

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



Hepzibah. W^{1*}, Vajida. J² and Balaji.M³

¹Department of Botany, Women's Christian College, Chennai-600006, Tamil Nadu, India.

²Department of Botany, Ethiraj College for women, Chennai, Tamil Nadu, India.

³Senior Bioinformatician, Akshaya computational Medicine, Chennai-600005, Tamil Nadu, India.

Abstract

Bacterial food poisoning refers to the diseases caused by microorganisms by the ingestion of toxins and infecting the host through the intestinal tract. The most commonly occurring food poisoning is caused by the ingestion of the enterotoxin formed in food during growth of certain pathogenic bacterial strains. 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 positive 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 gram positive bacteria by using Agar Well Diffusion method and further confirmed with the help of Disc Diffusion method. After analysing the treated sample showed activity against *Bacillus cereus* and *Streptococcus faecalis*. The untreated sample showed activity against *Bacillus pumilus* and *Staphylococcus aureus* whereas all the gram positive bacteria showed activity against Chloramphenicol.

Keywords: *Pithecellobium dulce*, Antibacterial agents, *Staphylococcus aureus*.

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*, *Bacillus cereus*, *Streptococcus faecalis*, *Bacillus pumilus* 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. 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.

To whom correspondence should be addressed:

Hepsibah W

Email: hepsibah.joseph27@gmail.com

Plants in Indian Medicinal Systems

Nature has provided a complete store-house of remedies to cure all ailments of mankind. Almost all the medicines used were from the plants, the plant being man's only chemist for ages [2]. The plant has been traditionally noted for its medicinal and food values. Medicinal plants represent a rich source from which antimicrobial agent may be obtained [3]. Plants are used medicinally in different countries and are source of many potent and powerful drugs [4]. Medicinal plants are growing wild in all parts of the world, especially in the tropical countries and have been widely used in India, China and the Middle Eastern countries. Today, India is one among the oldest countries which evolved and improved several medicines from plant. The major traditional medicinal system that are followed in India are

Ayurvedha

Ayurvedha system of medicine is one of the few systems of medicine taking mental, emotional and spiritual well being into account. According to Ayurvedha, health is a state of balance between the body, mind and consciousness [5].

Siddha

The Siddha system of medicine emphasize health as the perfect state of physical psychological, social and spiritual component of a human being [6].

Unani

The Unani system of medicine aims at combating disease and preserving and promotion of health through curative, preservative and primitive measure [7].

Homeopathy

In this System, the cause of disease itself plays a significant role in the treatment of patient. The drugs are extracted in the form of mother tincture, which is diluted in terms of decimal. The drug treatment is not specified and the choice of drug depends on symptoms and clinical conditions of the patient [8].

Aromatherapy

Aromatherapy is one of the most ancient healing arts and traces its origin to 4500 B.C. Different essential oils from various parts of plants are massaged into skin to treat a range of diseases, as

well as to have an effect on the mind and emotions [9].

Plant Profile

Pithecellobium Dulce (Roxb.) Benth

Family	: Leguminosae
Subfamily	: Mimosoideae
Genus	: <i>Pithecellobium</i>
Species	: <i>dulce</i>

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 [10].

Distribution of the plant

It is native to Mexico through Central America to Colombia and Venezuela. Introduced in southern Florida, Cuba, Jamaica, Puerto Rico and St.croix. Widely planted and naturalized in tropical regions. *Pithecellobium dulce* is listed as a common weed in Hawaii, found throughout the Philippines at low or medium altitudes .It is also cultivated throughout the plains of India and in the Andaman.

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 and attempts were

made to authenticate the drug botanically, chemically and biologically as per the standard procedures. The objective of the present research investigation is on the antibacterial activity of *Pithecellobium dulce* against Gram positive 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 was taken and made into a coarse mixture and then filtered. The extract is 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 evaporate.

Microorganisms

Gram positive bacteria

(Isolated from stool culture sample)

- a. Staphylococcus aureus
- b. Streptococcus faecalis
- c. Bacillus cereus
- d. Bacillus pumilus
- e. Listeria monocytogenes

ANTIBACTERIAL ASSAY

The effect of plant extract on the gram positive 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 is 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 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 were 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 agar plates. The agar plates were then incubated at 37°C. After 16 to 18 hours of incubation, each plate was examined. The resultant zone of inhibition was uniformly circular with a confluent lawn of growth. The diameter of the zone of complete inhibition was measured, including the diameter of the disc.

RESULTS AND DISCUSSION

The following results were observed during the Antibacterial Assay on food pathogens are listed in the table 1 & 2.

Table 1. Antibacterial sensitivity test using agar well diffusion method against food pathogens

S.NO	Microorganisms	Zone of inhibition		
		Treated Ethyl acetate(mm)	Fresh water (mm)	Chloramphenicol (mm)
1	<i>Bacillus cereus</i>	0.1	-	3.7
2	<i>Bacillus pumilus</i>	-	1.5	1.8
3	<i>Listeria monocytogenes</i>	-	-	2.8
4	<i>Streptococcus faecalis</i>	1.0	-	3.1
5	<i>Staphylococcus aureus</i>	-	1.8	2.2

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

S.NO	Microorganisms	Zone of inhibition		
		Treated Ethyl acetate(mm)	Fresh water (mm)	Chloramphenicol (mm)
1	<i>Bacillus cereus</i>	-	-	3.0
2	<i>Bacillus pumilus</i>	-	0.9	1.3
3	<i>Listeria monocytogenes</i>	-	-	2.6
4	<i>Streptococcus faecalis</i>	-	-	3.4
5	<i>Staphylococcus aureus</i>	-	1.1	1.9

Antibacterial sensitivity test – plates showing zone of inhibition



Bacillus cereus



Bacillus pumilus



Listeria monocytogenes



Streptococcus faecalis



Staphylococcus aureus

Gram positive Pathogenic bacteria were isolated. Antimicrobial susceptibility testing was done using the aril portion of the *Pithecellobium dulce* in order to determine the activities of five Gram positive pathogenic bacteria isolated from stool sample. The

zone of inhibition was measured. The treated sample showed activity against 2 organisms namely *Bacillus cereus*, and *Streptococcus faecalis*. The zone of inhibition of each bacteria was measured. The untreated sample showed activity against *Bacillus*

pumilus and *Staphylococcus aureus*. Therefore the zones were observed both in treated sample and the untreated sample.

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. 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 positive bacteria isolated from stool sample. The methods of extraction used 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 the zone of inhibition was measured. The treated sample showed activity against 2 organisms namely *Bacillus cereus*, and *Streptococcus faecalis*. The zone of inhibition of each bacteria was measured. The untreated sample showed activity against *Bacillus pumilus* and *Staphylococcus aureus*. Therefore the zones were observed both in treated sample and the untreated sample.

CONCLUSION

Herbs can be very effective in programs for resolving bacterial gastroenteritis infections .

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Antibiotic resistance is an important tool for genetic engineering. We focus on how the naturally occurring chemicals inhibit the Multi-drug Resistance (MDR) Human pathogens. Medicinal plants are richest bio-resource of drugs for traditional systems of medicine, modern medicine, nutraceuticals, food supplements, folk medicines, pharmaceutical 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. The results shown states that the treated and untreated sample showed activity against the food pathogens isolated from stool sample. Thus, we identified that the chemical compounds in the plant products are best agents to reduce the problems associated to food poisoning. The identified compounds have antioxidant activity whereas these results clearly show that the compounds also have anti-bacterial properties based on zone of inhibition.

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CONFLICT OF INTEREST

No Conflict of Interest.