ANTIMICROBIAL ACTIVITY OF ETHANOLIC LEAF EXTRACTS OF OCIMIUM SANCTUM, MANGIFERA INDICA AND Hibiscus rosa S. INENSIS

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ABSTRACT

Plants are very useful and utilised as medicine due to their medicinal properties. The aim of present study was to investigate antibacterial activity of extracts of three medicinal plants Ocimum sanctum, Mangifera indica and Hibiscus rosa sinensis. The three extracts of medicinal plants evaluated for activity against bacteria such as staphylococcus aureus Bacillus subtilis (gram positive) Eschereria coli, Klebsiella pneumonia (gram negative) & Fungal strain Candida albicans. The invito antimicrobial activity was performed by cup plate method. The Ethanolic extract of Hibiscus rosa sinensis shown maximum activity when compare to remaining two plants.

KEYWORDS: Antimicrobial activity Ocimum sanctum, Mangifera indica and Hibiscus rosa sinensis.

INTRODUCTION

Practice of healing is known as medicine which is an important branch of science. From the time unmemorable human beings (animals and birds also) self medicated themselves with natural sources of animal and plant origin to get rid of their illness, use of herbs has been practiced for centuries in all parts of the world in various systems of medicine like Ayurveda, Siddha, Unani and Naturopathy etc. A reference to a number of herbal remedies has been made in Vedas (Rigveda and Atharvanaveda). An antimicrobial is an agent that kills microorganisms or inhibits their growth. Tulsi is an important symbol of the Hindu religious
tradition. Found in most of the Indian homes and worshipped, its legend has Permeated Indian ethos down the ages. Known in English as Holy Basil and botanically called *Ocimum sanctum*, Tulsi belongs to plant family Lamiaceae. It has made important contribution to the field of science from ancient times as also to modern research due to its large number of medicinal properties. Traditionally, *Ocimum sanctum* L. is taken in many forms, as herbal tea, dried power or fresh leaf. For centuries, the dried leaves of Tulsi have been mixed with stored grains to repel insects (Biswas NP et al).

The herb *Hibiscus rosa-sinensis* Linn. belonging to the family Malvaceae and is commonly known as Jasvand (Kirtikar et al.1987). Flowers are used in all kinds of inflammation, internally they are prescribed in the form of decoction of bronchial catarrh, as a becenic and sudorific roots are mucilaginous and demulcent, valuable in cough (Caius et al.1992). The buds have cooling and astringent effect and it removes burning sensation of the body (Kirtikar et al.1987). The extract of the leaves is used to relieve pain.

Mangoes belong to genus *Mangifera* which consists of about 30 species of tropical fruiting trees in the flowering plant family Anacardiaceae. According to ayurveda, varied medicinal properties are attributed to different parts of mango tree. The antimicrobial activities of methanolic extracts of *P. guajava* and MI have been investigated. The results show that *P. guajava* and MI extracts exhibited antimicrobial activities at a concentration of 20 mg/ml. Overall, *P. guajava* extract show more antimicrobial activity than MI extract against tested organisms (Akinpelu DA et al.,2006).

The present study aims to investigate antibacterial activity of extracts of three medicinal plants *Ocimum sanctum*, *Mangifera indica* and *Hibiscus rosa sinensis*.

**MATERIALS AND METHODS**

**Plant Collection**

The leaves of *Ocimum sanctum* (OS), *Mangifera Indica* (MI) and *Hibiscus rosa-sinensis* (HS) were collected from the Tirumala hills Sri Venkateswara University, Tirupati.

**Drying of plant material**

The leaves of these plants were washed with distilled water to remove any impurities and finally dried under shade. Then the dried leaves were ground into a powder with warring Commercial laboratory blender and further milled (mesh size 850 um).
Preparation of extracts
The extraction was performed in Analytical department, Therdose Pharma. The solvent used for the extraction ethanol 99.8% (Fisher Scientific, Thermo fisher scientific India Pvt, Mumbai). The solvent selection was made depending on the several investigations (Nagaraju and Rao, 1989, Nagaraju, 1992).

Chemicals and Equipments
Multer-Hinton agar, Sabouraud Dextrose Agar, petridishes, sterile cork borer, Gentamycin, Flucanazole.

Test organism used
Bacterial strains: The strains of staphylococcus aureus ATCC  BAA 1026, Bacillus subtilis ATCC 11774, Eschereria coli ATCC 10536, Klebsiella pneumonia ATCC33495 Fungal strain. Candida albicans

ANTIMICROBIAL ACTIVITY: WELL DIFFUSION METHOD
In Vitro Antibacterial Activity
Standardization of Micro-Organisms
One loop full of micro-organisms were inoculated into 1000ml of sterile medium and incubated for 24hrs at 37ºc for bacterial culture and for 48hrs at 27ºc for fungal culture. After 24h/ 48hr of incubation, 1ml of broth containing the microorganisms was added to 9ml of peptone water. 10 fold serial dilutions were made in the range of 10^-1 to 10^-10 100µl of the dilutions ranging from 10^-5 to 10^-8 were spread over the sterile nutrient agar (SDA) plates and kept at 37 and 27ºc for 24/48 hours respectively. The number of colony forming units (CFU) was counted and number of micro-organisms per 1ml of stock culture was calculated.

Antibacterial Activity (cup plate method)
Each Petri dish containing Multer-Hinton agar medium was inoculated with one bacterial culture by spreading the suspension of the organism with a sterile glass rod with a bended tip. In each plate cups of 6mm diameter were made at equal distances using sterile cork borer. Gentamycin (std) solution was prepared at a concentration of 25 µg/ml. The extracts of three plants were tested. All plates were kept in the refrigerator for 30 minutes to allow the diffusion of sample to the surrounding agar medium. The Petri dishes were incubated at 37ºC for 24 hrs. Diameter of the zone of inhibition was measured and the average diameter for
each sample was calculated. The diameter obtained for the test samples were compared with standard Gentamycin.

**In Vitro Antifungal Activity**

**Principle and Interpretation**

Sabouraud Dextrose Agar is Carliers modification\(^1\) of the formulation described by Sabouraud\(^2\) for the cultivation of fungi (yeasts, moulds), particularly useful for the fungi associated with skin infections. This medium is also employed to determine microbial contamination in food, cosmetics, and clinical specimens.\(^3\) Mycological peptone provides nitrogenous compounds. Dextrose provides an energy source. High dextrose concentration and low pH favours fungal growth and inhibits contaminating bacteria from test samples.\(^4\) Some pathogenic fungi may produce infective spores which are easily dispersed in air, so examination should be carried out in safety cabinet. For heavily contaminated samples, the plate must be supplemented with inhibitory agents for inhibiting bacterial growth with lower pH.

**Procedure for anti fungal activity (cup-plate method)**

The agar well diffusion method was performed to determine the antifungal activity of three plant extracts. Fungal strain of *candida albicans* was used and maintained on Sabouraud dextrose agar media in plates maintained at 27\(^\circ\)c for 48h. Sterilised Sabouraud dextrose agar media was poured into sterile petri dishes. The respective clinical strain was spread separately on the agar medium. The wells were created using a stainless steel sterilized cork borer under aseptic conditions. The ethanolic extracts of three plants were tested at a concentration of (20mg/ml, 10 mg/ml). Flucanazole was used as standard at a concentration of 10mg/ml. The Plates were incubated at room temperature for 48 h and zones of inhibition (Pande A.and Saxena V.K., et al 1987) were measured.

**RESULTS**

The leaf extracts of three plants were studied for antimicrobial activity employing standard cylinder method. Microbes used were *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa Escherichia coli and Candida albicans*. Both gram-positive and gram-negative bacteria were sensitive to the extracts. The zone of inhibition recorded for various organisms was recorded in Table 1. Activity of Ethanollic leaf extract of the *Hibiscus rosa sinensis* was comparable to that of Reference Standard drug Gentamycin (25μg/ml). Ethanollic leaf extract
of the *Hibiscus rosa sinensis* exhibited good antibacterial activity and mild Antifungal Activity and results were tabulated in Table 1.

### Table 1: Antimicrobial Activity of Ethanolic leaf Extracts of Three Plants

<table>
<thead>
<tr>
<th>S.no</th>
<th>Compounds</th>
<th>Concentration</th>
<th><em>S.aureus</em></th>
<th><em>B.subtilis</em></th>
<th><em>E.coli</em></th>
<th><em>P.aeruginosa</em></th>
<th><em>C.albicans</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HS</td>
<td>100 mg/ml</td>
<td>10</td>
<td>9</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>300 mg/ml</td>
<td>14</td>
<td>16</td>
<td>15</td>
<td>14</td>
<td>20 mg/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 mg/ml</td>
<td>12</td>
<td></td>
<td>11</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>MI</td>
<td>100 mg/ml</td>
<td>10</td>
<td>8</td>
<td>9</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>300 mg/ml</td>
<td>11</td>
<td>12</td>
<td>10</td>
<td>10</td>
<td>20 mg/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 mg/ml</td>
<td>11</td>
<td></td>
<td>12</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>OS</td>
<td>100 mg/ml</td>
<td>9</td>
<td>8</td>
<td>10</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>300 mg/ml</td>
<td>11</td>
<td>12</td>
<td>8</td>
<td>9</td>
<td>30 mg/ml</td>
</tr>
<tr>
<td>4</td>
<td>Gentamicin 25µg/ml (Reference standard)</td>
<td>16</td>
<td>15</td>
<td>17</td>
<td>16</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Flucanozole 10mg/ml</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>18</td>
</tr>
</tbody>
</table>

### DISCUSSION

Plants are an 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 (Tona L., 1998). Many reports are available on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants (Samy R.P 2000, Palombo, E.A 2001, Kumaraswamy, Y., 2002). 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 antibacterial activity of the Ethanolic leaf extracts of three plants was related to their chemical composition. The diameters of the inhibition zones were measured in millimetre.

The preliminary phytochemical components of the plants were including Carbohydrates, Proteins and Amino acids. Several studies indicates that the presence of these bioactive compounds in plant materials to antibacterial activity. The presence of these secondary metabolites in plants, produce some biological activity in man and animals and it is responsible for their use as herbs. These compounds also serve to protect the plant against infection by microorganisms, predation by insects and herbivores, while some give plants against their odours and or flavours and some still are responsible for their pigments (Ketkar et al., 1995). In some cases, the activity has been associated with specific compounds of classes of compounds. These active constituents can be used to search for bioactive lead
compounds that could be used in the partial synthesis of more useful drugs (Ogbonnia, S.O et al., 2008).

Several authors have reported that plant extracts are most effective against gram positive than gram negative bacteria and attributed this to the different in this cell wall structures. (Yao, J et al., 1995). The quantity of the active ingredients required to effect kill may not matter since medicinal plants have been reported to have little or no side effects. The presence of antibacterial substances in the higher plant is well established. Successive isolation of botanical compounds from plant material is largely dependent on the type of solvent but we found in this study the plant extracts by ethanol provided more consistent antibacterial activity compared to those extracted by water. This might have resulted from the lack of solubility of the active constituents in aqueous solutions while ethanol extracts showed some degree of antibacterial activity.

The demonstration of antibacterial activity against both gram positive and gram negative bacteria may be indicative of the presence of broad spectrum antibiotic compounds. *Hibiscus rosa sinensis* Ethanolic leaf extract has shown excellent antibacterial activity against gram negative organism compared to that of gram positive which is evident from the Table 1. This will be of immense advantage in fighting the menace of antibiotic refractive pathogens that are so prevalent in recent times. However, actual ingredient antibacterial ingredients need to be extracted and identified also its tolerable levels in the human body as well as any toxic effects on human and animal tissues be investigated accordingly.

**CONCLUSION**

In this present study an attempt has been made to identify the biological potentiality of the plants to evaluate for their biological activities and for this study the leaf extracts of *Ocimum sanctum*, *Mangifera indica* and *Hibiscus rosa-sinensis* were collected from the Tirumala hills. After preparation of extracts, three selected plants they were subjected for screening of invitro antimicrobial. Based on the significant results obtained the following conclusions were postulated. Activity of Ethanolic leaf extract of the *Hibiscus rosa sinensis* was comparable to that of Reference Standard drug Gentamycin (25μg/ml). Ethanolic leaf extract of the *Hibiscus rosa sinensis* exhibited good antibacterial activity and mild Antifungal Activity.
REFERENCE