Studies on Antibacterial activity of *Pithecellobium dulce* (Roxb.) Benth against food pathogens – Gram negative bacteria

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Abstract

Bacterial food intoxication refers to food borne illnesses caused by the presence of food pathogens. The food borne diseases are caused by the entrance of bacteria into the body through ingestion of contaminated foods and the reactions of the body to their presence or to their metabolites. Herbal remedies have been used for many thousands of years in many different cultures. Today herbs have become a growing alternative for establishing a healthy body environment. Molds, actinomycetes and bacteria are the chief sources of antibiotics. Anti-bacterial agents are also present in some higher plants. The anti-bacterial agents include all classes of secondary metabolites such as alkaloids, steroids, tannins, phenolic compounds, flavonoids, resins and fatty acids which are capable of producing definite physiological actions on body. The present investigation is aimed to understand the antibacterial activity of *Pithecellobium dulce* against the food pathogens concentrating only on Gram negative bacteria. *Pithecellobium dulce* is a well-known Indian medicinal plant. Infusions of different parts of *Pithecellobium dulce* have been traditionally used to treat diseases, for example, skin of the stem is used for dysentery, leaves for intestinal disorders and seeds for ulcers. The experimental procedure employed in the present study is to analyse the aril part of *Pithecellobium dulce* for their antibacterial properties. The methods of extractions used were Maceration and Soxhlet method. The extracts were now tested for their antibacterial activity against the food pathogens by using Agar Well Diffusion method and further confirmed with the help of Disc Diffusion method. After analysing it showed that the treated sample only showed activity against the pathogens. The untreated sample did not show activity against any of the food pathogens, whereas the treated sample showed activity against gram negative food pathogens isolated from stool sample.

Keywords: *Pithecellobium dulce*, Soxhlet, Molds, Actinomycetes.

INTRODUCTION

Food borne illness is an ever present threat that can be prevented with proper care and handling of food products. It is estimated that between 24 and 81 million cases of food borne diarrhea disease occur each year in USA [1]. Chemicals, heavy metals, parasites, fungi, viruses and bacteria can cause food borne illness. Bacteria related to food poisoning is most common, but less than 20 of the many thousands of different bacteria actually are the culprits. More than 90 percent of the cases of food poisoning each year is caused by *Staphylococcus aureus*, *Salmonella*, *Clostridium perfringens*, *Listeria monocytogenes*, *Vibrio parahaemolyticus*, *Salmonella enterica*, *Klebsiella pneumoniae*, *Shigella flexneri*, *Pseudomonas aeruginosa* and *Escherichia coli*. These bacteria are commonly found on many raw foods. This infection may also occur after consuming food or water contaminated with bacteria or improper cleaning of storage and preparation areas and unclean utensils cause contamination of raw and cooked foods. Mishandling of raw and cooked foods allows bacteria to grow. The plants have been traditionally noted for its medicinal and food values. Medicinal plants represent a rich source from which antimicrobial agent may be obtained [2]. Plants are

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used medicinally in different countries and are a source of many potent and powerful drugs. Clinical microbiologists have great interest in screening of medicinal plants for antimicrobial activities and phytochemicals as potential new therapeutics. The active principles of many drugs found in plants are secondary metabolites. The antimicrobial activities of plant extracts may reside in a variety of different components, including aldehyde and phenolic compound [3]. The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a research for new anti-microbial substances from other sources including plants [4]. In recent years, phytochemical investigations of Indian medicinal plants have been progressing steadily and assumed extraordinary importance due to the use of indigenous drugs.

**PLANT PROFILE**

**Pithecellobium Dulce (Roxb.) Benth**

- Family: Leguminosae
- Subfamily: Mimosoideae
- Genus: *Pithecellobium*
- Species: *dulce*

Vernacular name of this plant known as in Tamil (Kodukkapuli), Hindi (Vilayati Babul), Kannad (Kottampuli), Bengal (Dekhani Babul), Marathi (Vilayati hinch), Malayalam (Korukkapuli), Telugu (Simachinta).

**PLANT DESCRIPTION**

*Pithecellobium dulce* is a small to medium sized evergreen and spiny tree that grows up to 15-20m in height; the trunk is up to 2m thick. It is often grown as avenue tree and also for its edible aril. They are broad spreading with regular branches. The plant bears bipinnately compound leaves which are alternate and spiral. The pinnules (leaflets) are in pairs and are oblong oblanceolate and 4cm in length. Thin spines are in pairs at the base of each leaf and range from 2-15mm in length, though some specimens are spineless. Leaves are deciduous but foliage in persistent, as the new leaves appear, the old ones are being shed; so that the tree looks like an evergreen tree. The flowers are small, spherical, glomerules white heads and 1cm in diameter. Each flower has a hairy corolla and about 50 thin stamens united in a tube at the base, surrounded by the green calyx. Flowering begins in 3-4 years and is seasonal (January-March) and the fruit ripens from April to July. The pods are pinkish, 1-1.5cm wide, about 12cm long and become spiral as they mature. Seeds are about 10 per pod, black and shiny, hanging on a reddish pod from the pod. The pod splits along both margins. The aril is white and turns purplish red on maturation. The bark is smooth, grey with yellowish white lenticels [5].

The present investigation of the plant, *Pithecellobium dulce* is aimed to understand controversies existing in the market and literature. Present work is taken up to scientifically evaluate the proper source for this drug. Attempts are made to authenticate the drug botanically, chemically and biologically as per the standard procedures. The objectives of the present research investigation are on the Antibacterial activity of *Pithecellobium dulce* against the food pathogens only on Gram negative bacteria.

**MATERIALS AND METHODS**

The experimental procedure employed in the present study to analyse the aril part of *Pithecellobium dulce* for their antibacterial properties

**Collection of the plant material**

The aril part of the *Pithecellobium dulce* were collected from Tambaram, Chennai and it was assayed for their antibacterial activity.

**Preparation of the extracts**

**Maceration**

In this process 10g of the plant sample is taken and it is made into a coarse mixture and then filtered. The liquid fresh extract is now stored in a container for the analysis of antibacterial activity.

**Hot Continuous Extraction (Soxhlet)**

In this method, the finely ground crude drug is placed in a porous bag or “thimble” made of strong filter paper, which is placed in the chamber of the Soxhlet apparatus. The extracting solvent ethyl acetate in flask is heated and its vapours condense in condenser. The condensed extract drips into the thimble containing the crude drug, and the level of the liquid in the chamber rises to the top of siphon tube into flask. This process is continuous and is carried out until a drop of solvent from the siphon tube does not leave residue when evaporated.

**Microorganisms (Gram negative bacteria isolated from stool samples)**
1. **Shigella flexneri**: It is a gram negative bacteria that can cause diarrhea in humans. Several different serogroups of Shigella are described; S. flexneri belongs to group B. S. flexneri infections can usually be treated with antibiotics, although some strains have become resistant.

2. **Klebsiella pneumoniae**: It is a Gram-negative, non-motile, encapsulated, lactose-fermenting, facultative anaerobic, rod-shaped bacterium. Although found in the normal flora of the mouth, skin, and intestines, it can cause destructive changes to human and animal lungs if aspirated (inhaled), especially to the alveoli (in the lungs) resulting in the bloody sputum.

3. **Salmonella enterica**: It is a rod-shaped, flagellated, facultative anaerobic, Gram-negative bacterium and a member of the genus Salmonella. Most cases of salmonellosis are caused by food infected with S. enterica, which often infects cattle and poultry, though also other animals such as domestic cats and hamsters have also been shown to be sources of infection to humans.

4. **Escherichia coli**: It is a Gram-negative, rod shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms. Most E. coli strains are harmless, but some serotypes can cause serious food poisoning in humans, and are occasionally responsible for product recalls due to food contamination.

5. **Pseudomonas aeruginosa**: It is a common Gram negative, rod shaped bacterium that can cause diseases in human. It is found in soil, water, and skin surfaces also. It can also cause serious food poisoning in humans, and are occasionally responsible for product recalls due to food contamination.

**ANTIBACTERIAL ASSAY**
The effect of the plant extract on the several bacterial strains were assayed by Agar well diffusion method and further confirmed by Disc diffusion method.

**Agar- well diffusion method**
The antimicrobials present in the plant extract are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zone of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeters.

**Procedure**
Petriplates containing 20ml Muller Hinton medium were seeded with 24hr culture of bacterial strains. Wells were cut and 20µl of the plant extracts (namely fresh, treated and antibiotic) were added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well. Chloramphenicol disc was used as a positive control.

**Disc Diffusion Method**
Paper discs impregnated with specific antibiotics or the test substances are placed on the surface of the Muller Hinton agar medium inoculated with the target organisms, which is recommended for the diffusion of antimicrobial agents as described in NCCLS approved standard. The plates are incubated and the zones of inhibition around each disc are measured.

**Procedure**
Muller Hinton Agar plates were prepared and the test microorganisms were inoculated by the spread plate method. Filter paper discs approximately 8mm in diameter were soaked in 100µl of the plant extract and placed in the previously prepared agar plates. The agar plates were then incubated at37°C. After 16 to 18 hours of incubation, each plate was examined. The resultant zones of inhibition were uniformly circular with a confluent lawn of growth. The diameters of the zones of complete inhibition were measured, including the diameter of the disc where the Chloramphenicol was used as control.

**RESULTS AND DISCUSSION**
The following results were observed during the Antibacterial Assay on food pathogens are listed in the table 1 & 2.
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Table 1. Antibacterial sensitivity test using agar well diffusion method against food pathogens

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Microorganisms</th>
<th>Zone of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treated Ethyl acetate (mm)</td>
<td>Fresh water (mm)</td>
</tr>
<tr>
<td>1</td>
<td>Escherichia coli</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Salmonella enterica</td>
<td>2.5</td>
</tr>
<tr>
<td>3</td>
<td>Klebsiella pneumonia</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>Shigella flexneri</td>
<td>2.7</td>
</tr>
<tr>
<td>5</td>
<td>Pseudomonas aeruginosa</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 2. Antibacterial confirmatory test using disc diffusion method against food pathogens

<table>
<thead>
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<th>S.NO</th>
<th>Microorganisms</th>
<th>Zone of inhibition</th>
</tr>
</thead>
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<tr>
<td></td>
<td>Treated Ethyl acetate (mm)</td>
<td>Fresh water (mm)</td>
</tr>
<tr>
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<td>Escherichia coli</td>
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<tr>
<td>3</td>
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<td>4</td>
<td>Shigella flexneri</td>
<td>3.2</td>
</tr>
<tr>
<td>5</td>
<td>Pseudomonas aeruginosa</td>
<td>-</td>
</tr>
</tbody>
</table>

Antibacterial sensitivity test – plates showing zone of inhibition

**Escherichia coli**

**Salmonella enterica**

**Klebsiella pneumonia**

**Shigella flexneri**

**Pseudomonas aeruginosa**

Antibacterial Studies

The antibacterial studies were carried using the aril portion of the Pithecellobium dulce. The treated sample showed activity against 3 organisms namely Shigella flexneri, Salmonella enterica, and Klebsiella pneumonia. The zone of inhibition of each gram negative bacteria was measured. The untreated sample did not show any activity against the bacterial strains. The organism Pseudomonas aeruginosa is resistant to Chloramphenicol since it
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did not have any zone of inhibition. Therefore the zones were observed only in the treated sample and the untreated sample did not show any activity.

SUMMARY

Pithecellobium dulce (Roxb.) Benth of Mimosoideae (Leguminosae) is a common tree taxon; economic values of the tree in timber industries, ornamental purpose and proved both in folklore as well as tribal fields [9]. In this present study “Antibacterial studies of Pithecellobium dulce (Roxb.) Benth. against the food pathogens” particularly focuses on the antibacterial activity of the plant against gram negative bacteria isolated from stool sample. The methods used for extraction are maceration and soxhlet method. The extracts were now tested for their antibacterial activity against the food pathogens by using Agar Well Diffusion method and further confirmed with the help of Disc Diffusion method. After analysing it showed that the treated sample showed activity against the pathogens. The untreated sample did not show activity against any of the food pathogens. The positive control Chloramphenicol showed activity against gram negative bacteria. It did not show activity against Pseudomonas aeruginosa.

CONCLUSION

Medicinal plants are richest bio-resource of drugs for traditional systems of medicine, modern medicine, nutraceuticals, food supplements, folk medicines, pharmaceuticals intermediates and chemical entities for synthetic drugs. The plant Pithecellobium dulce used for the study, is a well known Indian medicinal plant. It has been commonly used for fencing and tanning, as fodder for feed and pods for food. Infusions of different parts of the plant have been traditionally used to treat diseases, for example, skin of the stem is used for dysentery, leaves for intestinal disorders and seeds for ulcers. Antibiotic resistance is an important tool for genetic engineering. Hence, our focus was on antibacterial activity and how the naturally occurring chemicals in the plant inhibit the Multi-drug Resistance (MDR) in food pathogens (Escherichia coli, Salmonella enterica, Klebsiella pneumoniae, Shigella flexneri, Pseudomonas aeruginosa). The results shown states that the untreated sample did not show any activity against the food pathogens whereas the treated sample only showed activity against the pathogens.

ACKNOWLEDGEMENT

None.

CONFLICT OF INTEREST

No Conflict of Interest.

REFERENCES