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

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**INTRODUCTION**

Foodborne 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 foodborne diarrhea disease occur each year in USA [1]. Chemicals, heavy metals, parasites, fungi, viruses and bacteria can cause foodborne 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 enteric*, *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
nicobid 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 . 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><em>Escherichia coli</em></td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td><em>Salmonella enterica</em></td>
<td>2.5</td>
</tr>
<tr>
<td>3</td>
<td><em>Klebsiella pneumonia</em></td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td><em>Shigella flexneri</em></td>
<td>2.7</td>
</tr>
<tr>
<td>5</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>-</td>
</tr>
</tbody>
</table>

Table 2. Antibacterial confirmatory test using disc 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><em>Escherichia coli</em></td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td><em>Salmonella enterica</em></td>
<td>2.8</td>
</tr>
<tr>
<td>3</td>
<td><em>Klebsiella pneumonia</em></td>
<td>1.5</td>
</tr>
<tr>
<td>4</td>
<td><em>Shigella flexneri</em></td>
<td>3.2</td>
</tr>
<tr>
<td>5</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>-</td>
</tr>
</tbody>
</table>

Antibacterial sensitivity test – plates showing zone of inhibition

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 pneumoniae*. 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
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