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PHYSICO-CHEMICAL ANALYSIS AND PHYTOCHEMICAL EVALUATION OF *PITHECELLOBIUM DULCE* LEAVES

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ABSTRACT

The present research work deals with determination of physicochemical analysis and phytochemical investigation of *Pithecellobium dulce* leaves. Standardization of crude drug extracted from plant plays an important role in identifying the quality and purity of drugs. The present research analysis reveals standardization of crude drug that includes moisture content, total ash, acid insoluble ash, water soluble ash, and different soluble extractive values were estimated. The highest extractive values were recorded in water soluble extract of crude drug, the bioactive principles present in medicinal plants and that may lead to drug discovery and development. In the present study deals with phytochemical constituents of the *Pithecellobium dulce* leaves medicinal plant of family *Leguminosae* were identified in order to relate their presence with bioactivities of the plants. The present research find highlights that methanolic extracts of *Pithecellobium dulce* leaves had the highest number of phytochemicals compared to other solvent extracts. Hence, methanolic extracts of *Pithecellobium dulce* leaves holds the greatest potential to treat various human diseases and has profoundmedical applicability.

KEYWORDS: *Pithecellobium dulce* leaves, Physico-chemical analysis, Phytochemical screening.

INTRODUCTION

In many developing countries, major proportion of the population are depends on traditional practitioners and using medicinal plant products in order to get their health in good conditions.[1]

The use of medicinal products and supplements has increased exceedingly over the past decades, not less than 80% of world population are depends on medicinal plant products for their primary health. *Pithecellobium dulce* Benth belongs to family *Leguminosae*, which widely distributed in the greater part of India and is also found in Southeast Asia. This is commonly referred as manila tamarind, as its sour resembles tamarind. *Pithecellobium dulce* is a small to medium sized evergreen spiny tree height which grows up to 18-20 m. Medicinal plants are the rich source of different natural constituents with extensive pharmacological activities. In recent years, nutraceuticals have acquired huge attention as nutritional supplements for their positive physiological effects in the human beings[2], from villages to developed cities, traditional ways of natural medication consecutively becoming popular. Afzelin (kaemperol-3-O-α-L-rhamopyranoside) was isolated from *Pithecellobium dulce* leave which have anti mycobacterial property. The leaves also reported to have anti inflammatory, analgesic, antioxidant and antidiabetic activities.[3-5] The *Pithecellobium dulce* fruits have also been studied for anti-inflammatory activity due to saponin content, free radical scavenging and gastro-protective, antidiabetic and hepatoprotective effects.[6-9] Various phytochemical constituents with medicinal properties to cure various health illnesses have been revealed every day by researchers.[10-14] These derived plant products have the enormous potentiality to treat diseases like diabetes, cancer, inflammation, etc. Although, there are numbers of allopathic drugs which have been reported every day since, the permanent recovery from the diseases and the secondary complications aroused during the medication.

Understanding about physicochemical and preliminary phytochemical screenings of plants is needed in order to discover and develop novel therapeutic agents with improved efficacy. A number of plant species that thrive in hostile environment replete with bacteria, fungi or virus synthesize defensive natural products against these pathogens, they may also exhibits bactericidal, fungicidal or virucidal activity in human beings.[15,16] The present research work deals with determination of physicochemical analysis and phytochemical screening of *Pithecellobium dulce* leaves.

MATERIAL AND METHODS

Collection of the Plant Material

Pithecellobium dulce leaves were collected from the college ground, Mother Teresa Pharmacy College, Sathupally, Khammam, Telangana.

Preparation of Pithecellobium dulce leave powder

Plant leaves are collected and air dried because to prevent it from direct sunlight impact to minimize undesirable chemical reactions of plant metabolites. Dry conditions are crucial to prevent the formation of artifacts as a result of microbial fermentation and subsequent degradation of the plant metabolites. Hence in the present study, leaves are dried in shade and then powder with a mechanical grinder. The powder was passing through sieve number 44 and stored in an airtight container for further studies.

Physicochemical Analysis

Physicochemical analysis includes moisture contents, ash values and extractive values are determined to study the quality and purity of the powder of *Pithecellobium dulce* leaves.

Moisture Content

5g of the air-dried sample was weighed it was noted as (W_b), into a pre-dried and weighed (W_a) soiled porcelain crucible. The sample was dried in a hot air oven at 100°C until two consecutive weighing's (W_c) do not vary by more than 5mg. The moisture content of the sample was deliberated with reference to crude air dried drug.

$$\text{Moisture (\%)} = \frac{(W_b - W_C)}{(W_b - W_a)} \text{ x } 100$$

Total Ash Value

A silica crucible was heated until it becomes redness and cooledin a desiccator and weighed (W_1). About 5g of air- dried sample was placed to the silica crucible and weighed alongwith the contents accurately (W_2). Sample was burst into flame gradually in an electrical muffle furnace, increasing the heat to 450°C until it is white, indicating the absence of carbon. Itwas cooled in desiccators and reweighed (W_3), and the total ash content was determined as in equation.

$$\text{Total ash (\%)} = \frac{(W_3 - W_1)}{(W_2 - W_1)} \text{ x } 100$$

Acid-insoluble Ash

To the silica crucible containing the total ash 10ml of 2*M* HCl were added, covered with a watch-glass and boiled lightly for 5min. The watch-glass was cleaned with 5ml of hot water and the washings were added to the crucible. The insoluble matter was filtered on an ash less filter-paper and cleaned with hot water until the filtrate is neutral. The filter-paper containing the insoluble content was kept into the original crucible, dried on a hotplate and ignited to

constant weight (W_4). The residue was allowed to cool in desiccators for 30min, and then it was weighed. Acid-insoluble ash content was determined as in equation.

$$\text{Acid-insoluble ash (\%)} = \frac{(W_4 - W_1)}{(W_2 - W_1)} \times 100$$

Extractive Values

The extractive values of *Pithecellobium dulce* in various solvents like petroleum ether, benzene, chloroform, ethyl acetate, methanol, ethanol and water were estimated by employing the method of analysis described in Pharmacopoeia of India.[18] About 5g of air-dried *Pithecellobium dulce* leave powder was taken in a stopper conical flask. 100ml of the respective solvent were mixed, shaken well and allowed to stand for 24h with occasional shaking. Then it was filtered, 50ml of the filtrate were pipette out into a clean, previously weighed china dish and evaporated on a water bath. Lastly it was dried at 100ºC in an oven, cooled in a desiccator and weighed. The percentage of solvent soluble extractive with reference to the air-dried content was calculated.

Water Soluble Ash

25ml of water was mixed to the crucible containing the total ash and boiled for 5min. The insoluble components were collected on an ash less filter-paper. The filter was cleaned with hot water and then ignited in a crucible for 15min at a temperature not exceeding 350°C. The residue was confessing to cool in desiccators for 30min, and then re-weighed (W_5), calculations were finished according to equations.

Weight of residue, W_6 (g) = W_5 - W_1Weight of ash W_7 (g) = W_3 - W_1Water-soluble ash value (g) = W_7 - W_6

$$\text{Water-soluble ash (\%)} = \frac{(W_7 - W_6)}{(W_1)} \times 100$$

Preliminary Phytochemical ScreeningPreparation of Plant Extract

The coarse powder of the plant material was weighted (20g) and placed into the earthy colored glass bottles. The coarse powder was exposed to extraction in 250ml every one of petroleum ether, chloroform, and methanol solvents independently. At that point the solvents were added to it. At that point the containers were fixed with aluminum foil and kept in research center shaker at room temperature, and the flasks were shaken for 5 days. At last the concentrate was sifted through numerous layers of muslin fabric for coarse filtration. The coarse filtrate was than separated through Whatman filter paper number 1. They got filtrate was vanished in a vacuum turning evaporator under decreased pressing factor at 40ºC until

the filtrate was diminished to 33% of the beginning filtrate volume and the concentrated concentrates were additionally dissipated to get dry concentrates. A piece of dry concentrates were re-disintegrated in dimethyl sufloxide (DMSO) and were put away in plug glass bottles and another part was kept as such in hermetically sealed containers at 4ºC for additional examination.

Phytochemical Screening

The phytochemical screening establishes regarding the presence of different compounds possessing therapeutic effects. The different solvent extracts of *Pithecellobium dulce* leaves were used for screening the presence of carbohydrate, glycosides, alkaloids, flavonoids, steroids, coumarin, tannins, saponins, phenol, protein, xanthoprotein, catachin, quinone, anthroquinone, sugar and terpenoids according to standard procedures.[17]

Screening for Carbohydrates (Molisch Test)

3 drops of α-naphthol (20% in ethanol) was added to 2ml of extract sample. Then 1ml of concentrated sulphuric acid was added by side of the test tube. Reddish-violet ring was observed at the junction of the two layers indicated the presence of carbohydrates.

Screening for Glycosides (Borntrager's test)

50mg of extract powder was mixed with 5ml of concentrated H_2SO_4, it was heated for 5min, and it was filtered. The filtrate was mixed with 0.5ml of 10% NaOH and kept a side for 5min. Production of reddish brown precipitate indicates the presence of glycosides.

Screening for Alkaloids (Dragendroff's test)

2ml of the extracted samples were mixed with 8ml of 1% HCl, heated and filtered. Then the filtrates were mixed with solution of Potassium Bismuth Iodide (Dragendroff's reagent). Red color precipitate was observed, indicates the presence of alkaloids.

Screening for Reducing Sugar

50mg of the extract powder was taken in a test tube and equal volume of Fehling reagents (A and B) were added and boiled. Production of brick- red precipitate indicates the presence of reducing sugar.

Screening for Terpenoids (Salkowski test)

5ml of solvent extract was treated in 2ml of chloroform, and then added of 3ml concentrated sulfuric acid (H_2SO_4). Production of reddish brown color was formed at the interface that

indicates the presence of terpenoids.

Screening for Steroids (Liebermann Burchard test)

Extracted samples were treated with chloroform and filtered. The filtrates were mixed with few drops of acetic anhydride, heated and cooled. By addition of concentrated sulphuric acid, it produced brown ring at the junction indicates the presence of phytosterols.

Screening for Tannins

In 25ml distilled water, 50mg of various solvent extracted powder was placed and filtered. 1% aqueous ferric chloride ($FeCl_3$) solution was added to the filtrate. The appearance of different colors like green, purple, blue or black that indicate the presence of tannins in the test samples.

Screening for Saponin

In 25ml distilled water, 50mg of the various solvent extracted powders were boiled in boiling water bath and filtered. 10ml of the filtrate was treated with 5ml of distilled water and was shaken vigorously to the formation of stable persistent froth. The frothing was treated with 3 drops of olive oil and shaken vigorously for the production of emulsion thus a characteristic of saponins.

Screening for Flavonoids (Shinoda Test)

To 5ml of the extracted solution, few fragments of magnesium ribbon and concentrated HCl were added drop wise. Production of red or orange red color indicates the presence of flavonoids.

Screening for Quinone

1ml of the extract was treated with 1ml of concentrated H_2SO_4; production of red color shows the presence of Quinone.

Screening for Anthroquinone (Borntrager's test)

50mg of extract powder sample was placed into a test tube, to this 5ml of chloroform added and shaken for 5min. The extract was filtered through Whatman No 1 filter paper and to the filtrate equal volume of 10% ammonia solution was added and shaken for 5min. A pink violet or red color at lower layer of ammonia solution indicates the presence of anthroquinone.

Screening for Phenols

50mg of extract powder was dissolved in 5ml of distilled water. Few drops of 10% ferric chloride solution were added to this. Production of blue or green color indicates the presence of phenol compounds.

Screening for Protein

50mg of extract sample powder was dissolved in 10ml of distilled water and filtered through Whatman No. 1 filter paper. 1ml of 40% NaOH was added to the filtrate. Then, added 1 or 2 drops of 2% copper sulfate solution. Production of violet color indicates the presence of proteins.

RESULTS AND DISCUSSION

The physicochemical parameters are mainly used to get more information about the purity and quality of the drug. The extracted powder was evaluated for its physiochemical parameters like moisture content, total ash, water soluble ash, acid soluble ash, sulphated ash and different solvent extractive values (Table 1). Moisture content is one of the major factors responsible for the degradation of drugs and herbal formulations. The moisture content promotes the degradation processes caused by enzymes, development of microorganisms, oxidation and hydrolysis reactions. The residues endure after incineration of plant material is known as total ash or ash value. A high ash value is symbolic of contamination, substitution or adulteration by mineral components. Ash value constitute both physiological ash and non-physiological ash, physiological ash is obtained from plant tissue due to biochemical processes while non-physiological ash consist of residue of the extraneous matter (sand, soil etc.) deliberately or non-deliberately adhering to plant samples. Physiological ash disintegrates in the dilute acid and non-physiological ash remains same. In the total ash content, water-soluble ash value is also part of it, which is soluble in water. In the total ash content, acid insoluble ash measures the amount of silica present especially as sand and siliceous earth in the samples. These values indicate the magnitude of presence of phosphates, oxalates, carbonates, oxides and silicates. Hence, these values are indices of excellence of herbal remedies.

Table 1: Physicochemical analysis of *Pithecellobium dulce* leave.

Constants	**Percentage**
Moisture contents	8.45 ± 0.15
Total ash contents	0.70 ± 0.05
Acid soluble ash	1.50 ± 0.05
Water soluble ash	2.35 ± 0.05
Extractive values	
Methanol	2.1±0.03
Chloroform	1.3±0.05
Petroleum ether	2.1±0.02

Preliminary phytochemical screening of plants was predominant to the detection of bioactive principles which is a new source of therapeutically and industrially valuable compounds that may lead to the discovery of new drugs. In the present study, the presence of phytochemicals were screened with the petroleum ether, chloroform, and methanol extracts of the *Pithecellobium dulce* leaves and the results are shown in Table 2. Crude extracts and medicines are manufactured based on the principles of natural compounds even by pharmaceutical companies, may lead to large scale exposure of humans to natural products. Presence or absence of important bioactive compounds in an extracts were identified by color reactions with specific chemicals, this procedure is simple for preliminary pre-requisite before going to phytochemical investigation. Hence, in the present work, the crude extracts obtained by petroleum ether, chloroform, and methanol solvents were screened for the presence of phytochemicals. The methanol extract shows the presence of steroids, saponins, flavonoids, phenols, proteins, glycosides and terpenoids. Saponins have health benefits such as lower cholesterol, antimicrobial, anti-inflammatory and anticancer properties.[18] Many herbal medicinal researches have established saponins as the active components and their contributions to the health benefits of foods such as soybeans and garlic.

Table 2: Preliminary phytochemical screening of *Pithecellobium dulce* leave.

Test	**Petroleum ether**	**Chloroform**	**Methanol**
Alkaloids	+	+	+
Steroids	-	-	+
Tannins	-	+	+
Saponins	+	+	+
Phenols	+	-	+
Flavonoids	+	+	+
Terpenoids	-	+	+
Glycosides	-	+	+
Proteins	-	-	-

+ indicates the presence of the phytochemical;

- indicates the absence of the phytochemical

Phenolic compounds have biological and pharmacological properties such as anti-inflammatory, antioxidant, and antimutagenic and anticarcinogenic activities. Flavonoids are secondary metabolite having various pharmacological properties such as anti-oxidative, anti-fungal, anti-inflammatory and diuretic actions.[19,20] This research finding highlights that methanolic extracts of *Pithecellobium dulce* leave had the highest number of phytochemicals compared to other solvent extracts. Hence, methanolic extracts of *Pithecellobium dulce* leaves holds the great potential to treat various human diseases and has profound medical applicability.

CONCLUSION

The physicochemical parameters gave information about the purity and quality of the drug. The presence of phytoconstituents, such as phenols and flavonoids in plants, indicates the possibility of antioxidant activity and this activity will help in preventing a number of diseases through free radical scavenging activity. Since the plant *Pithecellobium dulce* leaves has been used in the treatment of different ailments, the medicinal roles of this plant could be related to identify bioactive compounds. The present analyses suggest that *Pithecellobium dulce* leaves contain potentially health-protective phytochemical compounds with a potent source of natural antioxidants and antibacterial activities that may be clinically promising.

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