Clinical and Medical Research and Studies

www.alcrut.com

Research Article | Clinical and Medical Research and Studies | Open Access

Phytochemical Screening and Evaluation of Antimicrobial Activity of Various Extracts of "Pithecellobium Dulce

Himabindu K[1] and Challa Stalin Reddy[2]

Department of Pharmacognosy, Vagdevi College of Pharmacy, Warangal, Telangana, India.

Deportment of Pharmaceutics, Jangaon Institute of Pharmaceutical Sciences, Jangaon, Telangana, India.

***Corresponding Author:** Himabindu K, Department of Pharmacognosy, Vagdevi College of Pharmacy, Warangal, Telangana, India.

Received Date: August 02,2024; **Accepted Date:** August 18,2024; **Published Date:** August 24,2024

Citation: Himabindu K, Phytochemical Screening and Evaluation of Antimicrobial Activity of Various Extracts of "Pithecellobium Dulce, J Clinical and Medical Research and Studies, V (3)I(2).

Abstract

Pithecellobium dulce is one of the important medicinal plants belonging to the family Fabaceae. Ayurveda and Unani medicinal systems use it for the treatment of several aliments. *Pithecellobium dulce* has Antidiabetic, Anti oxidant, Anti inflammatory, and Aborifacient activities. As per World Health Organization many people are suffering from microbial infections. The present study was designed to evaluate the anti- microbial activity of pithecellobium dulce.The preliminary phytochemical studies determine the various secondary metabolites like alkaloids, saponins, flavanoids, steroids, tannins, aminoacids are present. The different extracts were screened for their anti microbial activity against one pathogenic fungal organism and three pathogenic bacterial organisms by agar cup-plate method. In anti fungal screening pyridine+glacial aceticacid(1000µg/ml) extract showed the significant activity against Aspergillus niger (15mm ZOI) . The activity index is 15mm(ZOI) compared with a standard drug Cotrimoxazole. After performing anti-bacterial screening pyridine + glacial acetic acid (20mg/ml) showed the significant effect against Lacto bacillus (15mm ZOI) compared with the standard drug Chloramphenicol.

Keywords: Omission; environment; multinationals

Introduction

PLANT DESCRIPTION:

Pithecellobium dulce is a species of flowering plant in the pea family, Fabaceae, (*subfamily Mimosoideae*) locally known as Jangal Jalebe and with English name as Manila Tamarind. It is a small to medium sized, evergreen, spiny woody legume tree up to 18 m height.[16]

Fig.no:1 Pithecellobium dulce plant

Fig.no:2 Pithecellobium dulce leaf

Synonyms:

Acacia oblique folia, Albizia dulcis, Feuillea dulcis (Roxb)kuntze ,Mimosa dulcis Roxb, Mimosa edulis gagnep , Mimosa pungens , Mimosa unguis-cati blanco , Pithecellobium unguis-cati , Pithecellobium littorale.

VERNACULAR NAMES:

English:Monkey pod

Telugu: Seema chinta

Hindi: Jangal jalebi

Tamil: Kodukkappuli

Kannada: Seeme hunase

Marathi: Vilayatnchinch

HABITAT:

Dry, brushy or thinly forested plains or hill sides, often in coastal thickets, at elevations from sea level to 1500 meters. The tree is drought resistant and can survive in dry lands. It is native to Mexico, Central America, South America, India , Bangladesh . [15]

TAXONOMY:

Pithecellobium dulce

Kingdom: Plantae

Sub kingdom: Viridiplantae

Infra kingdom: Streptophyta

Super division: Embryophyta

Division: Tracheophyta

Class: Magnoliopsida

Order: Fabales

Family:Fabaceae

Genus: Pithecellobium

Species: Pithecellobium dulce (Roxb) Benth

MACROSCOPY DESCRIPTION:

Pithecellobium dulce forms a small to medium-sized tree up to 15-20 m in height with a dbh of 30-50 cm, or even 100 cm.

Branches:

It is usually multiple-stemmed, sometimes forming only a bush, but often forming a branchy tree with an irregular rounded crown and flexuous and pendulous branches.

A few slender whip-like branches often straggle well beyond the rest of the crown.

Bark:

The bark on the branches and in younger trees is smooth, pale whitish-grey, lenticellate, often with horizontal ribs encircling the trunk and branches, becoming rougher and fissured on older boles.

Shoots:

The shoots are randomly armed at nodes with pairs of straight, stout, stipular spines, 4-13 mm long, but are occasionally thornless.

Leaves:

The leaves are abruptly bipinnate with a single pair of pinnae per leaf and two pairs of leaflets per pinna, i.e. 4 leaflets in all per leaf.

Leaflets:

The leaflets are 25-56 mm long and 9-32 mm wide, obliquely elliptic or oblong elliptic with 4-7 pairs of pinnate veins, deep olive green above, paler grey-green below, with small glands, 0.3-0.8 mm high and 0.4-0.7 mm in diameter, at the tip of the petiole and the tip of the pinnular rachis.

Flowers:

The flowers are grouped in small, dense, sub-spherical heads, 7-12 mm in diameter, with 20-30 flowers per head, the heads arranged in fascicles of 2-4 in axils of leaves.

The flowers are pale whitish-green and the stamen filaments are white. There are 5 sepals and 5 petals fused into a tube, with 22-42 stamens per flower, also basally united into a staminal tube.

Fruits:

The fruits are distinctive in shape and color being spirally curved or coiled into 1-2 circles, noticeably constricted between the seeds, and green tinged red, turning bright rose or bright red as they ripen, and reddish-brown after dehiscence.

Pods:

The unripe pods are fleshy, becoming dry and papery after opening. The pods open along both sides to reveal 8-12 seeds which persist after the pods open, attached by the fleshy white, pale pink or occasionally red, aril. The seeds are shiny black, compressed, lentiform, 7-13 x 6-11 x 2-4 mm in size.[16]

TRADITIONAL USES:

Leaves

The leaves of *Pithecellobium dulce* can also be used to treat certain conditions. The astringent and anti-inflammatory effects of the leaves have led to their use as a treatment for both open and closed wounds. In order to use the leaves for this purpose, they must be crushed and spread over the affected area, where they are said to relieve pain and promote healing. Genital herpes has been treated with a compress made from the leaves of this tree, and though they may help with a current outbreak, they will not kill the virus responsible for this disease.

A toxin in the leaves of *Pithecellobium dulce* has led them to be used to induce abortions. Despite its use for this purpose in folk medicine, there is no published medical research to support claims that *Pithecellobium dulce* can be used for this purpose. Pregnant women who consume the leaves of this tree, however, may experience unintended medical consequences including serious illness.

Wood

The wood is used locally for construction, panelling, boxes, crates, agricultural implements, and cart wheels. Irregular growth habit and branchiness prohibit use as a sawn timber and the wood has never been used commercially, except in some areas for fuel. However, as a fuelwood it is not of very high quality, having only low to moderate calorific value, being thorny and burning with a very smoky flame . Nevertheless, the wood is used as a domestic fuel in many areas where firewood is in short supply and as fuel for brick kilns in India.

Part of Plant used	Type of extract	Activity
Leaves	Benzene,chloroform,aceton e, Methanol extracts	Antimicrobial activity.[9,10,13]
Leaves	Alcoholic extract	Anti tubercular activity.[19,20]
Leaves	Aqueous extract	Hypolipidemic activity.[9]
Leaves	Aqueous and Alcoholic Extracts	Anti diabetic activity.[11]
Leaves	Hexane, chloroform and alcohol extract	Anti tubercular activity.[12,13]
Leaves	Alcoholic extract	CNS depressant.[13]
Fruits	Methanolic extract	Anti inflammatory activity.[12]
Fruits	Aqueous and hydroalcoholic Extracts	Anti oxidant activity.[11]
Bark	Benzene, chloroform, methanol, acetone extracts	Antimicrobial activity.[14]
Bark	Aqueous extract	Anti venom activity.[5]
Seed	Methanolic and aqueous Extract	Antifungal activity.[18]
Seed	Benzene ,methanol extract	Protease inhibitor activity.[10]
Root	Alcoholic extract	Aborficient activity.[4]

Table no: 1: **Reported Pharmacological Activities**

Fruits

P. dulce is perhaps best-known for its sweet edible aril, which is eaten fresh, as an infusion, or macerated in water to make a lemonade-like beverage . The species name 'dulce' derives from this use. The aril is small, fleshy, sweet, but often rather astringent, and has been the focus of selection in the Philippines to produce superior clones with sweeter, redder arils. The pods are often harvested for local consumption, pods are harvested in larger quantities and sold in local markets. The fruits do not store for long and must be eaten within a few days.

Seed

The seeds themselves are also edible, eaten in curries in India. They also contain 17% oil, light-coloured, as thick as castor oil, which is extracted in some areas, and the resulting pressed seed cake residue is rich in protein (30%) and can be used as a seed meal for stock feed.

Pod

The pods are also relished by livestock and chickens . The leaves contain 29% crude protein and the young shoots are used for

livestock fodder in some areas , either browsed directly or by lopping branches and allowing the leaflets to dry and drop off. Hedge trimmings are often used in this way as fodder for goats in parts of India . However, it is rarely considered an important fodder and there has been only limited evaluation of its nutritive value.

Flowers

The flowers of P. dulce are a high quality nectar and pollen source producing excellent quality honey . Tannin, used to soften leather, can be extracted from the bark, seeds and leaves. The tree also produces a reddish-brown, water-soluble exudate gum similar to commercial gum arabic from Acacia senegal. P. dulce has numerous minor medicinal uses.

Materials And Methods

Procurement of plant material:

For the present investigation, pithecellobium dulce leaves were collected in the month of January 2016 from Thimmapur village of the karimnagar district. The plant was identified and authenticated by BSI/DRC/16-17/Tech/08. The leaves were dried in shade and stored at 25 ° c. It was powdered, passed through sieve no.40 and stored in air tight bottles.

Drugs and chemicals

Cotrimoxazole, Chloramphenicol Ethanol, Acetone, Pyridine, Glacial acetic acid, Ethylacetate, n-Hexane, Dimethyl sulfoxide was used during the experiment.

Preparation of Various Extractions:

The dried powder of leaf material of *Pithecellobiumdulce* was extracted using water, acetone+ethanol(1:10), acetone+ethanol(1:20) and pyridine+glaciaaceticacid(1:10) using maceration.

The dried powder of leaf material of *Pithecellobium dulce* was extracted using ethanol, ethyl acetate, n-hexane using soxhalation.

Qualitative Phytochemical Screening

A spectrum of natural compounds like alkaloids, glycosides, tannins, and essential oils and similar other secondary metabolites which exert physiological activity are synthesized in the plant, in addition to the carbohydrates, proteins and lipids utilized by man as food articles. A systematic and complete study of crude drugs should include a through investigation of both primary and secondary metabolites derived as a result of plant metabolism. The different qualitative chemical tests are to be performed for establishing profile of a given extract for its nature of chemical composition. In the process of phytochemical screening, the crude extracts or isolated constituents are subjected to qualitative and quantitative chemical analyses. Qualitative chemical analysis includes the determination of nature of the constituents

In an extract or its fractions which lead to the isolation of the active lead compound. Quantitative chemical analysis includes the determination of the purity of isolated substances or group of substances in a mixture by finger printing and different analytical techniques [18]. The following tests were carried out on extracts of *"pithecellobium dulce"* to detect various phytoconstituents present in them.

Detection of alkaloids: -

About 50 mg of solvent free extract was treated with little quantity of dilute hydrochloric acid and filtered. The filtrate was tested carefully with various alkaloid reagents.

Mayer's reagent: - (solution of potassium mercuric iodide)

To a few ml of filtrate, two drops of Mayer's reagent was added along the sides of the test tube. The formation of creamy precipitate indicates the presence of alkaloids.

Wagner's test: - (solution of Iodide in potassium iodide)

To a few ml of filtrate, few drops of Wagner's reagent was added along the sides of the test tube. Formation of reddish brown precipitate confirms the presence of alkaloids.

Dragendroff's test:-(solution of potassium bismuth iodide)

To a few ml filtrate, 1 or 2 drops of Dragendorff's reagent was added; formation of a prominent reddish brown color precipitate indicates the presence of alkaloids.

Hager's reagent: - (saturated picric acid solution)

To a few ml of filtrate, 1 or 2 ml of Hager's reagent was added. Formation of a prominent yellow color precipitate indicates the presence of alkaloids.

Detection of carbohydrates: -

About 100 mg of the extract was dissolved in 5 ml of distilled water and filtered. The filtrate was tested for the presence of carbohydrates. [8]

Molish's test: - (solution of □ - naphthol in alcohol)

To 2 ml of filtrate, 2 drops of alcoholic solution of □ - naphthol was added. The mixture was shaken well and 1 ml of concentrated H_2SO_4 was added slowly along the sides of the test tube. The formation of a violet ring at the junction of two liquids indicates the presence of carbohydrates.

Fehling's test: - (solution of copper sulphate, potassium tartarate and sodium hydroxide)

Small amount of extract is hydrolyzed with dilute hydrochloric acid and neutralized with alkali and heated with fehling's

solution A and B. Formation of red precipitate indicates the presence of reducing sugars.

Barfoed's test: -

To 1 ml of filtrate, 1 ml Barfoed's reagent was added and heated on a boiling water bath for 2 minutes. Formation of red precipitate indicates the presence of sugars.

Benedict's test: - (solution of copper sulphate, sodium citrate and sodium carbonate.

To 0.5 ml of filtrate 0.5 ml of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 minutes. Formation of orange red precipitate indicates the presence of reducing sugars.

Detection of Glycosides: -

For detection of glycosides, about 50 mg of extract was hydrolyzed with concentrated hydrochloric acid for 2 hrs on a water bath, filtered and the hydrolyzate was subjected to perform the following tests.

Borntrager's test: -

To 2 ml of hydrolysate, 3 ml of chloroform was added and shaken. Chloroform layer was separated

And 10% ammonia solution was added. Formation of pink color indicates the presence of anthraquinone glycosides.

Keller-Killani test

About 50 mg of the extract was dissolved in 2 ml of glacial acetic acid and two drops of 5% ferric chloride solution and mixed. Then 1 ml of sulphuric acid was added. Reddish brown colour appear at the junction of the two liquid layers and upper layer appear bluish green colour indicates the presence of steroidal glycosides.

Legal's test:

About 50 mg of the extract was dissolved in pyridine. Sodium nitro prusside solution was added and made alkaline using 10% sodium hydroxide solution. Formation of pink color indicates the presence of glycosides.

Detection of proteins and amino acids: -

About 100 mg of extract was dissolved in 10 ml of distilled water and filtered through whatman no.1 filter paper and filtrate was subjected to tests for proteins and amino acids.

Million's test: -

To 2 ml of filtrate, 2 ml of million's reagent was added and heated to boil. The formation of white precipitate, which turns to red up on heating, indicates the presence of proteins / amino acids.

Biuret test: -

To 1ml of filtrate, 1 ml of 10% sodium hydroxide solution was added and heated to boil. To this a drop of copper sulphate solution was added. The formation of purple violet color indicates the presence of proteins.

Ninhydrin test: -

To the test solution, few drops of 0.5% Ninhydrin reagent was added and boiled for few minutes. The formation of violet / blue color indicates the presence of amino acids.

Detection of phyto sterols: -

Leiber mann – bur chards test: -

The extract in chloroform was treated with few drops acetic anhydride, few drops of concentrated sulphuric acid was added along the sides of the test tube. Red, pink or violet color at the junction of the liquids indicates the presence of steroids / tri terpenoids and their glycosides.[13]

Salkowski test: -

The extract in chloroform was treated with a few drops of concentrated $H_2SO_{4,\ shaken}$ well and allowed to stand. The formation of yellow colored layer indicates the presence of tri terpenes and formation of reddish brown colored layer indicates the presence of steroids.

Test for phenolic compounds and tannins

Ferric chloride test: -

About 50 mg of extract was dissolved in distilled water and to this few drops of neutral 5% Ferric chloride solution was added. Formation of blue, green black indicates the presence of phenolic compounds.

Lead – acetate test: -

A small quantity of extract was dissolved in distilled water and to this, 3 ml of 10% lead acetate solution was added. The formation of white precipitate indicates the presence of phenolic compounds.

Bromine water test

About 50 mg of the extract was dissolved in 2 ml of distilled water and to this 1 ml of bromine water was added and observed for the decoloration of bromine water. Decolouration of bromine water indicates the presence of phenolic compounds.

Iodine test

About 50 mg of the extract was dissolved in 2 ml of distilled water and to this 1 ml of iodine solution was added and observed for coloration. Formation of transient red color indicates the presence of phenolic compounds.[17]

Vanillin hydrochloric acid test: -

The test solution was treated with a few drops of vanillin hydrochloric reagent. The formation of pinkish red color indicates the presence of phenolic compounds.

Gelatin test: -

To the test solution add 1% gelatin solution containing sodium chloride and heated to boil. The formation of white precipitate indicates the presence of tannins.

Test for flavanoids

Shinoda test: - (magnesium –hydrochloride test)

A little quantity of extract disssolved in alcohol and few fragments of magnesium turnings and conc Hcl was added. Formation of magenta, crimson red color indicates the presence of fllavonoids.

Alkaline reagent test: -

An aqueous solution of extract was treated with 10% ammonium hydroxide solution. The formation of an intense yellow color which turns to color less on addition of a few drops of dilute acid indicates the presence of flavonoids.

Little quantity of extract was dissolved in 2 ml of alcohol and to the extract; increasing amount of sodium hydroxide was added. It shows yellow coloration, which decolorizes after addition of an acid if flavonoids are present.

Test for saponins :-

To plant extract add 2-3ml of distilled water. Shake the mixture. Formation of foams indicates the presence of saponins.

Antifungal Evaluation

Selection of fungal strains:

Medicinally important fungal strains used in the study were *Aspergillus niger*. These fungi served as test pathogens for antibacterial activity.

Standard reference antibiotic:

The reference antibiotic used is Cotrimoxazole obtained from himedia.

Antifungal activity:

The antifungal activity of the extract were determined by the well diffusion method. Czapek-Dox agar medium was prepared and autoclaved at 121º C, 10lb pressure for 20mins. Allow the medium to cool , before the media get solidify fungal spore suspension was added, and allowed to mix thoroughly with the media the then media was poured into the petriplates. The plates were kept aside for solidification. The all the plates were labeled accordingly. The antifungal activity was tested by agar well diffusion technique. Wells were punched into the agar using gel borer. 50µls of the acetone+ethanol(1:10) and pyridine+glacial acetic acid & aqueous extracts by maceration (10mg/ml in DMSO) were loaded into the wells as per the labeling and incubated at 25º C for 4-5days. The paltes were observed for zone of inhibition every day upto 5days and measured the zone of inhibition.

Name of the component	Weight
Sodium nitrate	2gms
Dipotassium hydrogen phosphate	1gms
Magnesium sulphate	0.5gms
Ferrous sulphate	0.01gms

Sucrose	30gms
Zinc sulphate	Trace
Copper sulphate	Trace
Agar	20gms
Distilled water	1000ml

Table No 2: **Antibacterial Evaluation**

Selection of bacterial strains:

Medicinally important bacterial strains used in the study were staphylococcus aureus, proteus vulgaris, lactobacillus procured from. These bacteria served as test pathogens for antibacterial activity.

Standard reference antibiotic:

The reference antibiotic used is Chloramphenicol.

Preparation of broth culture:

For the preparation of broth culture for bacteria, the liquid media was prepared as per given composition for broth culture. Afterthe sterilization of media the bacterial strains were inoculated under laminar air flow. The incubation of inoculate of media was carried out at 37 °C for 48 hours.

S.no	Name of the compound	Weight
1	Beef extract	1gms
2	Peptone	1gms
3	Sodium chloride	0.5gms
4	Distilled water	100ml
5	Ph	7.4

Table no 3: **Preparation and sterilization of media:**

The agar media was preaped as per the formula given. The nutrient media was prepared as per the formula given. The nutrient agar was taken in 500ml conical flask which is plugged with non absorbed cotton plugs and kept in autoclave(121 °C, 15lbs pressure) to sterilize the media for an hour.

S.no	Name of the component	Weight
1	Peptone	5gms
2	Beef extract	10gms
3	Sodium chloride	10gms
4	Agar	20gms
5	Distilled water	1000ml

Table no 4: **Plating the media**

Molten media was poured on to the petridish(pre-sterilized in oven for 2hours at 200 °C in order to avoid contamination). The plated petri dishes were kept on a plane surface to avoid non-uniform solidification of medium. All these operations were performed on a sterile room which was fitted with laminar air flow.

Bacterial culture preparations:

Bacterial cultures were inoculated in the freshly prepared nutrient broth (which are prepared prior and sterilized) and kept on rotary shaker for 24 hours and observed for growth(turbidity indicates the growth). One day old cultures are used for testing and determination of each extract.

Assay procedure:

The assay procedure was carried out by well diffusion method. The sterilized molten agar media was poured in to petridishes, and kept aside for solidification. Then 100microliters of broth culture of bacterial solution was spread over the solid agar plate. By the use of sterile borer small bores were made over the plate and it was filled with test solution, standard solution and diluting solution respectively for each bacterial plate.

Then plates were kept under incubation for 48hours at 37 °C. The zone of inhibition was measured using scale in millimeters.

Antibacterial activity:

The antibacterial activity of the extract were determined by the well diffusion method. Nutrient agar medium was used for the test. Under aseptic conditions, in the laminar air flow chamber nutrient agar medium was dispersed into pre sterilized petri dishes to yield a uniform depth of 4mm. The media was allowed to solidify.

The test microorganisms were seeded into media containing petri dishes, by spread plate method(100microlitres) with 24hours culturing of bacteria. The plates were kept for pre diffusion for 15mins before use. Wells were then punched with sterile cork borer(6mm diameter) and 50 microlitres of the chloroform and methanol extracts by both soxhlation and maceration(10mg, 20mg/ml in dmso) were placed into each well. A negative control was maintained using 50microlitres of dmso in a well and 50 microlitres of standard antibiotic (streptomycin at 10microlitre/ml) was the positive control. Triplicates were maintained for each extract. Finally the plates were incubated for 18-24 hrs at 37 °C. The diameter of zone of inhibition was indicated by clear area which was devoid of growth of microbes was measured.

Evaluation of Pithecellobium dulce

S.No	**Extract**	**%Dry Weight(W/W)**	**Color**	**Consistency**
1	Acetone+ethanol(1:4) (maceration)	1.6%	Dark green	Resinous

2	Pyridine+glacialaceti c acid(1:4)(maceratio n)	1.2%	Blackis h green	Resinous
3	Distilled water(maceration)	1.1%	Light brown	Non-Resinous
4	Ethanol (soxhalation)	1.2%	Green	Non-Resinous
5	Ethylacetate (soxhalation)	0.49%	Greenis h Black	Resinous
6	n-hexane (soxhalation)	0.9%	Green	Non-Resinous

Table no 6:

The results presented in the table indicate that the leaf powder upon successive soxhlation and also maceration produced highest percentage of ethanol extract upon soxhlation and acetone+ethanol(1:4) upon maceration. Whereas, a little percentage of aqueous extract was obtained by maceration process and ethylacetate extract by soxhalation process. The results indicate that the leaf powder contains more quantity of ethanol soluble constituents upon soxhlation and acetone+ethanol(1:4) upon maceration.

Screening:

Natural compounds or primary as well as secondary metabolites like glycosides, volatile oil, alkaloids, tannins, steroids, and other secondary metabolites which exert physiological activity were synthesized in the plant, in addition to the carbohydrates, lipid and proteins utilized by man as food articles. A sytematic and complete study of crude drugs includes a thorough investigations of both primary and a secondary metabolites derived as a result of plant metabolism. Different qualitative chemical a tests were performed to investigate the phytochemical profile of prepared extracts.

In the present investigation, seven different solvent extracts were prepared from the leaf powder of *Pithecellobium dulce*. Each of these extracts was subjected to a battery of phytochemical tests for detection of various chemical constituents like alkaloids, glycosides, carbohydrates, proteins and amino acids, steroids and terpenoids, flavonoids and tannins.

MACERATION EXTRACTS

Phytochemicalconstituents	Acetone+ethanol (1:4) extract	Pyridine+glacial aceticacid(1:4)	Aqueous Extract
Alkaloids	+	+	+
Carbohydrates	_	_	_
Glycosides	_	_	_
Aminoacids	+	+	+
Steroids	+	_	+
Phenoliccompounds&tannins	+	+	_
Flavanoids	+	+	+
Saponins	+	+	+
Terpenoids	+	+	+

Resins	+	+	+

Table No 7:

'+' - Present '-' –Absent

The maceration extracts Acetone+ethanol(1:4) extract of the powdered leaves of *Pithecellobium dulce* showed presence of alkaloids, tannins, flavonoids, steroids, amino acids , saponins, resins and terpenoids but showed negative results to tannins and flavonoids. Pyridine+glacial acetic acid(1:4) extract of the leaves showed the presence of the alkaloids, amino acids, saponins, tannins, flavanoids, but gave negative results indicating absence of carbohydrates, glycosides, steroids. Aqueous extract gave positive results indicating alkaloids, aminoacids, steroids, flavanoids, and saponins but showed negative results to carbohydrates, glycosides, phenolic compounds ,steroids.

Soxhalation Extracts:

Phytochemical constituents	Ethanol extract	Ethylacetate extract	n-hexane extract
Alkaloids	+	-	+
Carbohydrates	-	-	-
Glycosides	-	-	-
Aminoacids	+	-	+
Steroids	+	-	-
Phenolic compounds and tannins	+	-	+
Flavanoids	-	-	+
Saponins	+	-	+
Terpenoids	+	-	+
Resins	+	-	+

Table.no 8:

'+'-Present '-' Absent

The soxhalation extracts of the powdered leaves of pithecellobium dulce showed presence of carbohydrates, glycosides, alkaloids, tannins, steroids, glycosides, proteins and aminoacids ,terpenoids, resins. The ethanolic extract gave positives results indicating the presence of alkaloids, proteins and amino acids, tannins, steroids, phenolic compounds and tannins and terpenoids but showed negative results to carbohydrates, glycosides, and flavonoids. Ethyl acetate extracts showed negative results for the presence of all chemical constituents. N-hexane extract of the leaves showed the presence of the alkaloids, proteins and amino acids, flavanoids, saponins,tannins and phenolic compounds and terpenoids but gave negavite results indicating absence of carbohydrates, glycosides, and steroids.

Comparative phytochemical evaluation between maceration and soxhalation studies:

When both maceration and soxhalation phytochemical constituents evaluations were compared unlike maceration extracts soxhalation extracts showed the presence of less phytochemical constituents. This indicates maceration process extracted the chemical constituents effectively than the soxhalation.

Phytochemical constituents	**Maceration extracts**	**Soxhalation extracts**
Alkaloids	+	+

Carbohydrates	–	–
Glycosides	–	–
Amino acids	+	–
Steroids	–	+
Phenolic compounds and tannins	+	+
Flavanoids	+	+
Saponins	+	+
Terpenoids	+	+
Resins	+	+

Table.no 9: **Antimicrobial activity of leaves extract of Pithecellobium dulce**

By results it was confirmed that leaves extract of pithecellobium dulce have antimicrobial activity. The different concentration of various extracts by maceration process showed antifungal activity against the tested organism aspergillus niger.

A comparitive study between various extracts had been carried out. Unlike aqueous extract, (pyridine+glacial acetic acid) extract obtained from maceration formed more zone of inhibition i.e., most of the phytoconstituents like alkaloids, steriods, tannins, flavanoids, terpenoids, saponins were extracted effectively by maceration process.

Over all Pyridine+glacial acetic acid(1:4) maceration extract (1000µg/ml) and (10mg/ml &20mg/ml) has showed significant effect towards fungi Aspergillus niger and bacteria Lactobacillus respectively

.

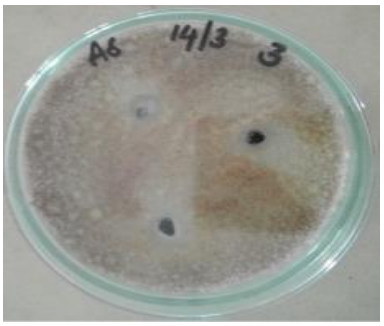


Fig No:3

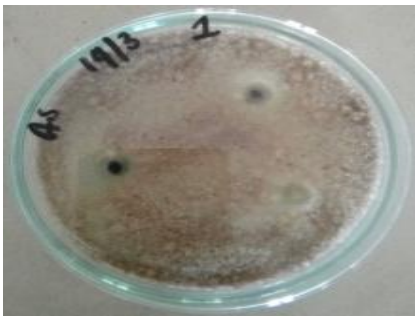


Fig No:4

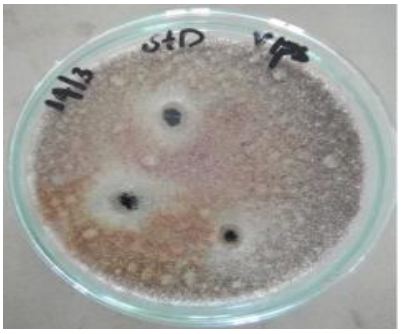


Fig No: 5

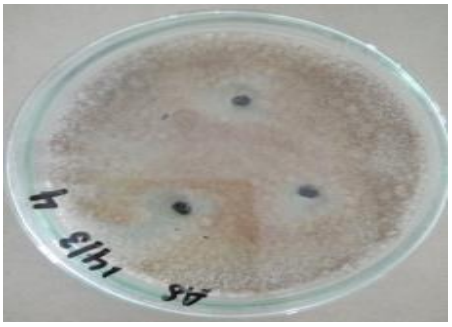
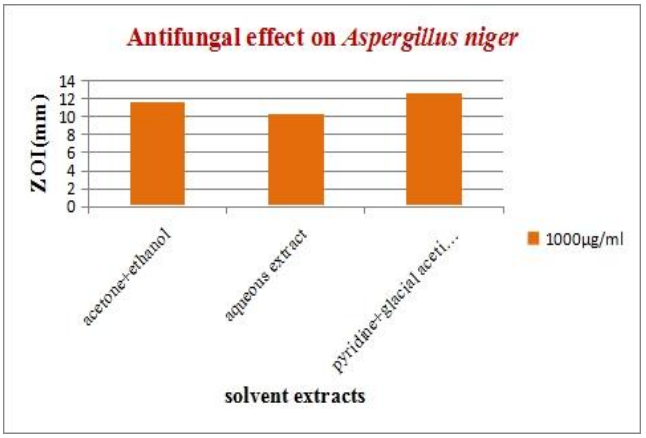


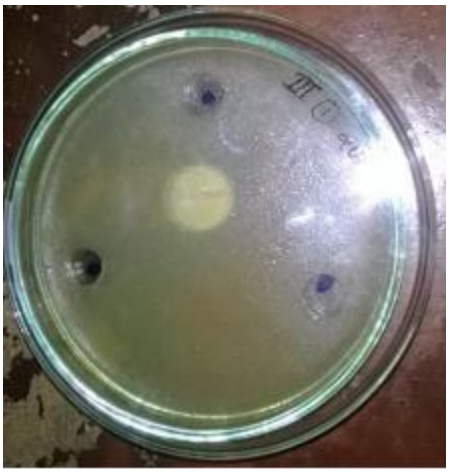
Fig No: 6

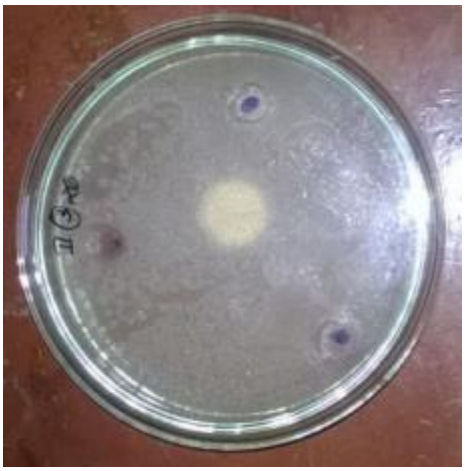
RESULTS

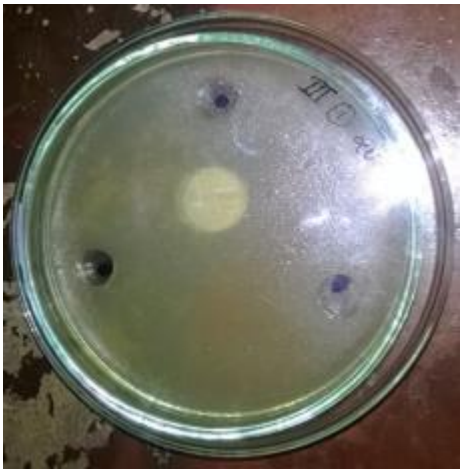
Different extracts(1000µg/ml)	***Aspergillus niger***
	ZOI(mm)
Acetone+Ethanol extract	11.6mm
Aqueous extract	10.3mm
Pyridine+Glacial aceticacid	12.6mm
Standard	12mm

Table.no:10









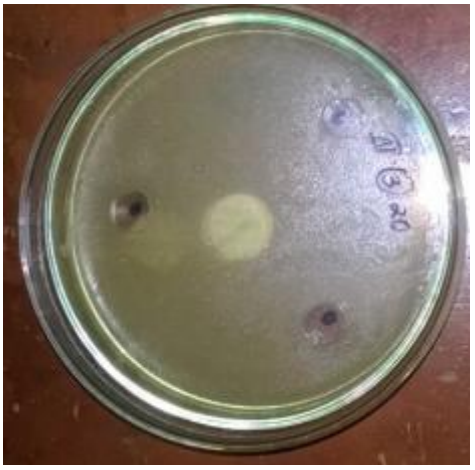


Fig No: 7 Fig No: 8

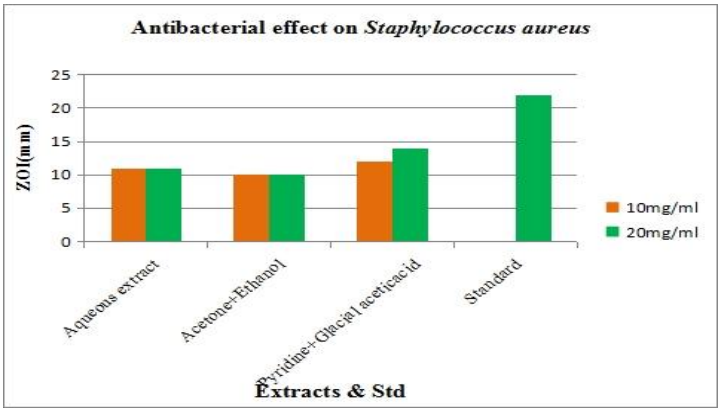
Fig No: 9 Fig No:10

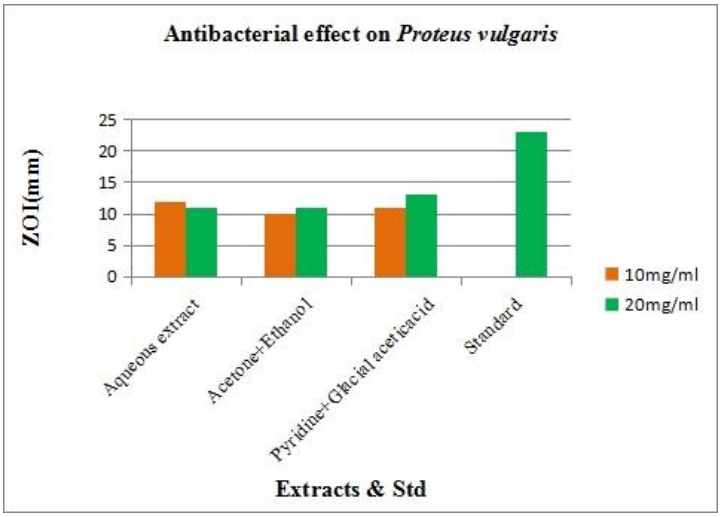
Fig No: 11

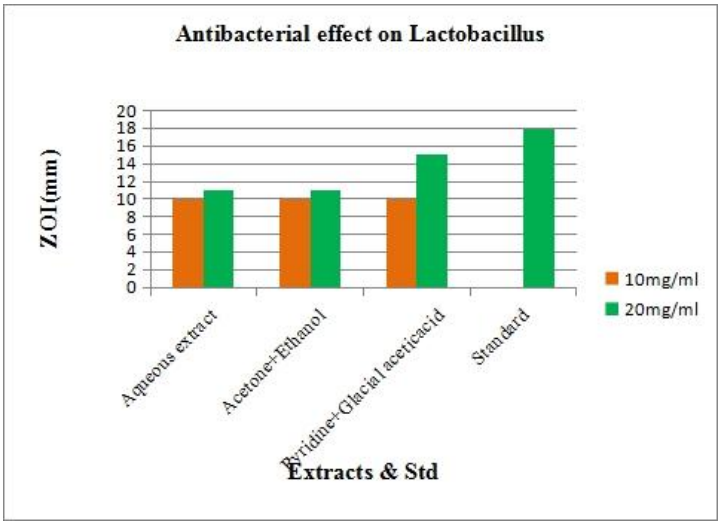
Fig No: 12

Staphylococcus aureus	11	11	10	10	12	14	22
Proteus vulgaris	12	11	10	11	11	13	23
Lacto bacillus	10	11	10	11	10	15	18

Table.no:11







Evaluation of Pithecellobium dulce

S.No	Extract	%Dry Weight(W/W)	Color	Consistency
1	Acetone+ethanol(1:4) (maceration)	1.6%	Dark green	Resinous
2	Pyridine+glacialacetic acid(1:4)(maceration)	1.2%	Blackish green	Resinous
3	Distilled water(maceration)	1.1%	Light brown	Non-Resinous

4	Ethanol (soxhalation)	1.2%	Green	Non-Resinous
5	Ethylacetate (soxhalation)	0.49%	Greenish Black	Resinous
6	n-hexane (soxhalation)	0.9%	Green	Non-Resinous

Table no:6

The results presented in the table indicate that the leaf powder upon successive soxhlation and also maceration produced highest percentage of ethanol extract upon soxhlation and acetone+ethanol(1:4) upon maceration. Whereas, a little percentage of aqueous extract was obtained by maceration process and ethylacetate extract by soxhalation process. The results indicate that the leaf powder contains more quantity of ethanol soluble constituents upon soxhlation and acetone+ethanol(1:4) upon maceration.

Screening:

Natural compounds or primary as well as secondary metabolites like glycosides, volatile oil, alkaloids, tannins, steroids, and other secondary metabolites which exert physiological activity were synthesized in the plant, in addition to the carbohydrates, lipid and proteins utilized by man as food articles. A sytematic and complete study of crude drugs includes a thorough investigations of both primary and a secondary metabolites derived as a result of plant metabolism. Different qualitative chemical a tests were performed to investigate the phytochemical profile of prepared extracts.

In the present investigation, seven different solvent extracts were prepared from the leaf powder of *Pithecellobium dulce*. Each of these extracts was subjected to a battery of phytochemical tests for detection of various chemical constituents like alkaloids, glycosides, carbohydrates, proteins and amino acids, steroids and terpenoids, flavonoids and tannins.

MACERATION EXTRACTS

Phytochemical constituents	Acetone+ethanol (1:4) extract	Pyridine+glacial aceticacid(1:4)	Aqueous Extract
Alkaloids	+	+	+
Carbohydrates	–	–	–
Glycosides	–	–	–
Aminoacids	+	+	+
Steroids	+	–	+
Phenoliccompounds&tannins	+	+	–
Flavanoids	+	+	+
Saponins	+	+	+
Terpenoids	+	+	+
Resins	+	+	+

Table no:7 **'+' - Present '-' –Absent**

The maceration extracts Acetone+ethanol(1:4) extract of the powdered leaves of *Pithecellobium dulce* showed presence of alkaloids, tannins, flavonoids, steroids, amino acids , saponins, resins and terpenoids but showed negative results to tannins and flavonoids. Pyridine+glacial acetic acid(1:4) extract of the leaves showed the presence of the alkaloids, amino acids, saponins, tannins, flavanoids, but gave negative results indicating absence of carbohydrates, glycosides, steroids. Aqueous extract gave positive results indicating alkaloids, aminoacids, steroids, flavanoids, and saponins but showed negative results to carbohydrates, glycosides, phenolic compounds ,steroids.

SOXHALATION EXTRACTS:

Phytochemical constituents	Ethanol extract	Ethylacetate extract	n-hexane extract
Alkaloids	+	-	+
Carbohydrates	-	-	-
Glycosides	-	-	-
Aminoacids	+	-	+
Steroids	+	-	-
Phenolic compounds and tannins	+	-	+
Flavanoids	-	-	+
Saponins	+	-	+
Terpenoids	+	-	+
Resins	+	-	+

Table.no:8

'+'-Present '-' Absent

The soxhalation extracts of the powdered leaves of pithecellobium dulce showed presence of carbohydrates, glycosides, alkaloids, tannins, steroids, glycosides, proteins and aminoacids ,terpenoids, resins. The ethanolic extract gave positives results indicating the presence of alkaloids, proteins and amino acids, tannins, steroids, phenolic compounds and tannins and terpenoids but showed negative results to carbohydrates, glycosides, and flavonoids. Ethyl acetate extracts showed negative results for the presence of all chemical constituents. N-hexane extract of the leaves showed the presence of the alkaloids, proteins and amino acids, flavanoids, saponins,tannins and phenolic compounds and terpenoids but gave negavite results indicating absence of carbohydrates, glycosides, and steroids.

Comparative phytochemical evaluation between maceration and soxhalation studies:

When both maceration and soxhalation phytochemical constituents evaluations were compared unlike maceration extracts soxhalation extracts showed the presence of less phytochemical constituents. This indicates maceration process extracted the chemical constituents effectively than the soxhalation.

Phytochemical constituents	**Maceration extracts**	**Soxhalation extracts**
Alkaloids	+	+
Carbohydrates	–	–
Glycosides	–	–
Amino acids	+	–
Steroids	–	+

Phenolic compounds and tannins	+	+
Flavanoids	+	+
Saponins	+	+
Terpenoids	+	+
Resins	+	+

Table.no 9: **Antimicrobial activity of leaves extract of Pithecellobium dulce**

By results it was confirmed that leaves extract of pithecellobium dulce have antimicrobial activity. The different concentration of various extracts by maceration process showed antifungal activity against the tested organism aspergillus niger.

A comparitive study between various extracts had been carried out. Unlike aqueous extract, (pyridine+glacial acetic acid) extract obtained from maceration formed more zone of inhibition i.e., most of the phytoconstituents like alkaloids, steriods, tannins, flavanoids, terpenoids, saponins were extracted effectively by maceration process.

Over all Pyridine+glacial acetic acid(1:4) maceration extract (1000µg/ml) and (10mg/ml &20mg/ml) has showed significant effect towards fungi Aspergillus niger and bacteria Lactobacillus respectively.

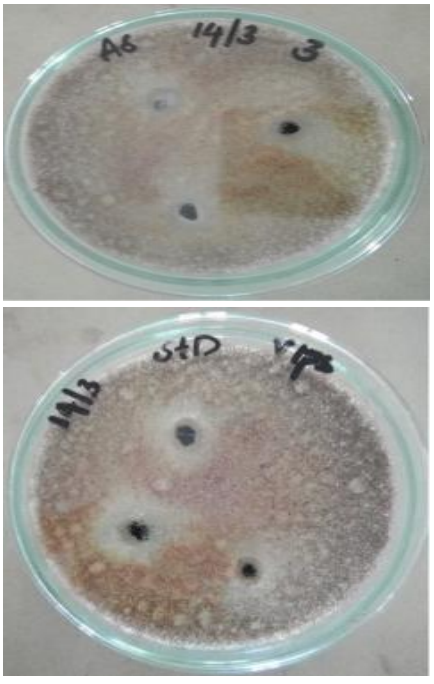


Fig No: 13 and 14

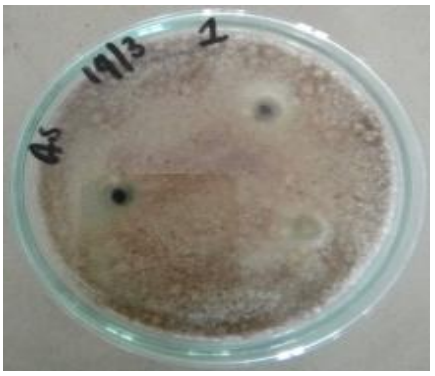
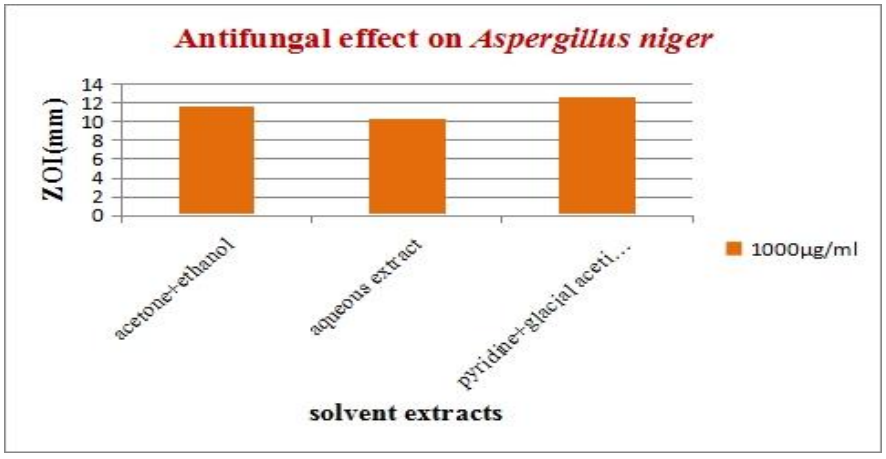


Fig No; 15 and 16

RESULTS

Different extracts(1000µg/ml)	*Aspergillus niger*
	ZOI(mm)
Acetone+Ethanol extract	11.6mm
Aqueous extract	10.3mm
Pyridine+Glacial aceticacid	12.6mm
Standard	12mm

Table.no:10



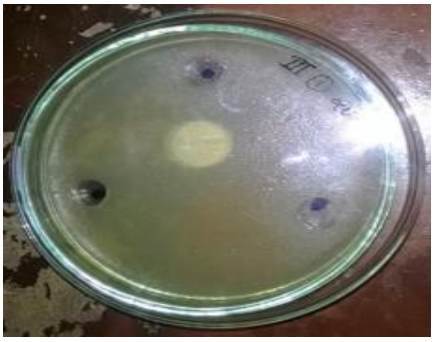


Fig No:12

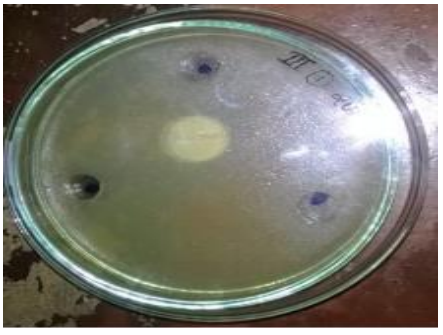
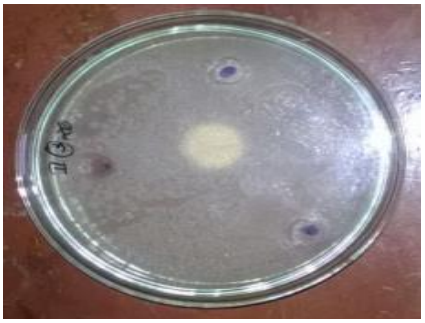


Fig No: 13



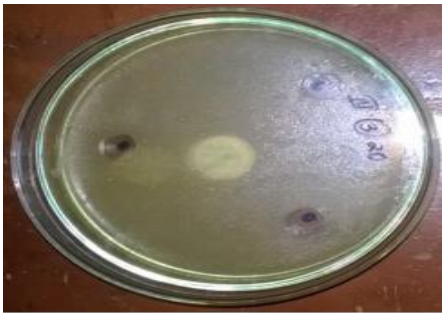


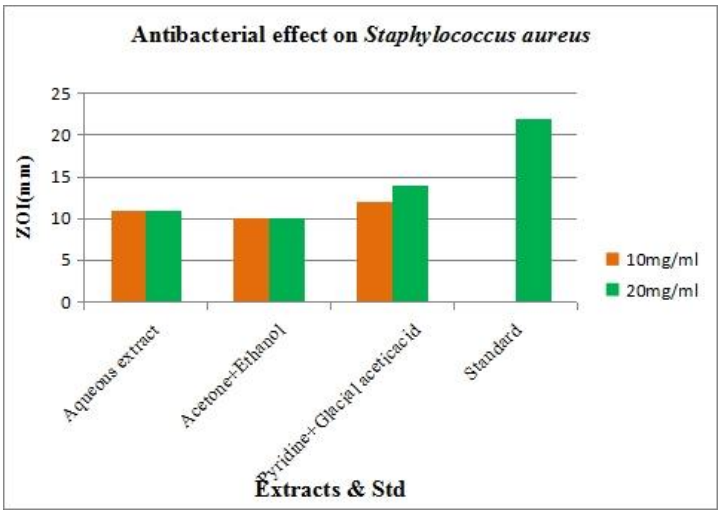
Fig No: 15

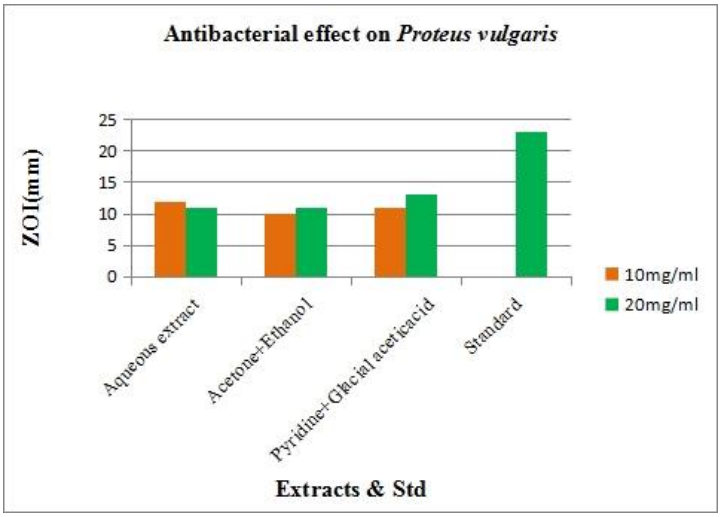
Fig No: 14

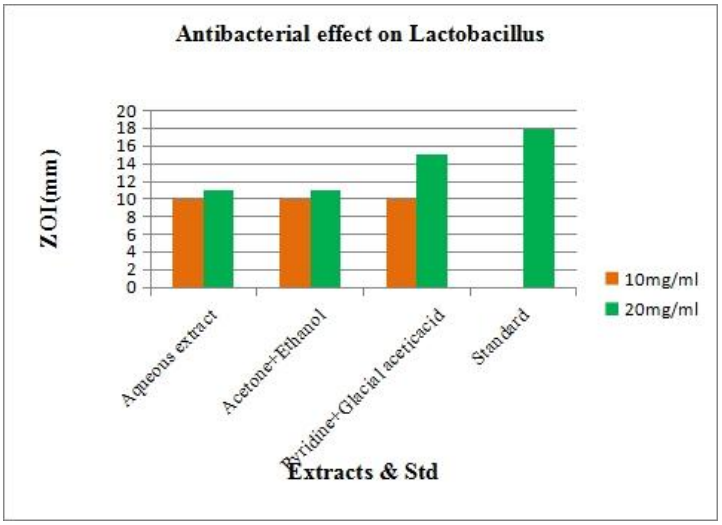
Name of the organism	Aqueous extract		Acetone+Ethanol		Pyridine+Glacial aceticacid	Standard	
	10mg/ml	20mg/ml	10mg/ml	20mg/ml	10mg/ml	20mg/ml	10mg/ml
Staphylococcus aureus	11	11	10	10	12	14	22
Proteus vulgaris	12	11	10	11	11	13	23
Lacto bacillus	10	11	10	11	10	15	18

Fig No: 16

Fig No: 17







References:

1. Acharya, Deepak and shrivastava anshu, 2008, Indegenious Herbal Medicines:Tribal formulations and traditional herbal practices, Aavishkar publishes Distributor, Jaipur- India. ISBI. International journal of Green pharmacy.2008,2,126-128.
2. Ali M S, Azhar I , Ahmad F, Ahmad A U, smanghani K U, 2001. Antimicrobial screening of Mimosaceous plants. Pharmaceutical Biology. 39(1): 43-46.
3. Bacteria(eubacteria). Taxonomy browser. NCBI. Retrived 2008-09-10.
4. Banarjee Anup, 2005. Studies on Alkylated resin from Pithecellobium dulce .J Indian Chem Soc. 82: 186- 187.
5. Barrera N L, Bravo L L, Bermudez T K, Garcia S F , Jimenez E M, Reyes C R, 2003. Antifungal activity against postharvest fungi by extracts and compounds of Pithecellobium dulce seeds (huamuchil). Acta Horticulturae. 628 (2): 761-766. Journal of Medical Pharmaceutical and Allied Sciences (2013) 05: 35-45 44.
6. Bautista B S , Garcia D E, Barrera N L, Reys C R, Wilson C L, 2003. Seasonal evaluation of the postharvest fungicidal activity of powders and extracts of huamuchil (Pithecellobium dulce): Action against Botrytis cinerea,Penicillium digitatum and Rhizopus stolonifer of straw berry fruit. Postharvest Biol. Technol. 29: 81-92.
7. Bhargva P Krishna, Gupta M B, Chittranjan Dr, 1970. Anti inflammatory activity of Saponins and other Natural Products. Indian J. Med. Res. 58: 724.
8. Bruneton J, 1999, Pharmacognosy, Phytochemistry Of Medicinal Plants, Second Edition, Intercept, 131-160.
9. Chandran P G R, Balaji S, 2008. phytochemical investigation and pharmocological studies of the flowers of Pithecellobium dulce . Ethnobotanical Leaflets. 12, 245-253
10. Delgado VF, Lopez V H E, Valdes R S, BlancoLabra, Chagolla L A, Lopez V E J. 2004. Isolation and properties of a Kunitz-type protein inhibitor obtained from Pithecellobium dulce seeds. J. Agricult. Food Chem. 52(20): 6115-21.
11. Fredrickson JK, Zachara JM, Balkwill DL, Kennedy D, Li SM, Kostandarithes HM, Daly MJ, Romine MF, Brockman FJ"Geomicrobiology of high-level nuclear waste-contaminated

vadose sediments at the Hanford site, Washington state". Applied and Environmental Microbiology *,2004.*

12. Govindarajan M, Sivakumar R, Rajeswary M, Yogalakshmi K, 2012. Adulticidal activity of Pithecellobium dulce (Roxb.) Benth. against culex quinquefasciatus. Asian Pacific Journal of Tropical Disease. 124- 128.
13. Harborne J.B,2005, Methods A Guide To Modern Techniques Of Plant Analysis, 3rd Edition, Springer Verlag, Berlin, 107-200.
14. Misra G, Nigam S K, Singh S, Nigam S G, Saxena RC, 1979. Steroid and Triterepenoid Saponins as Spermicidal agents. Indian Drugs. 17 (1): 6-8.
15. Mukesh kumar, kiranNehra(et.al 2014), Antimicrobial efficacy of crude extract of Pethacellobium dulci bark.
16. Mukesh kumar, kiranNehra(et.al 2013),phytochemical analysis & antimicrobial efficacy of leaf extracts of pethacellobium dulci.
17. Mukherjee Pk, 2002, Quality Control Of Herbal Drugs. An Approach, To Evaluation Of Botanicals, 1st Edition, Business Horizon Pharmaceuticals Publications, New Delhi, 525-533.
18. Rangari Vd,2004, Pharmacognosy and Phytochemistry, Career Publications, Part-1, First Edition, 129-150.
19. Shanmugakumaran S D, Amerjothy S, Balakrishna K, Vasantha kumar M S, 2005. Antimycobacterial properties of leaf extracts of Pithecellobium dulce Benth. Indian Drugs. 42(6): 392-395.
20. Shanmugakumaran S D, Amerjothy S, Balakrishna K, 2006. Pharmacognostical, Antibacterial and Antifungal Potentials of the Leaf extracts of Pithecellobium dulceBenth. Phcog. Mag. 7(2): 163- 167.
21. Whittaker R.H, 1969, new concepts of kingdoms of organisms science 163:150-161.