filter paper disc (6 mm) were impregnated with (1000μg/ml) of extract, dried and placed aseptically on seeded plates with the help of a sterile forceps. Later on these plates were kept at room temperature for 30min (pre diffusion time). The standard discs (6 mm) impregnated with antibiotics Streptomycin (2 μg/ml) and Fluconazole (2 μg/ml) were used as control. The plates were
incubated at 37°C for 24 h and 25°C for 48 h for bacteria and fungi, respectively. The diameter of the inhibition zone (mm) was measured and in each case the activity index was calculated. The experiment was repeated three times and the mean values calculated for conclusion.

Minimum inhibitory concentration of extract against tested microorganisms was determined by broth dilution method1. For broth dilution, 1 ml of standardized suspension of strain (10^6 cfu/ml) was added to each tube containing extracts at various concentrations in nutrient broth medium. The tubes were incubated at 37°C for 24 h (for bacterial strains) and 25°C for 48 h (for fungal strains) and observed for visible growth after vortexing the tube gently. The experiment was repeated two times. The minimum inhibitory concentration (MIC) is taken as the lowest concentration of the extracts at which there is turbidity after incubation.

RESULTS AND DISCUSSION
The antimicrobial efficacy of the extracts of Ocimum basilicum leaves quantitatively assessed on the basis of inhibition zone, activity index (Table – I) and minimum inhibitory concentration (Table –II). In the present investigation, all the extracts (hexane, methanol and chloroform) were found to be effective against tested pathogenic strains except aqueous extract. Methanol extract showed more pronounced antimicrobial activity than other extracts. Among the test bacterial strain, the most susceptible bacterium to the extract (hexane, methanol and chloroform) was Bacillus subtilis, which is known to play significant role in skin diseases38. It indicates that leaves of Ocimum basilicum may possess compounds with antimicrobial properties which are effective against infectious diseases. Earlier the extracts of Ocimum basilicum were shown to possess bioactive compounds having anti-oxidative, anti-mutagenic and hypoglycemic activities39.

Table 1 Antimicrobial activity of various organic extracts of leaves of Ocimum basilicum L.

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Hexane</th>
<th>Methanol</th>
<th>Chloroform</th>
<th>Aqueous</th>
<th>Streptomycin (2μg/ml)</th>
<th>Fluconazole (2μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IZ</td>
<td>AI</td>
<td>IZ</td>
<td>AI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>8.00</td>
<td>0.33</td>
<td>15.00</td>
<td>0.75</td>
<td>7.00</td>
<td>0.31</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>8.00</td>
<td>0.30</td>
<td>10.00</td>
<td>0.41</td>
<td>9.00</td>
<td>0.31</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>7.00</td>
<td>0.35</td>
<td>9.00</td>
<td>0.50</td>
<td>6.00</td>
<td>0.33</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>7.00</td>
<td>0.36</td>
<td>9.00</td>
<td>0.52</td>
<td>8.00</td>
<td>0.47</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>7.00</td>
<td>0.38</td>
<td>14.00</td>
<td>0.70</td>
<td>8.00</td>
<td>0.44</td>
</tr>
</tbody>
</table>

IZ = Inhibition Zone (mm); AI = Activity Index; - = No activity; Values are the mean of three replications.

Table 2 Determination of MIC of various organic extracts of leaves of Ocimum basilicum L.

<table>
<thead>
<tr>
<th>Organic extracts</th>
<th>Hexane</th>
<th>Methanol</th>
<th>Chloroform</th>
<th>Aqueous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus subtilis</td>
<td>0.313</td>
<td>0.078</td>
<td>0.625</td>
<td>-</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>0.628</td>
<td>0.312</td>
<td>0.312</td>
<td>-</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>0.628</td>
<td>0.628</td>
<td>0.628</td>
<td>-</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>0.628</td>
<td>0.312</td>
<td>0.628</td>
<td>-</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>0.628</td>
<td>0.156</td>
<td>0.628</td>
<td>-</td>
</tr>
</tbody>
</table>

MIC: Minimum Inhibitory Concentration (mg/ml) of the extract; Values are the means of two replications.

In antifungal screening, the methanol extract showed maximum inhibitory effect against Candida albicans (IZ = 14.00 mm; AI = 0.70) with MIC value of 0.156 mg/ml while hexane extract showed minimum inhibitory effect against Candida albicans (IZ = 7.00 mm; AI = 0.38) with MIC value of 0.628 mg/ml. Aqueous extract also showed no inhibitory effect against tested two fungal strains (Aspergillus niger and Candida albicans). The antifungal activity of leaves of M. koenigii is well documented12-13.

The results of the present study indicate that this plant extract could possibly use as antibiotics. The antimicrobial activity of leaf of Ocimum basilicum is due to the presence of carbazole alkaloids4. The present investigation supports the antimicrobial traditional use of this plant. In vitro antimicrobial activity of extracts against pathogens justifies the folk medicinal use of Ocimum basilicum leaf for the treatment of diarrhoea, dysentery and skin eruptions.

CONCLUSION
The present investigation revealed that the various extracts from the leaf of Ocimum basilicum exhibited antimicrobial properties which explain the basis for its use in traditional medicines to treat skin diseases. The methanol extracts exhibited significant inhibitory activity against pathogenic microorganisms. It showed maximum inhibitory effect against Salmonella typhi and Candida albicans, bacterial and fungal strains respectively.

In antimicrobial screening, the methanol extract showed maximum inhibitory effect against Bacillus subtilis (IZ = 15.00 mm; AI = 0.75) with MIC value of 0.078 mg/ml while hexane extract showed minimum inhibitory effect against Escherichia coli (IZ = 8.00 mm; AI = 0.30) with MIC value of 0.628 mg/ml. However, aqueous extract showed to inhibitory effect against tested three bacterial strains (Bacillus subtilis, Escherichia coli and Salmonella typhi).


********