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PHARMACOGNOSTICAL STANDARDIZATION OF LEAVES AND BARKS OF *AEGLE MAMELOS* (RUTACEAE)

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ABSTRACT

Herbs lay a vital role in Siddha system of medicine. Vilva ilai kudineer is one of the siddha formulation which controls the Diabetes mellitus. The present study is design to carry out the Pharmacognostical standardization of leaves and bark of *Aegle marmelos* (L.) *Correa* (Rutaceae) individually, which are the raw materials used in the preparation of Vilva ilai kudineer. The standardization parameter includes organoleptic evaluation, powder microscopic analysis, physiochemical evaluation and phytochemical screening. These kind of comprehensive data might be useful to standardize the Vilva ilai kudineer for further research.

Keywords: *Aegle marmelos*, Rutaceae, Vilva ilai kudineer, Pharmacognostical standardization.

INTRODUCTION

Plants have been utilized as a natural source of medicinal compounds since thousands of years. *Aegle marmelos* (L.) Correa (Rutaceae) is one of the important medicinal plant, popularly known as '*Bael*' is indigenous to India and is cultivated throughout the Indian subcontinent. The importance seems largely due to its medicinal properties [1]. All parts of this tree, viz., root, leaf, trunk, fruit and seed, are used for curing one human ailment or another such as anti-diabetic, astringent, anti-diarrhoeal, anti-dysentery, demulcent, anti-pyretic, haemostatic.

The present work was undertaken to study Organoleptic parameters, Physicochemical parameters, Microscopical evaluation and Phytochemical analysis of leaves and barks of *Aegle marmelos* individually which are the key ingredients used in the preparation of Vilva ilai kudineer. Hence, the present study is designed to pharmacognostical standardization of leaves and barks of *Aegle marmelos* used to prepare and standardize vilva ilai kudineer.

MATERIAL AND METHODS

Collection and authentication of plant material

The whole plant of *Aegle marmelos* (L.) Correa (Rutaceae) was collected from Mahatma Gandhi Dental College Campus (MGDCC), Gorimedu, Puducherry, India in the month of December 2013 and the plant was authenticated by Dr. B.R. Ramesh, Director of Research, Institute Franceais de Pondicherry, French Institute, Louis st., Pondicherry

The collected plant materials in fresh condition was used to study of macroscopic characters and air dried. Finally dried plant materials were subjected to size reduction to get coarse powder and then passed through sieve no. 80 to get homogenous powder. Then the powder was subjected for the study of microscopic characters, determination of ash values, extractive values, loss on drying and phytochemical screening.

Organoleptic parameters

The morphological evaluation was done using standard protocol organoleptic parameters like color; odor and taste were carried out [2].

Powder microscopy analysis

The powder samples was treated with phloroglucinol in 90% ethanol (2%w/v) and concentrated hydrochloric acid (1:1) and studied for their fragments of diagnostic value [3].

Physicochemical parameters

The physicochemical parameters like loss on drying, ash value (total ash, acid insoluble ash and water soluble ash) and extractive value (alcohol soluble extractive and water soluble extractive) were determined as per WHO guideline, 2002 [4].

Total ash

Place about 2 to 4 gram of the ground air-dried material, accurately weighed, in a previously ignited and tared crucible (usually of platinum or silica). Spread the material in an even layer and ignite it by gradually

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increasing the heat to 500-600^{0}C, until it is white, indicating the absence of carbon. Cool in desiccator and weigh.

Water-soluble ash

To the crucible containing the total ash, add 25 ml of water and boil for 5 minutes. Collect the insoluble matter in a sintered-glass crucible or on an ash less filter-paper. Wash with hot water and ignite in a crucible for 15 minutes at a temperature not exceeding 450^{0}C. Subtract the weight of this residue in mg from the weight of total ash. Calculate the content of water-soluble ash in mg per gram of air-dried material.

Acid insoluble ash

To the crucible containing the total ash, add 25 ml of hydrochloric acid (70g/l), cover with a watch-glass and boil gently for 5 minutes. Rinse the watch-glass with 5ml of hot water and add this liquid to the crucible. Collect the insoluble matter on an ash less filter-paper and wash with hot water until the filtrate is neutral. Transfer the filter-paper containing the insoluble matter to the original crucible, dry on a hot-plate and ignite to constant weight. Allow the residue to cool in a suitable desiccator for 30 minutes and then weigh without delay. Calculate the content of acid-insoluble ash in mg per gram of air-dried material.

Extractive values

Water soluble extractive value

Macerate 5 g of the air-dried drug, coarsely powdered, with 100 ml of water of the specified strength in a closed flask for 24 hrs, shaking frequently during the first 6 hrs and allow to stand for 18 hrs. Then filtrate carefully and evaporate 25 ml of the filtrate to dryness in a tarred flat bottomed shallow dish, dry at 105^{0}C and weigh. Then, finally calculate the % of extractive value for each solvent with reference to the air-dried drug.

Alcohol soluble extractive value

The air-dried sample macerates with various solvents like acetone, chloroform, petroleum ether, ethanol, methanol and water successively. Then, finally calculate the % of extractive value for each solvent with reference to the air-dried drug.

Loss on drying

Weigh a glass stoppered, shallow weighing bottle that should dry under the same conditions to be employed in the determination. Transfer to the bottle the quantity of the sample specified in the related monograph, cover it and accurately weigh the bottle and the contents. Distribute the sample as evenly as practicable by gentle sidewise shaking to a depth not exceeding 10 mm [5].

PRELIMINARY PHYTOCHEMICAL SCREENING

The different qualitative chemical tests can be performed for establishing the chemical profile of given extract knowing for its chemical composition. The following tests may be performed on extracts to detect various phytoconstituents present in them. The extract of leaves and bark were used for phytochemical screening **[6]**.

Detection of Alkaloids

Solvent free extract, 50 mg is stirred with few ml of dilute hydrochloric acid and filtered. The filtrate is tested carefully with various alkaloid reagents as follows

Mayer's test

To a few ml of filtrate, 1-2 drop of Mayer's reagent (Potassium mercuric iodide) are added by the sides of the test tube. A white or creamy precipitate indicates the test as positive.

Wagner's test

To a few ml of filtrate, few drops of Wagner's reagent (Iodine- potassium iodide) are added by the side of the test tube. A reddish-brown precipitate confirms the test as positive.

Hager's test

To a few ml of filtrate, 1 or 2 ml of Hager's reagent (saturated solutions of picric acid) are added. A prominent yellow precipitate indicates the test as positive.

Dragendorff's test

To a few ml of filtrate, 1 or 2 ml of Dragendorff's reagent (Potassium bismuth iodide) are added. A prominent yellow precipitate indicates the test as positive.

Detection of glycosides

A minimum quantity of the extract was hydrolysed with hydrochloric acid for few min on a water bath and the hydrolysate was subjected to the following tests.

Legal test

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

Borntrager's test

Hydrolysate was treated with chloroform and the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. Appearance of red color confirms the presence of glycosides.

Detection of phytosterols

A small quantity of extract was suspended in 5ml of chloroform separately. The chloroform solution was subjected to Libermann Burchard test and Salkowski test.

Libermann Burchard test

The chloroform solution of extract was treated with a few drops of concentrated sulphuric acid followed by 1ml of acetic anhydride solution. Appearance of bluish green color solution in the extract shows the presence of phytosterols.

Salkowski test

To the 1ml of chloroform solution, few drops of concentrated sulphuric acid was added. Appearance of brown ring with the extract indicates the presence of phytosterols.

Detection of saponins

Foam test

The extract was diluted with 20ml of distilled water and agitated in a graduated cylinder for 15min. Formation of foam indicates the presence of saponin.

Haemolysis test

About 2ml of human blood was taken in the test tube. To the test tube, an equal quantity of methanol extract was added. Formation of cleared liquid in the test tube indicates that the red blood corpuscles are haemolysed.

Detection of Flavanoids

Shinoda's Test

The extracts were dissolved in alcohol, to that one piece of magnesium is added followed by concentrated hydrochloric acid along the sides of the test tube drop wise. It is heated in a boiling water bath for few minutes. The appearance of magenta colour indicates the presence of flavanoids.

5ml of extract solution was hydrolysed with 10% v/v sulphuric acid and cooled. Then, it is extracted with diethylether and divided into three portions in three separate test tubes. 1ml of diluted sodium carbonate, 1ml of 0.1N sodium hydroxide and 1ml of strong ammonia solution were added to the first, second and third test tubes respectively. In each test tube, development of yellow colour demonstrated the presence of flavanoids.

Detection for Flavones

The extract was treated with 10% sodium hydroxide. Appearance of yellow to orange color shows the presence of Flavonones.

The extract was treated with Con. Sulphuric acid Appearance of orange to crimson red color, confirms the presence of Flavonones.

Detection of tannins and phenolic compounds

The ethanol extract was dissolved separately in minimum amount of water and filtered. The filtrate was subjected to the following tests.

Lead acetate test

To the filtrate a few drops of lead acetate solution was added. Formation of white color precipitate shows the presence of tannins in the extract.

The filtrate was treated with aqueous bromine solution. The appearance of white precipitate indicates the presence of tannins.

Gelatin test

To the filtrate 1ml of 1% solution of gelatin was added. Formation of white color precipitate shows the presence of tannins in the extract.

Ferric chloride test

To the filtrate a few drops of ferric chloride was added. Formation of violet color precipitate shows the presence of phenolic compound in the extract.

The filtrate was treated with 10% sodium chloride solution. The appearance of cream colour indicates the presence of phenols.

Detection of diterpenoids

A little quantity of the extract was treated with concentrated sulphuric acid. Formation of pink color indicates the presence of diterpenoids

Detection of triterpenoids

A small quantity of the extract was added with little amount of tin and thionylchloride. Formation of light red color solution with the extract shows the presence of triterpenoids.

Detection of gums and mucilage

Ruthenium red test

A small quantity of extract was suspended with water and ruthenium red solution was added. Appearance of pink color with the extract shows the presence of gums and mucilages.

Detection of Carbohydrates

A minimum amount of extract was suspended in5ml of distilled water. The suspension was subjected to the following chemical tests.

Molisch's test

The extract was treated with 2-3drops of 1% alcoholic α-naphthol and 2 of concentrated sulphuric acid were added along the sides of the test tube. Appearance of purple ring between two layers indicates the presence of carbohydrates.

Fehling's test

The extract was treated with mixture of Fehling's A (7% copper sulphate) and Fehling's B (25% potassium hydroxide and 35% sodium potassium tartarate) solution and heated for few min. Formation of red precipitate shows the presence of reducing sugar.

Benedict's test

The extract was treated with Benedict's reagent (10 % sodium carbonate, 17.3% sodium citrate and 1.73% copper (II) sulphate) and heated for few min. Formation of red precipitate shows the presence of reducing sugar.

Barfoed's test

The extract was treated with Barfoed's (Copper Acetate + Glacial Acetic Acid) reagent and heated in a boiling water bath for few minutes. The appearance of reddish orange color precipitate indicates the presence of non reducing sugars.

Seliwanoff's test

The extract was treated with few ml of Seliwanoff's reagent and boil on a water bath few minutes. The appearance of red colour precipitate is produced shows presence of reducing sugars.

Detection of proteins and amino acids

A small quantity of extract was dissolved in few ml of water and it was subjected to the following tests.

Millon's test

The extract was treated with Millon's reagent (mercuric nitrate in nitric acid containing a trace of nitrous acid) and boiled in a water bath for 5 min. Appearance of pink to red color indicates the presence of proteins.

Ninhydrin test

The extract was treated with Ninhydrin reagent. Appearance of purple color confirms the presence of amino acids.

Biuret test

To the extract equal volume of 5% sodium hydroxide solution and 1% copper sulphate solution was added. Appearance of violet color shows the presence of amino acids.

Detection of Coumarin

Place a small amount of sample in a test tube and cover the test tube with a filter paper moistened with dilute sodium hydroxide solution. Place the covered test tube on water bath for several minutes. Remove the paper and expose to UV light, the paper shows green fluorescence.

Detection of Volatile Oil

In a volatile oil estimation apparatus, 50 gram of powdered material (crude drug) is taken and subjected to hydro-distillation. The distillate is collected in graduated tube of the assembly, wherein the aqueous portion automatically separated out from the volatile oil.

RESULTS AND DISCUSSION

A. marmelos an important medicinal herb and extensively used in Siddha, Ayurveda, and other medicinal systems. Almost all parts of this plant such as leaf, fruit, seed, bark and root are used to cure a variety of diseases. Here, the organoleptic evaluation was investigated. The components such as oil glands, trichomes and crystals were observed in the leaves and Vessels, fibres, vessels were observed in the bark of *Aegle marmelos* (L.) Correa.

A. marmelos contains a number of phytoconstituents, which are the key factors in the medicinal value of this plant analyzed. A systemic research and development work should be undertaken for the development of products for their better economic and therapeutic utilization. The raw materials from A*egle marmelos* need to be further evaluated to explore its therapeutics importance in drug development process.

Table 1. Organoleptic evaluation of Raw materials

Sl. No.	Name of the drug & Parts	Parameters					
		Colour	Odour	Taste	Texture	Size	Shape
1.	***Aegle marmelos* (Leaves)**	Green	Aromatic	Bitter	Deciduous	4-10cm long, 2-5cm wide	oval
2	***Aegle marmelos* (Bark)**	Externally greyish and internally cream	Pleasant	Sweet at first, then irritation	Firm	10-15cm long 2-5cm thickness	Flattened

Physiochemical Analysis

Table 2. Determination of Total ash value, Acid insoluble ash & Water soluble ash

Sl.No	Name of the drug	Total ash (% W/W)	Acid insoluble ash (% W/W)	Water soluble ash (% W/W)
1	*Aegle marmelos* (Leaves)	12	9	2.5
2	*Aegle marmelos* (Barks)	4	3	1

Table 3. Determination of Extractive value

Sl.No	Name of the drug	Water soluble(% W/W)	Alcohol soluble(% W/W)
1	*Aegle marmelos* (Leaves)	18	12
2	*Aegle marmelos* (Barks)	8	6

Table 4. Determination of Loss on drying

Sl.No.	Name of the drug	Loss on drying (% W/W)
1	*Aegle marmelos* (Leaves)	13.28±0.08
2	*Aegle marmelos* (Barks)	14.15±2.02

Phytochemical Studies

Table 5. Preliminary phytochemical screening

Sl.No	Chemical groups	*Aegle marmelos* (Leaves)	*Aegle marmelos* (Barks)	Vilva ilai kudineer formulation
1	Alkaloids	+	+	+
2	Glycosides	-	-	-
3	Carbohydrates	+	-	+
4	Steroids	+	+	+
5	Triterpenoids	+	-	+
6	Flavanoids	+	-	+
7	Tannins	+	-	+
8	Phenols	+	+	+
9	Saponins	+	-	+
10	Fixed oils and fats	-	-	-
11	Proteins & free amino acid	+	+	+
12	Volatile oil	+	-	+
13	Mucilage and gums	+	+	+
14	Coumarin	+	+	+

(+) Present (-) Absent

Figure 1. Morphology of leaves of *Aegle marmelos* (L.) Correa (Rutaceae)

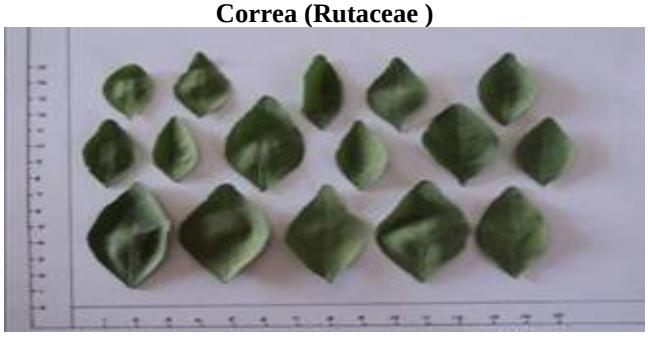

Figure 2. Morphology of barks of *Aegle marmelos* (L.) Correa (Rutaceae)

Figure 3. Powder microscopy of leaves of *Aegle marmelos* (L.) Correa

Figure 4. Powder microscopy of barks of *Aegle marmelos* (L.) Correa

CONCLUSION

Pharmacognostical standardization plays a vital role in herbal drug industry. Since the modern world established numerous standards for quality control of herbal drugs it is an essential step to prove the quality of a medicinal herb by using modern equipments and new procedures. The developed of botanical description, Physiochemical constants and Phytochemical test for both leaves and barks of *A. marmelos* could be worthy to future scientist.

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