SCREENING OF ANTIMICROBIAL ACTIVITY OF MOCHARASA (BOMBAX CEIBA L.)

Sanjivani Samadhan Shekokar1*, SV Shete2

1Associate Professor, Department of Dravya Guna, Government Ayurveda College, Nanded, Maharashtra, India
2H.O.D., Microbiology Dept., Netaji Subashchandra Bose Science College, Nanded, Maharashtra, India

*Corresponding Author: E-mail: drsanjivanisshekocar@rediffmail.com

Received: 08/03/2016; Revised: 01/04/2016; Accepted: 16/04/2016

ABSTRACT

Human being uses plants for food as well as for curing the natural vagaries like diseases since ancient times. Many drugs derived from plant molecules acts as antibacterial or antifungal drugs. Drugs like Mocharasa (gum of BOMBAX CEIBA L.) are being used to treat infectious diseases like diarrhoea, dysentery, cholera since many centuries. The study of antimicrobial activity of Mocharasa aqueous extracts invivo was assessed against certain microorganisms using the agar disc diffusion method. Samples of Mocharasa were collected from tree source. Antimicrobial activity of Mocharasa was carried out with different strains of bacteria such as Salmonella typhii, Staphylococcus aureus, Shigella dysentrae, and Escherichia coli. The diameter of inhibition zone was used as indicators of antimicrobial activity. Observation on the basis of diameter of zone of inhibition on the petri dishes was noted & the area was calculated and comparison was done. Staphylococcus aureus and Salmonella typhii were having a good zone of inhibition ranging between 15–17 mm in diameter whereas Shigella dysentrae and Escherichia coli were having no zone of inhibition. The study showed that Mocharasa is effective in the studied concentration in Staphylococcus aureus and Salmonella typhii and not in Shigella dysentrae and Escherichia coli.

KEY WORDS: Mocharasa, Pravahika, Atisara, Salmonella typhii, Shigella dysentrae, Escherichia coli, Staphylococcus aureus, Antibacterial activity, zone of inhibition

Cite this article:
INTRODUCTION

The use of herbs as medicine is the oldest form of healthcare known to humanity and has been used in all cultures throughout history (Barnes et al., 2007). Early humans recognized their dependence on nature for a healthy life and since that time humanity has depended on the diversity of plant resources for food, clothing, shelter, and medicine to cure myriads of ailments. Primitive human treated illness by using plants, animal parts, and minerals that were not part of their usual diet. Herbal medicines are in great demand in both developed and developing countries as a source of primary health care owing to their attributes having wide biological and medicinal activities, high safety margins and lesser costs. Herbal molecules are safe and would overcome the resistance produced by the pathogens as they exist in a combined form or in a pooled form of more than one molecule in the protoplasm of the plant cell (Lai & Roy, 2004). Even with the advent of modern or allopathic medicine, (Balick, 1996) have noted that a number of important modern drugs have been derived from plants used by indigenous people.

Due to the increasing drug resistance of the bacteria (Joseph Gangoué-Piéboj1 et al., 2009) by frequent use of antibiotics there is increased risk of diseases so now it’s time to explore new alternatives to the newer antibiotics from plant sources.

The antimicrobial compounds found in plants may prevent bacterial infections by different mechanisms than the commercial antibiotics and therefore may have clinical value in treating resistant microorganism strains (Eloff, 1999). The indiscriminate use of antibiotics has resulted in many bacterial pathogens rapidly becoming resistant to a number of originally discovered antimicrobial drugs (Barbour et al., 2004). There is, thus, a continuous search for new antibiotics, and medicinal plants may offer a new source of antibacterial agents.

Many antibiotics have lost effectiveness against common bacterial infections because of increasing drug resistance (Perez et al., 1990; Barie, 1998; Domin, 1998; Okeke et al., 2005). Indiscriminate, inappropriate, and prolonged use of antibiotics have selected out the most antibiotic-resistant bacteria (Van Waai & Nord, 2000; Petrosillo & Fantosti, 2002).

Microbiological assay is a process of analysing the changes of inhibition of growth of bacteria by measured concentration of the drugs to be examined. The inhibition of microbial growth under standardized conditions is generally utilized for demonstrating the therapeutic efficacy of antibiotics (R. Aanathanrayan, 2005).

Human beings have used plants for the treatment of diverse ailments for thousands of years (Sofowara, 1982; Hill, 1989). Ayurveda has references of use of plant drugs in many diseases which are now differentiated as bacterial, fungal, systemic, organic, etc. So, to assess the efficacy of plant drug in the diseases caused by bacteria a study was planned to assess the effect of Mocharasa (Gum of Bombax Ceiba L.) in bacterial invasion like Atisara (Diarrhoea), Pravahika (dysentery), Grahani (irritable bowel syndrome) and causative bacterial strains like Salmonella typhii, Shigella dysenterae, Staphylococcus aureus and Escherichia coli, commonly occurring in the intestine and causing similar symptoms were selected for the invitro antimicrobial activity study.

Mocharasa known as Shalmali Nirya, is described to be used preferably in diseases like Pravahika, Atisara, Grahani, Raktaja Atisara, etc (Chunekar, 2002) which shows similar symptoms to bacillary dysentery or diarrhoea. According to the modern concepts, these diseases are supposed to be bacterial in origin. Mocharasa has been proved to be a potent anti diarrhoeal drug so diarrhoea causing bacterial strains was selected to assess the efficacy (Shingh, 1982).
MATERIALS AND METHODS

Plant Materials

Various samples of Mocharasa were procured from various Market areas of India, out of these some were heavier in weight, reddish brown in colour, stout/solid and were opaque. Some samples were collected from tree source from forest area of Sitakhandi forest, Tq. Bhokar, Dist. Nanded (M.S.) and forest area of Kolhapur region (M.S.). All these samples were sent to National Institute of Science Communication And Information Resources, Raw Materials Herbarium and Museum, New Delhi (NISCAIR) for authentication.

Two samples authenticated by National Institute of Science Communication and Information Resources, Raw Materials Herbarium and Museum, New Delhi (NISCAIR) as Mocharasa, (dried gum of Bombax ceiba L.) were taken for the antibacterial activity study which were, dark brown, hollow and light in weight. These samples were collected from from Bhokar forest named as sample A₁ and from Kolhapur forest named as sample A₂.

Fig.1 shows various samples collected from different places for the antibacterial activity study.
Selection of Bacteria for antibacterial activity

Many different strains of bacteria (Duguid, 1975) are present in human gut and production of diseases depends mainly on either colonization or by previous or new acquisition of bacteria. The bacterial strains were selected on the basis of common symptoms caused by bacteria like diarrhoea, dysentery and also found in the intestinal tract such as *Salmonella typhii*, *Shigella dysenteriae*, *Staphylococcus aureus* and *Escherichia coli*.

All strains of bacterial cultures were procured from the Department of Microbiology, Netaji subash Chandra Bose, Science College, Nanded, Maharashtra. The antibacterial activity study was carried out in Microbiology dept, NSB College, Nanded, Maharashtra, India.

**METHODOLOGY**

**Preparation of extraction:**

The selected *Mocharasa* samples named as A₁ and A₂ samples were powdered and water extract was prepared by dissolving the powdered drug in water in shaker machine, kept shaking for 6 hours and standing for 18 hours, filtered after 24 hours and dried in petri dish to make 10% aqueous extract. Dried extracts of both the samples were taken for the study and DMSO i.e. Dimethyl sulphate was used as Control.

**Composition of media:**

Nutrient Agar media as - Peptone- 1 gm, 1Meat extract - 0.5 gm, Sodium Chloride (NaCl) - 0.3 gm, Distilled water (H₂O) – 100 ml (pH – 7.2), Agar–agar - 2.5 gm (Ananthnarayan, 2014)

**Preparation of nutrient agar**

An amount of 24.8 g of nutrient agar was weighed into a conical flask. One thousand millilitres of distilled water was added and the mixture was melted over a Bunsen flame. The mixture was then poured into test tubes, 20 ml each and plugged with cotton wool. The cotton wool was covered with cellophane and the test tubes were autoclaved at 1.1 kg/cm³ steam pressure for 15 min. The nutrient agar was then stabilized in an electric water bath at 45°C for 15 min before use.

**Microbiological assay of antibiotics**

Disc diffusion method was used for the antimicrobial assay (Ananthnarayan, 2005; Gislene, 2000).

Temperature control as required in different stages of a microbial assay was used during culturing of microorganism and preparing its inoculums, and during incubation in a plate assay.

Glass petri dishes (approximately 20 × 100 mm) having covers of suitable material were sterilized and used. For assay, holes of 5 to 8 mm in diameter were bored in the medium with a sterile borer.

The agar disc diffusion method was employed in the assay. Twenty millilitres of stabilized nutrient agar was seeded with microorganisms, palmed and poured into a Petri dish to solidify. A cork borer of 8 mm in diameter was used to make wells in the agar. With the aid of a syringe, the wells were filled with different concentrations of the plant extracts. The extract was allowed to diffuse for 30 minutes and the plates were incubated at 37°C for 24 h. The zone of inhibition of the extract, the clear area around the well was measured in millimetres (mm) using a ruler after 24 h of incubation.

The diameters of the circular inhibition zones were measured in mm with the help of a scale and the results were calculated.

Quantity of samples used- 0.1 ml
Dose of compound- 10% water extracts
Well size- 8 mm

The observations of the study are as shown in Fig.2 and obtained measurements of antibacterial activity as shown in table 1.
**Fig. 2 Antibacterial activity of Mocharasa**

A1- Bhokar and A2 - Kolhapur Samples 10% aqueous extract used

**OBSERVATIONS**

Table 1- Showing Diameter of inhibition Zones (DIZ) of the test samples on the bacteria used.

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Sample</th>
<th>Conc. Extracts</th>
<th>Escherichia coli</th>
<th>Salmonella typhi</th>
<th>Staphylococcus Aureus</th>
<th>Shigella dysenteriae</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>A1</td>
<td>0.1ml</td>
<td>–ve</td>
<td>15mm*</td>
<td>17mm*</td>
<td>–ve</td>
</tr>
<tr>
<td>2.</td>
<td>A2</td>
<td>0.1ml</td>
<td>–ve</td>
<td>15mm*</td>
<td>18mm*</td>
<td>–ve</td>
</tr>
</tbody>
</table>

*IZ = Inhibition zone (mm), –ve = No antibacterial activity
Results obtained were as follows, 
1) *Staphylococcus aureus* and *Salmonella typhii* were having a good zone of inhibition on bacteria. 
2) *Shigella dysentrae* and *Escherichia coli* were having no zone of inhibition. 

So antibacterial activity of *Mocharasa* was found positive on *Staphylococcus aureus* and *S. typhii* and negative on *E. coli* and *S. Dysentrae* (as shown in table 1). 

**DISCUSSION**

The extract of *Bombax ceiba* L. Gum i.e. *Mocharasa* showed some levels of inhibitory activity against *Salmonella typhii* and *Staphylococcus aureus* by inhibiting their growth. This suggests that the extract contained antimicrobial substances like tannin, Catechol, Tannic acid, Galic acid, (Hisanori Akiyama et al., 2001) which are responsible for the antibacterial activity. The effect of the plant extract varied from one microorganism to another. *Salmonella typhii* and *Staphylococcus aureus* were only susceptible to the extract than the rest of the microorganisms. The activity of the plant extract may be dependent on the increasing concentration. Although the antimicrobial activities for 10% extract occurred to be positive and good for the *Salmonella typhii* and *Staphylococcus aureus*. But, there was no significant effect on the inhibition of *Escherichia coli* and *Shigella dysentrae*. May be the increasing concentration of the extract may show inhibitory effect on these bacteria as *Mocharasa* is used in *Piccha basti* (a type of enema) in *Atisara* (diarrhoea) and *Pravihaka* (dysentery) very effectively.

Properties of *Mocharasa* are *kashaya rasa* (Astringent), *sheeta virya* which helps in for *shoshana* (reabsorption of fluids in the bowel), *stambhana* (preventing further loss of fluids in the form of liquid stools) in *Atisara* (diarrhoea) and *pravahika* (dysentery) (Sharma, 2006). The phyto constituents like Tannin, tanic acid may also have played role in inhibition of the above bacteria.

**CONCLUSION**

Out of the four types of bacteria used for study, *Staphylococcus aureus* and *Salmonella typhii* cultured plates were having a good zone of inhibition of bacteria whereas plates cultured with *Shigella dysentrae* and *Escherichia coli* were having no zone of inhibition. This shows *Mocharasa* extract is having antibacterial activity against *salmonella typhii* and *Staphylococcus aureus* with 10% extract calculated on the basis of increased diameter of inhibition zone but had no bacteria inhibiting activity in this concentration of drug. So this proves the efficacy of *Mocharasa* as an antimicrobial drug on *Staphylococcus aureus* & *Salmonella typhii* more strongly as compared to the other strains and hence *Mocharasa* can be a promising drug to be used in bacterial infections of the GIT.

**Further Scope of the study:**

1. The study is done using only two samples so number of samples and different strain of bacteria can be studied.
2. The study can be conducted by using modern allopathic drugs as control group.
3. 20%, 40%, 60% of extraction to be used to study the antibacterial activity in *Shigella dysentrae* and *Escherichia coli*.
4. Clinical trial should be conducted, to analyze its efficacy on clinical subjects.

**REFERENCES**


James Hamuel Doughari, Phytochemicals: Extraction Methods, Basic Structures and Mode of Action as Potential Chemotherapeutic Agents, page no. 8


Saleem R, Ahmad M, Hussain SA, Qazi AM, Ahmad SI,.....Husnain SN. (1999), Hypotensive, hypoglycemic and toxicological studies on the flavonol, c-glycoside shaminin from *Bombax ceiba*. Planta Med. Hamdard University Karachi, Pakistan, May; 65(4): 331–4


Us Pati & Kurude, (2000), Antibacterial activity screening methods for evaluation of natural products, regional station, Indian veterinary research institute, Palampur, HP.

Wang YC, Huang TL (2005), Screening of anti-Helicobacter pylori herbs deriving from Taiwanese folk medicinal plants, Dept. of Food Science, National Chung Hsing, University, 250, Kukuang Road, Taichung 40227, Taiwan, FEMS Immunol Med. Microbial, Feb 1; 43(2): 295–300.

The present study was conducted to investigate the phytochemical screening and antimicrobial activities of stem bark of Bombax ceiba L. The methanol extract was subjected to qualitative phytochemical screening using standard procedures. The results indicated the presence of alkaloids, tannins, glycosides, reducing sugar, saponins, phlobatanins and terpenoids. The antimicrobial activity was measured by disc diffusion method. Data revealed that Pseudomonas aeruginosa was inhibited by both methanol and ethanol extracts at the concentration of 2mg disc-1 (21.8mm (68.12%) and 21.3mm (66.56%)). Simi Bombax ceiba belonging to Family Bombacaceae, commonly known as salmali. It is widely distributed throughout India, in forest up to an altitude about 1500 m, also raised in plantation and found to be Malaya. In India, it is distributed from Rajasthan, and Andhra Pradesh. In Ayurveda, Bombax ceiba stem bark was reported to contain lupeol and β-sitosterol. Aqueous bark extracts of the Bombax ceiba was subjected to a preliminary screening for antimicrobial activity against Gram positive & Gram negative bacteria i.e. Bacillus subtilis, Bacillus aureus, Staphylococcus aureus, Escherichia coli, K pneumoniae and Pseudomonas aeruginosa. It was clear from Table-1.