Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Suppl 2, 2013

Research Article

EVALUATION OF PHYSICOCHEMICAL AND PHYTOCHEMICAL PARAMETERS OF *MELIA AZEDARACH.L* LEAVES (FAMILY: MELIACEAE)

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Received: 18 Jan 2013, Revised and Accepted: 26 Mar 2013

ABSTRACT

The present study was carried out to provide physicochemical and phytochemical detail about the leaves of *Melia azedarach.L*. The physicochemical results obtained can be used for the identification of the powdered drugs. In the phytochemical screening different type of extracts were prepared to find the presence of secondary metabolites. Preliminary qualitative chemical test for different extract shows the presence of alkaloids, glycosides, fixed oil and fats, phenolic compounds, protein and aminoacids,tannins,gums and mucilage,flavonoids and carbohydrates.

Keywords: *Melia azedarach.l* leaves Physicochemical, Phytochemical, Successive solvent extraction.

INTRODUCTION

World plant biodiversity is the largest source of herbal medicine and still about 60-80% world population rely on plant based medicine which being used since the ancient ages as traditional health care system. It is now clear that the medicinal value of this plant lies in the bioactive phytochemical constituents that produce definite physiological effect on human body. These natural compounds signify the base of modern drugs as we use today.

Phytoconstituents are the natural bioactive compounds found the plants. These phytoconstituents work with nutrients and fibers to from an integrated part of human defense system against various disease and stress condition. Phytochemicals are basically divided into two groups i.e. primary and secondary constituents according to their function in the plant metabolism. Primary constituents comprise common sugars, amino acids, proteins and chlorophyll. While secondary constituents consist of alkaloids, flavonoids, saponin, phenolics and so on.

Melia azedarach. L. belongs to the *family* Meliaceae is from west Asia. It is widely distributed in Himalayan region between the attitude of 700 to 1000m. A moderate –sized deciduous tree 9-12m in height with a cylindrical bole with dark grey bark having shallow longitudinal furrows; the leaves are bi – or tripinnate, pinnate opposite or alternative, ovate or lanceolate, serrate, acuminate, glabrous on both surface, slightly oblique at the base[1]. It has been used for various medicinal purposes[2]. The leaf juice is used as an anthelmintic. It is also to cure strangury,amenorrhoea, bronchitis, leprosy, eczema, asthma and as an antipyretic[3].The present study is designed to explore the preliminary phytochemical and physiochemical analysis of *Melia azedarach leaves,* which is responsible for its pharmacological properties.

MATERIALS AND METHODS

The leaves of *Melia azedarach.L* were collected from the Western Ghats of Anamalai Hills, Coimbatore district, Tamilnadu. The plant was identified and authenticated by Botanical survey of India (IBSI), Coimbatore, Tamilnadu, India.

Preparation of crude drug for extraction

The authenticated fresh leaves were dried under shade and used for the preparation of extract. These leaves was coarsely powdered with the help of mechanical grinder and passed through sieve no.60. The powder was stored in an airtight container for further use.

Preparation of the Extracts [6-9]

Method of extraction

Continuous hot percolation (successive solvent extraction) process by using soxhlet apparatus and cold maceration method.

Materials

i. Soxhlet apparatus
ii. Petroleum ether (60-80^{o}C)
iii. Chloroform
iv. Acetone
v. Methanol (95% v/v)
vi. Distilled water with chloroform (0.25%)
vii. Shade dried coarse powder of *Melia azedarach* leaves.

Extraction Procedure

Petroleum ether extract

The shade dried coarsely powdered leaves of *Melia azedarach* (1 kg) was extracted with petroleum ether (60-80^{o}C) until the extraction was completed. After completion of extraction, the solvent was removed by distillation. Dark green color residue was obtained. The residue was then stored in dessicator.

Chloroform extract

The marc left after petroleum ether extraction was dried and then extracted with chloroform (55-56^{o}C), until the extraction was completed. After completion of extraction, the solvent was removed by distillation. Dark greenish yellow colour residue was obtained. The residue was then stored in dessicator.

Acetone extract

The marc left after chloroform extraction was dried and then extracted with acetone (55-56^{o}C), until the extraction was completed. After completion of extraction, the solvent was removed by distillation. Dark brownish green colour residue was obtained. The residue was then stored in dessicator.

Methanol extract

The marc left after acetone extraction was dried and then extracted with methanol 95% v/v (75-78^{o}C), until the extraction was completed. After completion of extraction, the solvent was removed by distillation. Dark brown colour residue was obtained. The residue was then stored in dessicator.

Aqueous extract

The marc left after methanol extraction was dried and then extracted with chloroform water by cold maceration process for 7 days. At the end of 7th days, it was filtered through muslin cloth and the filtrate was concentrated. The remaining solution was evaporated by heating on a water bath. The brown colour residue was obtained. The residue was then stored in dessicator.

The extractive values of various extracts of *Melia azedarach leaves* were presented in **Table no.1**

Preliminary photochemical tests.[10-13]

All the extracts of *Melia azedarach* leaves were subjected to qualitative tests for the identification of various active constituents.

Test for Carbohydrates and Glycosides

A Small quantity of various extracts were dissolved separately in 4ml of distilled water and filtered. The filtrate was subjected to the following tests to detect the presence of carbohydrates and glycosides.

Molisch's Test

The filtrate was treated with 2-3 drops of 1% alcoholic alpha naphthol and 2ml of concentrated sulphuric acid was added along the sides of the test tube. Appearance of brown ring at the junction of two liquids shows the presence of carbohydrates.

Fehling's test

The filtrate was treated with each 1ml of Fehling's solution A and B and heated on a water bath. A reddish precipitate was obtained shows the presence of carbohydrates.

Another portion of extracts were hydrolyzed with dilute hydrochloric acid for few hours on a water bath and the hydrolysate was subjected to the following tests to detect the presence of glycosides.

Legal's test

To the hydrolysate 1ml of pyridine and few drops of sodium nitroprusside solution were added and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red colour shows the presence of glycosides.

Borntrager's test

Hydrolysate was treated with chloroform and the chloroform layer was separated. To this equal volume of dilute ammonia solution was added. Ammonia layer acquires pink colour shows the presence of glycosides.

Detection of Fixed Oils And Fats

Filter paper test

Small quantities of various extracts were pressed separately between the filter papers. Appearance of oil stain on the paper indicated the presence of fixed oils.

Saponification test

Few drops of 0.5M alcoholic potassium hydroxide was added to small quantities of various extracts along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 hours. Formation of soap indicates the presence of fixed oils and fats.

Detection of Proteins and Free amino acids

Small quantities of various extracts were dissolved in few ml of water and then they were subjected to the following tests.

Million's test

The above-prepared extracts were treated with Million's reagent. Red colour formed shows the presence of proteins and free amino acids.

Biuret test

To the above prepared extracts equal volume of 5% sodium hydroxide and 1% copper sulphate solution were added. Violet colour produced shows the presence of proteins and free amino acids.

Ninhydrine test

The extracts were treated with Ninhydrine reagent. Purple colour produced shows the presence of proteins and free amino acids.

Detection of Saponins

The extracts were diluted with 20ml of distilled water and it was agitated in a measuring cylinder for 15 minutes. The formation of 1cm layer of foam shows the presence of saponins.

Detection of Tannins and Phenolic Compounds

Small quantities of the various extracts were taken separately in water and test for the presence of phenolic compounds and tannins was carried out with the following reagents.

1) 5% Ferric chloride solution -Violet colour
2) 1% solution of gelatin containing 10% sodium chloride -White precipitate
3) 10% lead acetate solution -White precipitate

Above findings shows the presence of phenolic compounds and tannins.

Detection of Phytosterols

Small quantities of various extracts were dissolved in 5ml of chloroform separately. Then this chloroform solution was subjected to the following tests to detect the presence of phytosterols.

Salkowski test

To 1ml of above prepared chloroform solution, few drops of concentrated sulphuric acid was added. Brown colour produced shows the presence of phytosterols.

Libermann Burchard test

The above prepared chloroform solution was treated with a few drops of concentrated sulphuric acid followed by few drops of diluted acetic acid, 3ml of acetic anhydride. A bluish green colour appeared indicates the presence of phytosterols.

Detection of Alkaloids

Small quantities of various extracts were separately treated with few drops of dilute hydrochloric acid and filtered. The filtrates were used for the following tests.

1). Mayer's reagent -cream precipitate

2). Dragendroffs reagent – orange brown precipitate

3). Hager's reagent - yellow precipitate

4). Wagner's reagent - reddish brown precipitate

Detection of Gums and Mucilages

A small quantity of various extracts were added separately to 25ml of absolute alcohol with constant stirring and filtered. The precipitate was dried in air and examined for its swelling properties. No swelling was observed indicates the absence of gums and mucilage.

Detection of Flavonoids

1) Small quantities of various extracts were dissolved separately in aqueous sodium hydroxide. Appearance of yellow colour indicates the presence of flavonoids.
2) To the small portion of each extract, concentrated sulphuric acid was added. Yellow orange colour was obtained shows the presence of flavonoids.
3) **Shinoda's test:** Small quantities of the extracts were dissolved in alcohol. To that piece of magnesium followed by concentrated hydrochloric acid was added drop wise and heated. Appearance of magenta colour shows the presence of flavonoids.

The phytochemical constituents present in various extracts were presented in **Table 2.**

Physicochemical parameters [9]

The powder of *Melia azedarach* leaves was subjected to evaluate its total ash, acid insoluble ash, water soluble extractive value, alcohol soluble extractive value and moisture content. Each determination

was carried out three times and the average value was taken. Each result is reported in table form.

Ash Values

Total ash value was found by incinerating at temperature 450° C until freed from carbon and then cooled. The weight of total ash was taken and the percentage of it was calculated with reference to the air dried drug.

Water soluble ash

The total ash obtained was boiled with water and the insoluble matter was collected on ash less filter paper, washed with hot water and ignited. The difference in weight represents the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried drug.

Acid insoluble ash

The total ash obtained was boiled with 2N hydrochloric acid, filtered and the insoluble matter was collected on ash less filter paper. It was washed with hot water, ignited in tarred crucible, cooled and the residue obtained was weighed and the percentage of acid insoluble ash was calculated with reference to the air dried drug.

Alcohol soluble extractive value

Coarsely powdered leaves were macerated with alcohol in a closed flask, it was filtered rapidly and precautions were taken against loss of alcohol. 25ml of the filtrate was evaporated to dryness, dried at 105°C and weighed. The percentage of alcohol soluble extracts were calculated with reference to the air dried drug.

Water soluble extractive value

Coarsely powdered leaves was macerated with chloroform water (2.5ml chloroform in 1000ml water) in a closed flask it was filtered, and filtrate was evaporated to dryness at 105°C and weighed. The percentage of water soluble extractive value was calculated with reference to the air dried drug.

Moisture content

Known weight of the powdered leaves was taken and dried in oven for 30 minutes, cooled and the percentage of moisture content was then calculated with reference to the air dried drug.

RESULTS AND DISCUSSION

The phytoconstituents were extracted by using different solvents of increasing polarity like petroleum either, chloroform acetone, ethanol and water. The extractive values were given in **Table 1**

Table 1: Data showing the extractive values of *Melia azedarach* leaves

Plant Name	Part used	Method of extraction	Percentage yield				
			Petroleum ether	Chloroform	Acetone	Ethanol	Aqueous
Melia azedarach leaves	Leaves	Continuous hot percolation and cold maceration process (successive solvent extraction)	2.7g	2.9g	3.4g	3.9g	4.8g

The phytoconstituents were identified by chemical tests which showed the presence of various phytoconstituents (Table 2) mainly in the following extracts.

Table 2: Data showing the preliminary phytochemical screening of the various extracts of *Melia azedarach* leaves:

Sr. No.	Constituents	Petroleum Ether Extract	Chloroform Extract	Acetone Extract	Methanol Extract	Aqueous Extract
01	Alkaloids	-	-	-	+	+
02	Sterols	-	-	-	+	+
03	Glycosides	-	+	+	+	+
04	Fixed oil and fats	+	+	+	+	+
05	Phenolic compounds	+	+	+	+	+
06	Protein & amino acids	+	+	+	+	+
07	Tannins	-	-	-	+	+
08	Gum & mucilage	+	+	+	+	+
09	Flavonoids	-	-	+	+	+
10	Carbohydrates	-	-	-	+	+
11	Saponins	-	-	-	-	-

'+' Presence, '-'Absence

Methanol Extract

Alkaloids, Fixed oil and fats, Carbohydrate, sterols, proteins, amino acids, tannins, glycosides, phenolic compounds and flavonoids, Gum and mucilage.

Aqueous Extract

Alkaloids, Fixed oil and fats, carbohydrate, sterols, proteins, amino acids, tannins, glycosides, phenolic compounds flavonoids, Gum and mucilage.

In the above stated extracts, aqueous and methanol extracts showed the same types of constituents. Hence methanol and aqueous extracts were selected for pharmacological studies. Methanol extract was selected for the isolation of the available active constituents, because ethanol being a bipolar solvent, which can dissolve a wide range of phytoconstituents, whereas the aqueous extract contains polar compounds. The results show that the plant has a number of chemical constituents, which may be responsible for the many pharmacological actions.

The results of physicochemical parameters of leaves were reported in table 3.

Table 3: Physicochemical parameters of *Melia azedarach* leaves

Physical Parameters	Values (in % w/w)
Total ash value	5.19
Water soluble ash value	3.47
Acid insoluble ash value	1.72
Alcohol soluble extractive value	3.26
Water Soluble extractive value	7.83
Moisture content	4.64

The physico-chemical evaluation of drugs is an important parameter in detecting adulteration or improper handling of drugs. Moisture content of drugs could be at minimal level to avoid microbial growth during storage. Ash values are used to determine quality and purity of crude drug. It indicates the presence of various impurities like carbonate, oxalate and silicate. The presence of silicates and the earthy materials is indicated in acid insoluble ash and the inorganic

elements present in the drug is indicated in water soluble ash value present in drugs. Extractive values determine which are primarily useful for the determination of exhausted or adulterated drugs. Physico-chemical parameter can serve as valuable source of information and provide appropriate standards to establish the quality of this plant material in future study or application.

CONCLUSION

The preliminary phytochemical and physicochemical evaluation of studies on *Melia azedarach* leaves were done the Phytochemical constituents were extracted by successive solvent extraction and identified by chemical tests. These tests showed the presence of various phytochemical constituents like Alkaloids, Fixed oil and fats, Carbohydrate, Proteins, Amino acids, Tannins, Glycosides, Phenolic compounds and Flavonoids, Gum and mucilage.

Methanolic and aqueous extracts shows the presence of majority of phyto constituents. Hence it was selected for the pharmacological studies.

The Methanol extracts which has the polarity in between the acetone and aqueous has been selected for isolation of the available active constituents.

The present study on preliminary phytochemical and physicochemical evaluation of *Melia azedarach* leaves could be used as the diagnostic tool for the standardization of medicinal plant. However, the plant needs further pharmacological study to develop useful drugs from the plant.

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