

PHYTOCHEMICAL STUDIES AND ANTHELMINTIC ACTIVITY OF THE LEAF EXTRACT OF PITHECOLOBIUM DULCE, BENTH.

Safna Nazar N and Oommen P. Saj*

Dept. of Botany, University College, Thiruvananthapuram, Kerala, India, 695003

Article Received on
18 Aug 2015,

Revised on 11 Sept 2015,
Accepted on 05 Oct 2015,

***Correspondence for
Author**

Oommen P. Saj

Dept. of Botany,
University College,
Thiruvananthapuram,
Kerala, India, 695003,

ABSTRACT

Pithecolobium dulce, Benth is an evergreen tree widely distributed in the greater part of India. The present study was aimed to reveal anthelmintic, antioxidant activity and identification and quantification of crude phytochemical constituents present in the plant. Plant material was collected and leaves were used to make the extracts. The polar solvent methanol was used to make extracts by the distillation method using soxhlet apparatus. The shade dried leaves was powered and used to obtain extract after continuous heating of 8 hours at boiling point of 65°C. The extract was concentrated to dryness and the residue were transferred to preweighed bottle and stored for further studies. Initial part of the study was focus on the phytochemical screening of

methanolic leaf extract that revealed the presence of reducing sugar, glycosides, flavanoids, tannins, terpenoids, alkaloids, coumarins and steroids where as saponins showed negative results. Moisture content of *Pithecolobium dulce* Benth. was determined and the result revealed the presence of 26.17 % moisture content. The result revealed various concentration of biochemicals present in the plant tissue. Result showed that the least content was flavanoid which was only about 0.000037 mg/g. Protein was present in higher amount, about 10.54 mg/g. *P. dulce* plant was found to be a rich source of proteins and fattyacids. On HPLC analysis 13 compounds were detected of which the major constituent was found to be gallic acid, a phenolic compound. Another compound, Tridecanoate, a saturated fatty acid was also found in large quantity. The presence of tridecanoate and gallic acid as major compounds may be the reason for its prominent biological activities like antioxidative and anthelmintic potentials.

KEYWORDS: *Pithecolobium dulce*, HPLC, anthelmintic, antioxidant, phytochemical studies.

INTRODUCTION

Pithecolobium dulce Benth. belongs to family Leguminosae and subfamily Mimosoideae. The generic name refers to the curly pod that mimics an ape's earring (*pithekos- ellobium*) and the species name-*dulce* refers to the sweet pod. It is distributed widely in the greater part of India and is also found in Southeast Asia. 10 species of this genus are found in India.^[1] It is an evergreen medium sized, irregularly branched, spiny tree that reaches heights of about 22m. Greyish bark becoming rough, then furrowed. Leaves are paripinnate with one single pair of pinnae and one single pair of leaflets per pinna; leaflets are 2.0-3.5 cm long x 1.0-1.5 cm wide. There are small thorns 2.0-15.0 mm long in axillary pairs inserted on each side of the leaves' pedicels. Leaves are deciduous but foliage is persistent, as the new leaves appear while the old ones are being shed; so that the tree looks like an evergreen. Flowers are disposed in small spherical glomerules of 1 cm in diameter, forming short axillary panicles of 5-30 cm in length. Flowers are white-greenish slightly fragrant 1.0-1.5 mm in diameter, with a hairy corolla, 50 thin stamens, connate in a tube at their basis, surrounded by the green calyx. Legumes are greenish-brown to red or pinkish, rather thin, 10-15 cm long x 1-2 cm wide. There are 10 seeds per pod; pods are irregular in shape and flattened, set in a spiral of 1 to 3 whorls and strangled between the seeds (lomentaceous). Seeds are black and shiny with 1 cm in diameter, hanging in the pods by a red funicle. The pod is dehiscent on both sides. It has fair cold and heat tolerance.^[2]

The bark of the plant is used as astringent in dysentery, febrifuge and it is also useful in dermatitis and eye inflammation.^[3] The leaves possess abortifacient, antidiabetic, emollient, anticonvulsant and antiulcer properties. The leaves can be applied as plasters for pain and venereal sores. Decoctions of leaves used for indigestion, intestinal disorder, earache, toothache, leprosy and larvicide.^[4] Various parts of plant are used for different purposes like, leaf as astringent, seed oil as spermicidal, anti-inflammatory, anti-oedema, fruit and seed as edible and bark for tannin extraction.^[5] In this scenario, the present study was concentrated on "Phytochemical Studies and Anthelmintic activity of the leaf extract of *Pithecolobium dulce* Benth."

MATERIALS AND METHODS

Pithecolobium dulce Benth was collected from Palayam, Trivandrum District. Fresh materials were collected in the early morning, washed with water and wiped with a cheese cloth to remove dust and dirt.

Preparation of Extracts

The phytochemicals present in the leaf of the plant material was extracted by the distillation method using Soxhlet apparatus. Methanol solvent system was used for the separation of chemicals. About 250g of fresh leaves were weighed and shade dried. The dried sample was powdered and 25g of powdered sample was packed into a thimble and kept in Soxhlet apparatus. Solvent was taken for extraction and powdered sample was siphoned by three times. The whole apparatus was kept over a heating mantle and was heated continuously for 8 hours at boiling point 65⁰C. The extract was concentrated to dryness and the residue was transferred to a preweighed sample bottle and was stored in a desiccators for further studies.^[6]

Phytochemical Screening of Plant Extract

Different biochemical parameters like reducing sugars, glycosides, flavonoids, alkaloids, tannins, terpenoids, steroids, coumarins, saponins were qualitatively estimated by standard procedure.^[6]

Test for Reducing Sugars (Fehling's Test)

Fehling solution was boiled and ethanol extract was added to it in a test tube. Presence of reducing sugars was indicated by the development of brick red colour.

Test for Flavonoids (Shinoda Test)

A few magnesium turnings were added to 1ml of extract, followed by the addition of concentrated HCl drop by drop. Presence of pink colour indicated the flavonoids in the extract.

Test for Terpenoids (Liebermann–Burchard Test)

Two ml of dry extract was dissolved in chloroform and added a few drops of acetic anhydride and conc. H₂SO₄ to it and kept undisturbed for few minutes. Formation of green colour indicated the presence of terpenoids.

Test for Steroids

To the extract evaporated in dryness, add a few drops acetic anhydride and concentric H₂SO₄, an array of color changes from yellow, green and brown to black indicates the presence steroids.

Test for Tannins

Two drops of 5% FeCl_3 was added to 1ml of extract. Development of dirty green precipitate indicated the presence of tannins.

Test for Coumarins

One ml of extract was taken and dissolved in methanol, then a few drops of alcoholic NaOH was added to it. Concentrated HCl was added along the sides of the test tubes. The appearance and disappearance of yellow colour indicated the presence of coumarins.

Test for Alkaloids

Ten ml of 2% H_2SO_4 was added to 5ml of extract and warmed for 2 minutes and filtered. 1ml of its aliquot was then treated with a few drops of Dragendorff's reagent. Orange brown precipitate indicated the presence of alkaloid.

Test for Saponins

Five ml of distilled water was added to 5ml of the extract and shaken well. The mixture was heated to the boiling point. Frothing indicated the presence of saponins.

Test for Glycosides (Keller-Killiani Test)

0.5g of extract was diluted to 5ml with distilled water. 2ml of glacial acetic acid containing one drop of ferric chloride solution was added to it. This was then treated with 1ml of Conc. H_2SO_4 . A brown ring at the interface indicated the presence of glycosides.

Test for Anthraquinones

A known quantity of powdered material was mixed with 10 ml of 1% hydrochloric acid and boiled for 5 minutes. The mixture was filtered, cooled, added with equal volume of chloroform. Separate the chloroform layer, mixed well and added with equal volume of 10% ammonia solution, allow the layer to separate. A delicate pink colour indicated the presence of anthraquinones.

Test for Phlobatannins

The extract was warmed with 5 ml of 1% hydrochloric acid and red precipitate showed the presence of phlobatannins.

Test for Iridoids

The extract was added to 1 ml of reagent (10 ml acetic acid, 0.2% copper sulphate solution and 0.5 ml concentrated hydrochloric acid). The mixture was heated over a small flame. Development of a light blue colour indicated the presence of iridoid.

Quantitative Test**Determination of Moisture**

5g of the material was taken in a preweighed petridish. The petridish was placed without lid into an oven at 1100C for three hours. The petridish was taken out and closed immediately with the lid. The dish was cooled in a desiccator and weighed out. The amount of moisture of the material was calculated from the difference in weight.^[7]

Estimation of Total Phenol

Estimation of total phenols was performed by the method of Mayr et al.^[8] Weighed amount of fresh tissue was chopped and refluxed in boiling methanol (80%) for 10 minutes. The refluxed mixture was homogenized in 80% methanol. The homogenate was filtered and centrifuged at 10,000 rpm for 10min. The supernatant was collected and the volume was made up to 20ml using 80% methanol. 0.5ml of Folin-Ciocalteu reagent was added to it followed by 2ml of 20% sodium carbonate to an aliquot of sample and mixed thoroughly. The reaction of phenol with phosphomolybdic acid in Folin-Ciocalteu reagent in alkaline medium produced a blue coloured complex. The tubes were kept in a boiling water bath for one minute, cooled and centrifuged, supernatant was taken and the absorbance was measured at 650nm.

Estimation of Total Proteins

The total protein content of the test material was estimated by following the method (Bradford).^[9] The Bradford reagent was prepared by dissolving 60 mg of Coomassie Brilliant Blue G-250 in 1 L 3% per chloric acid. 1 g of tissue was taken and homogenized with 10 ml of phosphate buffer, filtered through double layered cheese cloth and the filtrate was subjected to centrifugation at 10,000 rpm for 5 minutes. Supernatant was collected and made up to a known volume. 0.5 ml of protein was made up to 1.5 ml with homogenizing buffer and mixed with Bradford reagent and kept for 5 minutes. The absorbancy was recorded at 595 nm against the blank containing reagent and buffer without protein. The protein content was estimated using the standard graph of Bovine Serum Albumin (BSA).

Estimation of Lipids

Total lipids in the sample tissues were estimated by the method of Bligh and Dyer.^[10] with some modifications. 1 g of plant tissue was homogenized in 10 ml of chloroform / methanol (2:1). The homogenate was filtered using filter papers and the extraction was performed three times and the filtrates were pooled together, transferred to a separating funnel after adding a few drops of chloroform and 1 ml of saturated NaCl solution and water. The mixture was shaken well and allowed to settle. Subsequently the lower fraction was released into a pre-weighed petridish and kept overnight at 600 °C for evaporation and the lipid was estimated gravimetrically.

Total Carbohydrate

Weighed amount of fresh leaves was homogenized with distilled water. The homogenate was filtered using a double layered cheese cloth. The filtrate was then centrifuged at 10,000 rpm for 10min. The supernatant was collected and the volume was made up to 20ml using distilled water. An aliquot of the sample was pipetted out and 2ml of DNS reagent was added. It was then kept in a boiling water bath for 10min. The amount of total carbohydrates present in it was determined using the standard graph of glucose.^[11]

Estimation of Chlorophyll

Weighed amount of fresh leaves was homogenized with 80% acetone. The homogenate was filtered using a double layered cheese cloth. The filtrate was then centrifuged at 5000 rpm for 5min. The supernatant was collected and made up to a known volume using 80% acetone. An aliquot of sample was pipetted out and made up to a known volume using 80% acetone. The absorbance was measured at 490, 645 and 663nm.

Estimation of Total Flavonoids

Aluminum chloride colorimetric method was used for the determination of the total flavonoids in the plant extract. The aluminum chloride reagent was prepared by dissolving 133 mg of crystalline aluminum chloride and 400 mg of crystalline sodium acetate in 100 ml of 80% methanol. Water (400 microliter) and aluminum chloride reagent (1 ml) were added to 2 ml of the extract. Absorbance was measured at 430 nm against blank without aluminum chloride reagent. From the stock solution of quercetin (1 mg/ml prepared in 80% methanol), various dilutions of quercetin (5-25 microgram/g) were prepared in methanol and a standard curve was plotted. The amount of flavonoids was calculated as quercetin equivalent from the calibration curve of quercetin (5-25 microgram/g).^[12]

Estimation of Amino acids

The amount of amino acids present in the sample was estimated by (Moore and Stein, 1948).^[13] A known quantity of fresh tissue was chopped and put in boiled 80% methanol for 10 minutes and refluxed. The refluxed matter was homogenized; homogenate was filtered and centrifuged at 7,500 rpm for 10 minutes. The supernatant was collected and made up to a known volume by 80% methanol. An aliquot was pipetted, added 5 ml of Ninhydrin-reagent mixed thoroughly and boiled in a water bath for 10 minutes. Cooled under running water, read the absorbance at 570 nm against the blank and expressed in mg g-l.

Estimation of Tannins

The amount of tannins was determined by (Schanderl, 1970)¹⁴. A known quantity of the powdered sample was transferred to a 250 ml conical flask. 75 ml water is added, boiled for 30 minutes, centrifuged at 2,000 rpm for 20 minutes and collected the supernatant in 100 ml volumetric flask and made up the volume. 1ml of the sample was transferred to a 100 ml volumetric flask containing 75 ml water. 5 ml of Folin–Denis reagent, 10 ml sodium carbonate solution were added and diluted to 100 ml with water. Shaken well and read the absorbance at 700 nm after 30 minutes. The amount of tannins present in the sample was calculated using standard graph of tannic acid and expressed in mg g-l.

Estimation of proline

Proline was estimated colorimetrically by following the method of Bates (1973).^[15] 1 g fresh tissue was homogenized in 10 ml of 3% aqueous Sulfosalicylic acid. The homogenate was filtered through whatman No.2 filter paper. 2 ml of the filtrate was mixed with 2 ml of Acid ninhydrin and 2 ml of glacial acetic acid in test tube and kept for 1 hour at 90°C. The reaction was terminated in an ice bath. The reaction mixture was extracted with 4 ml Toluene, and mixed vigorously for 10-50 seconds. The layer containing toluene was aspirated from the aqueous phase and warmed to room temperature and the absorbance was recorded at 520 nm using toluene as the blank. The proline concentration was determined from the standard curve of proline.

HPLC Analysis**Apparatus and Chromatographic conditions**

A supercritical fluid extractor SFE-2 (Applied Separation, USA) which is capable of pressure up to 680 bar and temperature up to 240°C, static and dynamic extraction with flow from 0 to 10 L/min (gaseous carbon dioxide) and extraction vessels from 5 ml to 1l were used. An

Agilent 1200 liquid chromatograph system (Agilent technologies, CA, USA) consisting of binary pump, an auto-sampler and diode-array detector was used. The column configuration consisted of an Agilent Zorbax Extend reversed-phase C18 column (250 mm × 4.6 mm, 5 µm). Detection wavelength was set at 220 nm. The mobile phase consisted of A (methanol) and B (deionized water), using a linear gradient: 0-40 min (85% A), 40-60 min (85% A-95% A). The flow rate was 1.0 ml/min. The column temperature was maintained at 30 °C.

Preparation of standard solutions–*Pithecolobium dulce* Benth.

A mixed standard stock solution containing a few standard compounds was prepared in methanol. Working standard solutions were prepared by diluting the mixed standard solution with methanol to give six different concentrations. The standard solutions were filtered through a 0.45 µm membrane prior to injection. The standard stock and working solutions were stored at 4°C.

Preparation of sample solutions

The extract sample of *Pithecolobium dulce*, Benth. under optimized conditions (extraction pressure: 30 Mpa; extraction temperature: 35°C; extraction time: one hour; 20 ml 95% ethanol modifier) was used. . After evaporating ethanol to dryness by a rotary evaporator, residue was dissolved in methanol in a 25 ml flask, and then filtrated through a 0.45 micro-m millipore filter before HPLC.

Antioxidant Assay

DPPH (1, 1-Diphenyl-2-Picrylhydrazyl) radical scavenging activity

DPPH (0.006% w/v) was prepared in 95% methanol. The methanol extract of the leaves was mixed with 95% methanol to prepare the stock solution (1mg/ml). Freshly prepared DPPH solution was taken in test tubes and extract was added followed by serial dilutions (1.25-20µg/ml) to every test tube such that the final volume was 2ml and discoloration was measured at 517nm after incubation for 30minutes in the dark (thermo UV1 spectrophotometer, thermo electron corporation, England, UK). Measurements were performed at least in triplicate. Ascorbic acid was used as a reference standard and dissolved in double distilled water to make the stock solution with the same concentration (1mg/ml). The control sample was prepared, which contained the same volume without any extract and 95% methanol was used as the blank. Percentage scavenging of the DPPH free radical was measured using the following equation.^[16]

CALCULATION

Percentage of scavenging activity = $\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$

Anthelmintic Studies

Worm Collection

The Indian earth worm *Pheretima posthuma* (Annelida) was collected from moist soil of Botanic Garden, Department of Botany, University College, Trivandrum. Adult earthworms collected were washed with distilled water to remove all matters was used for the study.

Anthelmintic Activity

Leaf extract of *Pithecolobium dulce*, Benth. was dissolved in minimum amount of DMF and the volume was adjusted to 10 ml with saline water. Drug and extract solutions were freshly prepared before starting the experiment. In each case, six earthworms were released into 10 ml of desired formulations as follows; vehicles (5% DMF in normal saline), Albendazole (20 mg/ml), different concentrations (40mg/ml, 60mg/ml, 80mg/ml) of methanolic leaf extracts of *Pithecolobium dulce*, Benth. in normal saline containing 5% DMF were used. Observations were made for the time taken to paralysis and death of individual worm. Paralysis was said to occur when the worms were not able to move even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body colors as our previous method.^[17] a thimble and kept in Soxhlet apparatus. Solvent was taken for extraction and powdered sample was siphoned by three times. The whole apparatus was kept over a heating mantle and was heated continuously for 8 hours at boiling point 65°C. The extract was concentrated to dryness and the residue was transferred to a preweighed sample bottle and was stored in a desiccators for further studies.^[6]

RESULTS AND DISCUSSION

Medicinal plants play the most important role in the traditional medicines in various developing countries. Most of the flora remain virtually of the medicinal utilizing through traditional eastern system of medicines strongly upholds the use of elements for curing many diseases.^[18]

Table:1 Phytochemical screening of Methanol leaf extract of *Pithecolobium dulce*, Benth.;

Sl No	Qualitative tests	Methanol
1	Anthraquinones	+++
2	Glycosides	++
3	Flavonoids	++
4	Alkaloids	+
5	Tannins	++
6	Terpenoids	++
7	Saponins	-
8	Sterols	++
9	Reducing sugars	++
10	Coumarins	+
11	Phlobatannins	-
12	Iridoids	++

(+) Presence of phytochemical constituents, (-) Absence of phytochemical constituent

Table:2 Quantitative analysis of various biochemical parameters of *Pithecolobium dulce*, Benth.;

Sl No	Quantitative test	Mg/g(FW)
1	Total Carbohydrates	0.554
2	Total Phenol	0.01
3	Total Protein	10.54
4	Chlorophyll –a	0.0871
5	Chlorophyll-b	0.0879
6	Total chlorophyll	0.175
7	Carotenoids	0.3
8	Tannin	0.001
9	Total Aminoacids	0.84
10	Flavanoids	0.000037
11	lipid	8.06
12	proline	0.0046

Mg/g FW: Milligram/gram Fresh weight, DW: Dry weight

Table: 3 HPLC Identified compounds in the methanolic leaf extract *Pithecolobium dulce*, Benth.

Sl No	Compounds identified in Methanol leaf extract of <i>Pithecolobium dulce</i> , Benth.	Percentage detected
1	Dulcitol	4.89
2	Octadecanate	3.85
3	Tetradecanote	1.34

4	Methyl di hexacosenate	4.39
5	Catechol	7.56
6	Oleate	6.89
7	Kaempferol	2.67
8	Gallic acid	16.13
9	Palmitate	7.75
10	Tridecanoate	12.45
11	Quercetin	4.31
12	Afezilin	1.78
13	Geddlic acid	1.79

Table: 4 Anthelmintic Activity of methanolic leaf extract of *Pithecolobium dulce*, Benth.

Group	Sample (concentration mg/ml)	* Time taken for paralysis (min)	* Time taken for Death (min)
I	Control	No paralysis (upto 180min)	No death (upto 180min)
II	Albendazole (20mg/ml)	3.14	10.46
III	methanolic leaf extract (40mg/ml)	2.38	3.32
IV	methanolic leaf extract (60mg/ml)	1.37	2.13
V	methanolic leaf extract (80mg/ml)	0.76	1.72

* Results are expressed as Mean from four observations; Control worms were alive up to 7 hrs of Observation.

Table:5 Effect of Methanol leaf extract of *Pithecolobium dulce*, Benth.; and positive control (L-Ascorbic Acid) on in vitro Free Radical Scavenging Activity by DPPH Assay

Sl No	Concentration(g/ml)	Methanol plant extract (% of inhibition)	L-Ascorbic Acid (% of inhibition)
1	1.25	30.96	34.29
2	2.50	36.41	38.72
3	5	43.29	39.42
4	10	51.12	49.67
5	20	53.49	58.97

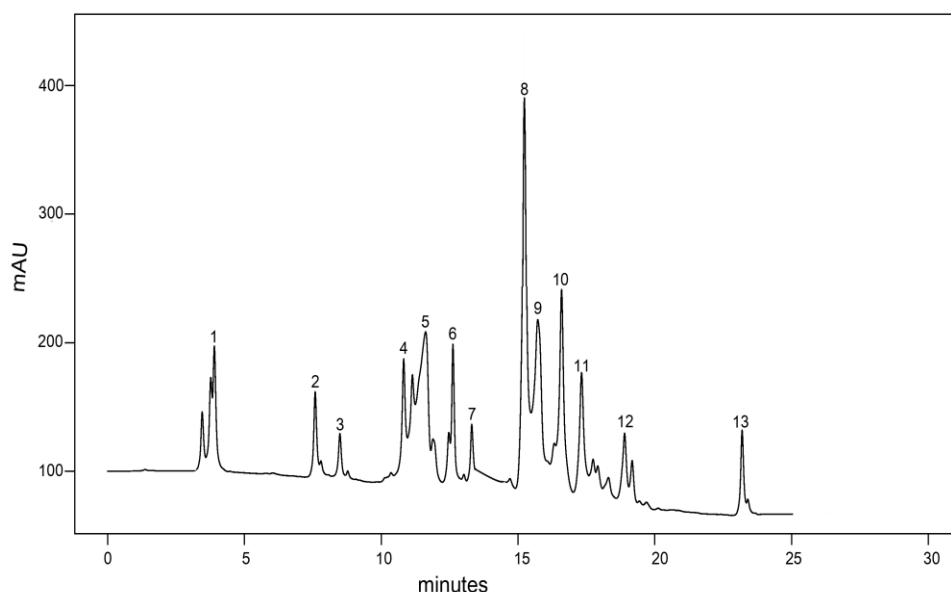


Figure: 1 HPLC Identified compounds in the methanolic leaf extract *Pithecolobium dulce*, Benth.

Phytochemistry

Qualitative analysis

Initial part of the study was focus on the phytochemical screening of methanolic leaf extract that revealed the presence of reducing sugar, glycosides, flavanoids, tannins, terpenoids, alkaloids, coumarins and steroids where as saponins showed negative results (Table 1). Mukesh et al.,^[19] reported the phytochemical analysis of Leaf extracts of *Pithecolobium dulce* Benth. showed the presence of alkaloids, anthraquinones, cardiac glycosides, proteins, tannins, terpenoids, and sugars. Saponins were found to be absent in the tested extracts; while flavonoids and sterols were found to be present in 50% of the extracts. The relative anthelmintic and antioxidant activity of leaf extract may not be easily correlated with any individual component but with a mixture of compounds. Sharma et al.,^[20] reported the Screening of Phytochemical constituents of Hydro ethanolic extracts of Aerial parts of *Pithecolobium dulce* and *Ricinus communis*. Tannins suggesting the ability to play a major role for the treatment of sore throat, wound healing, antidiarrhoea and antihaemorrhagic agent,^[21,22] Tannins are also reported for the antimicrobial degradation of dietary proteins of semen.^[23,24] Flavanoids are reported to inhibit the initiation, promotion and progression of tumors. Phenols and flavanoids, both are potent water soluble antioxidants.^[25] These phenolic compounds also possess some biological properties such as antiapoptosis, anti-septics, anticarcinogen, antiageing, antiinflammation, antiatherosclerosis, improvement of endothelial function and cardiovascular protection.^[26,27] These phenolic compounds are also capable for

inhibition of angiogenesis and cell proliferation activities^[27]. The anti-oxidative or free radical scavenging activity for schemes degenerative diseases are reported.^[28] Terpenoids have carboxylic acid group due to which their presence is responsible for the activity of organic extracts.^[29] Presence of glycosides makes the plants responsible for lowering the blood pressure as previously they have been known to lower blood pressure.^[30]

Alkaloids are one of the major phyto-constituent present in traditional plants known for anti-fungal, anti-microbial and anti-inflammatory activity.^[31] Presence of alkaloids in the methanolic extract of the plant tested makes them responsible for the treatment of several diseases. Alkaloids are also reported for its cytotoxicity.^[32] antispasmodic.^[24] antibacterial and analgesic activity^{6,24}. Presence of saponins in traditional plants known to possess reduction in cholesterol level and can manage cardiovascular diseases in humans.^[24,32] Saponins are also reported for their use as emulsifying agents and having anti-fungal, anti-inflammatory activity.^[33] coagulating and precipitating property in red blood cells.

Quantitative analysis

Moisture content of *Pithecolobium dulce* Benth. was determined and the result revealed the presence of 26.17 % moisture content. The result revealed various concentration of biochemicals present in the plant tissue. Result showed that the least content was flavanoid which was only about 0.000037 mg/g. Protein was present in higher amount, about 10.54 mg/g. *P. dulce* plant was found to be a rich source of proteins and fatty acids were reported.^[34] Proteins are complex nitrogenous organic substances that are one of the most important plant products to man. A part from this, the protein hydrolytes from various sources are reported to possess antioxidant activity.^[32,34] Amino acids (0.84 mg/g), Carbohydrates (0.55 mg/g), Carotenoids (0.3 mg/g), Reducing sugar (0.06 mg/g), Chlorophyll a (0.0871 mg/g), Chlorophyll b (0.0879 mg/g), Total Chlorophyll (0.175 mg/g), Phenol (0.01 mg/g). Plant phenols are groups of natural products with variable structure that are well known for their beneficial effects on health possess significant antimicrobial and antioxidant activities.^[32,33,34]

Tannin (0.001 mg/g), Proline (0.0046 mg/g), Lipid (8.06 mg/g) are present in the methanolic leaf extract of *Pithecolobium dulce*, Benth. (Table-2 and Fig-2).

HPLC Analysis

HPLC analysis of the methanol leaf extract of *Pithecolobium dulce*, Benth. is given in (Table-3, figure-3). 13 peaks were obtained from HPLC analysis where all phytoconstituents were

identified (Table-3). 13 compounds were identified from this plant. They are Tridecanoate (12.45%), Octadecanoate (3.85%), Tetradecanoate (1.34%) Afezilin (1.78%), Palmitate (7.75%), Catechol (7.56%), Dulcitol (4.89%), Geddlc acid (1.79%), Quericitin (4.31%), Oleate (6.89%), Gallic acid (16.13%), Methyl di hexacosenate (4.39%) and Kaempferol (2.67%) were detected from the methanolic leaf extract of *Pithecolobium dulce*, Benth. by using HPLC. The major constituent was found to be gallic acid, a phenolic compound at retention time of 15 minutes. The high concentration in leaf, make it potentially useful in the medicines because they exhibit antioxidant and antimicrobial activities. Tridecanoate, a saturated fatty acid is found at retention time of 17 minutes. Tridecanoate present in the leaf of *Pithecolobium dulce*, Benth. gives nutritional property. The presence of tridecanoate and gallic acid as major compound has been reported from the plant.^[34]

Tridecanoate is used in preparation of drugs for dermatological disorders and anti-inflammation. Octadecanoate used in anti-acne drugs, anti-inflammatory drugs, antibiotics and also used in anti aging preparations. Tetradecanoate is also called myristic acid. Myristic acid is used in cosmetic and tropical medicinal preparations where good absorption through the skin is desired.. Afezilin is used in preparation of drugs for abortion. 3-Trans-Isocamphylcyclohexanol, widely used as a replacement for sandalwood oil, is prepared from catechol. Palmitate act as a long-acting release carrier medium when injected intramuscularly. Along-acting antipsychotic medication, paliperidone palmitate (marketed as INVEGA Sustenna), used in the treatment of schizophrenia, has been synthesized using the oily palmitate. Dulcitol is used to treat urinary tract infection. Gallic acid was found to show cytotoxicity against cancer cells, without harming healthy cells. Gallic acid is used as remote astringent in cases of internal haemorrhage. Gallic acid is also used to treat albuminuria and diabetes. Quericitin is used for treating several conditions of the heart and blood vessels including —hardening of the arteries (atherosclerosis), high cholesterol, heart disease, and circulation problems. It is also used for diabetes, cataracts, hay fever, peptic ulcer and schizophrenia, small amounts of oleic acid are used as an excipient in pharmaceuticals. Kaempferol has been shown to reduce growth in pro-myelocytic leukemia cells through altering the cell cycle.^[34,35,36]

Antioxidant Activity

The DPPH assay is a very simple method form screening small anti-oxidant molecules, because the ratio can be observed visually and intensity can be analyzed using common

spectrophotometric assay. The stable radical DPPH has been used widely for the determination of primary antioxidant activity. The DPPH antioxidant assay is based on the ability of DPPH a stable free radical, to decolorize in the presence of antioxidants. The Figure-4 showed percentage of DPPH scavenging activity of methanolic extract of leaf of *P. dulce*. The highest percentage of inhibition is 53.49% at a concentration of 20 μ g by DPPH assay compared with the standard ascorbic acid. The strong antioxidant and free radical scavenging activity was previously reported in *Pithecolobium dulce*, Benth. Leaves.^[36]

The results indicate that *P. dulce* plant extract contains significant amounts of flavonoids and phenolic compounds. Both these classes of compounds have good antioxidant potential and their effects on human nutrition and health are considerable.^[36] Phenolic compounds are also very important plant constituents because their hydroxyl groups confer scavenging ability.^[34,35] The result found from the experiment confirms that medicinal plant *P. dulce* are potential enough to prevent oxidative stress and other diseases.^[36]

Anthelmintic Activity

It is evident from the experimental data that, methanolic leaf extract of *Pithecolobium dulce*, Benth. showed significant anthelmintic activity at 80mg/ml. Results were comparable with the standard drug, Albendazole at a concentration of 20 mg/ml. Table-6 reveals that methanol extract of leaf of *Pithecolobium dulce*, Benth. Showed better anthelmintic activity. These extracts required the least time for causing paralysis and death of the earthworms compared with the standard. The function of the anthelmintic drugs like Albendazole is to cause paralysis of worms so that they are expelled in the faeces of man and animals. The extracts not only demonstrated this property, they also caused death of the worms, especially at 80 mg/ml as compared with the Albendazole. In conclusion, *Pithecolobium dulce*, Benth. have been confirmed to display anthelmintic activities. Methanolic leaf extracts shows over all potency for anthelmintic activity. Methanol leaf extract were tested for the presence of phytochemicals with different tests and shows presence of tannins, flavonoids, anthraquinones, glycosides, iridoids, coumarins, terpenoids and alkaloids (Table1). These phytoconstituents may be responsible for the said activities. Sugumaran et al.,^[4] previously reported that the aqueous extract is more potent than the alcoholic extract and both the extracts are endowed with significant anthelmintic property which was comparable with the reference drug, piperazine citrate.

CONCLUSION

Medicinal plants are a major source of raw material for the traditional system like Ayurveda, Sidda and Unani. Even the modern system of medicine has more than 25 percent of drugs in use, which are either plant based or plant derived. Although several tree possess various medicinal properties, among them *Pithecolobium dulce*, Benth is an evergreen tree widely distributed in the greater part of India and is also found in Southeast Asia.

The bark of the plant is used as astringent in dysentery, febrifuge and it is also useful in dermatitis and eye inflammation. The leaves have been reported to possess abortifacient, antidiabetic properties, emollient, anticonvulsant, and antiulcer. The leaves can be applied as plasters for pain and venereal sores. Decoctions of leaves have been reported to be used for indigestion, intestinal disorder, earache, toothache, leprosy, and larvicide. Various parts of plant are used for different purposes like leaf as astringent, seed oil as spermicidal, anti-inflammatory, anti-oedema. Thus the present study was aimed at finding 7anthelmintic, antioxidant activity and identification and quantification of crude phytochemical constituents present in the plant *Pithecolobium dulce*, Benth.

Plant material was collected and leaves were used to make the extracts. The polar solvent methanol was used to make extracts by the distillation method using soxhlet apparatus. About 650gms of leaves was weighed and shade dried for 45 days. The dried material was powdered and was used to obtain extract after continuous heating of 8 hours at boiling point of 65°C. The extract was concentrated to dryness and the residue was transferred to preweighed bottle and stored for further studies.

- *Pithecolobium dulce* leaves contain a number of pharmaceutically important phytochemicals like reducing sugar, alkaloids, coumarins, flavanoids and tannins.
- Quantitative analysis showed the presence of Carbohydrates, Phenol, Protein, chlorophyll, Carotenoids, Aminoacids, Tannin and Flavanoids. Plant leaves were found to be a rich source of proteins and fattyacids.
- 13 compounds were analyzed by HPLC analysis. The major constituent was found to be gallic acid, a phenolic compound at retention time of 15 minutes and the another major compound was Tridecanoate, a saturated fatty acid is found at retention time of 17 minutes.
- Antioxidant activity of methanol extract of leaves was estimated using DPPH assay and the extract was found to possess concentration dependent scavenging activity on DPPH

radicals. The highest percentage of inhibition is 53.49% at a concentration of 20µg/ml by DPPH assay compared with the standard ascorbic acid.

- Further the study concentrated on anthelmintic activity of the extract of plant leaves. It showed better activity compared with the drug Albendazole.

From the results it can be concluded that the methanol leaf extract of *Pithecolobium dulce* has a potential source of compounds with antioxidant and anthelmintic properties. Further studies are required to identify and isolate the active principle responsible for its pharmacological activities and exploited as alternative food source for human and animal consumption.

REFERENCES

1. Trease GE and Evans WC. Pharmacology 11 Ed. Bailliere Tindall Ltd, London, 1985; 9: 60-75.
2. Aubreville A. Flore forestieresoudano-guinene: AOF-Cameroun-AEF Societed Editions Geographiques, Maritimes, Coloniales, Paris, 1950; 40: 1347-1352.
3. Chopra RN, Nayar SL and Chopra IC. Glossary of Indian Medicinal Plants. Council of Scientific and Industrial Research. New Delhi, 1992; 195-196.
4. Shanmugakumaran SD, Amerjothy S and Balakrishna K. Pharmacognostical, antibacterial and antifungal potentials of the leaf extracts of *Pithecolobium dulce*, Benth. Pharmacognosy Magazine, 2006; 7: 163-167.
5. Mohammed S, Kasera PK and Shukla JK. Exploited plants of potential medicinal value from the Indian Thar desert. Natural Product Radiance, 2004; 3: 69-74.
6. Harbone JB. Phytochemical methods, A Guide to Modern techniques of plant analysis. Chapman and Hall; London, 1973; 7- 126.
7. Sadasivam S and Balasubramanian T. Introduction to Practical Manual in Biochemistry. Tamilnadu, agriculture university, Coimbatore, 1987; 14-27.
8. Mayr VD, Trutter C, Santos DH, Baner and Senchitt W. Developmental changes in phenol concentrations of golden delicious apple fruits and leaves. Phytochemistry, 1995; 38(5): 1151-1155.
9. Bradford MM. Binding Assay for Protien. Analytical Biochemistry, 1976; 72: 248-254.
10. Bligh EG and Dyer WJA rapid method for total lipid extraction and purification. Canadian. Journal of Biochemistry and Physiology, 1959; 37: 911-917.
11. Rose JH. Determination of food carbohydrates. DAT sathgate, Uni, canbr, England. 1965; 258.

12. Zhinshen J, Mengeheng T and Jianming W. The determination of flavanoid contents in mulberry and their by scavenging effects on super oxide radicle, 1999; 122-127.
13. Mooore S and Stein WH. Methods in Enzymology. Academic Press, NewYork. 1948; p468.
14. Schanderl SH. Methods in Food Analysis. Academic Press, NewYork, 1970; 709.
15. Bates LS, Waldren RP and Teare ID. Rapid determination of free proline for water stress studies. Plant Soil, 1973; 39: 205-207.
16. Saeed N and Shabbier MR. Antioxident activity, total phenolic and total flavanoid contents of whole plant *Torilis leptophylla* L. BMC Complementary and Alternative Medicine, 2012; 12: 1-12.
17. Girme AS, Bhalke RD, Ghogare PB, Tambe VD, Jadhav RS and Nirmal SA, Comparative in vitro anthelmintic activity of *Mentha piperita* and *Lantana camara* from Western India. Pharmaceutical science, 2006; 5(1): 5-7.
18. Kaneez AF, Qadirruddin M, Kalhor MA, Khaula and Badar Y. Determination of major trace elements in *Artemisia elegantissima* and *Rhazaya stricta* and their uses. Pakistan Journal of Scientific and Industrial Research, 1988; 45(5): 291-293.
19. Mukesh K, Kiran N and Duhani JS. Phytochemical analysis and Antimicrobial Efficacy of Leaf Extracts of *Pithecolobium dulce* Benth. Asian Journal of Pharmaceutical and Clinical Research, 2013; 6(1): 70-76.
20. Sharma S, Verma N and Garg V. Screening of Phytochemical constituents of Hydro ethanolic extracts of Aerial parts of *Pithecolobium dulce* Benth and *Ricinus communis*. Research Journal of Chemical Sciences, 2014; 4(8): 54-57.
21. Asquith TN and Butler LG. Interaction of condensed Tannins with selected proteins. Photochemistry, 1986; 25(7): 1591-1593.
22. Hatano T, Edamatsu R, Hiramatsu M, Mori A, Fujita Y and Yashuara D. Effects of interaction of tannins with co-existing substances. VI. Effects of tannins and related polyphenols on superoxide anion radical and on DPPH radical. Chemical and Pharmaceutical Bulletin, 1989; 37: 2016-2021.
23. Aletor VA. Allelochemicals in plants and feeding stuffs. Nutritional, biochemical and physiopathological aspects in animal production. Veterinary and Human Toxicology, 1993; 35: 57-67.
24. Kim SY, Kim JH, Kim SK, Ohandy MJ and Jung MY. Antioxidant activities of selected oriental herb extracts. Journal of the American Chemical Society, 1994; 71: 633-640.

25. Okwu DE and Okwu ME. Chemical composition of *Spondias mombin* Linn. Plant parts. *Journal of Sustainable agriculture and the Environment*, 2004; 6(2): 140-147.
26. Han X, Shen T and Lou H. Dietary Polyphenols and their biological significance. *International Journal of Molecular Sciences*, 2007; 950-988.
27. Vani T, Rajani M, Sarkar S and Shishoo CJ, Antioxidant properties of ayurvedic formulation triphala and its constituent. *International Journal of Pharmacology*, 1997; 35(5): 313-317.
28. Antherden LM. *Textbook of Pharmaceutical Chemistry*. 8th edn. Oxford University Press, London, 1969; 813-814.
29. Nyarko AA and Addy ME. Effects of aqueous extract of *Adenia cissampeloides* on blood pressure and serum analyte of hypertensive patients. *Phytotherapy Research*, 1990; 4(1): 234-246.
30. Nobori T, Miurak K, Wu DJ, Takabayashik LA and Carson DA. Deletion of cyclin dependent kinase-4 inhibitor gene in multiple human cancers. *Nature*, 1994; 46: 753- 756.
31. Kanazawa H, Nagata Y, Matsushima Y, Tomota M and Takai N. Determination of acidic saponins in crude drugs by high-performance liquid chromatography on octadecylsilyl porous glass. *Journal of Chromatography-A*, 1993; 30: 408-414.
32. Just MJ, Recio MC, Giner RM, Cueller MU, Manez S, Billia AR and Rios JL. Anti-inflammatory activity of unusual lupine saponins from *Bupleurum frutescens*. *Planta Medica*, 1998; 64: 404-407.
33. Samina KK, Wazir S and Syed A. Phytochemical studies on *Pithecolobium dulce* Benth. A Medicinal plant of Sindh, Pakistan. *Pakistan Journal of Botany*, 2013; 45(2): 557-561.
34. Aiyer HS, Warri AM, Woode DR, Hilakivi L and Clarke R. "Influence of Berry-Polyphenols on Receptor Signaling and Cell-Death Pathways: Implications for Breast Cancer Prevention". *Journal of Agricultural and Food Chemistry*, 2012; 60(23): 569-708.
35. Shankar DK, Mahesh KS. Antioxidant and free radical scavenging activity of *Pithecolobium dulce*, Benth wood bark and leaves. *Free Radicals and Antioxidants*, 2012; 2: 62-73.
36. Rice ECA, Miller NJ, Howell PG, Bramley PM and Pridhan JB. The relative antioxidant activity of plant derived poly phenolic flavonoids. *Free Radical Research*, 1995; 22: 375-383.