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VARIOUS EXTRACTION METHOD AND PHYTOCHEMICAL INVESTIGATION OF *SYZYGIUM CUMINI* LEAF

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ABSTRACT

The purpose of this study was to extract phytochemical constituents' *syzygium cumini* (L) leaf by using various extraction method and various organic solvents. Here we utilized distillation, maceration, percolation and soxhlet extraction method. The organic solvents used for extraction in polar solvents (Methonol, Ethanol, Water, Acetone) and non-polar solvents (n-hexane, ether, choloroform). The various extracts *of Syzygium Cumini* (L). Obtained were subjected to qualitative analysis to test the presence of various phytochemical constituents like alkaloids, carbohydrates, glycosides, flavonoids, steroids, amino acid, phenols, proteins, tannins etc. There are many solvents used among these Methanol extracts gives more productivity. *Syzygium Cumini* (L). Are important sources of natural antioxidants, which might be useful as preventive agents against oxidative stress and hence currently the evaluation of *In-vivo* antioxidant activity and anti-diabetic of these extracts are in progress.

KEYWORDS

Syzygium Cumini (L), Distillation, Maceration, Percolation and Soxhlet extraction method, etc.

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INTRODUCTION

The study of medicinal plants is neglected by medical men all over the world, but more so in India. It is our misfortune that the chemistry and pharmacology of most of these plants have not been properly investigated. The ease and cheapness with which these are procurable the marvelous powers that are attributed to them in the cure of different diseases encourages us to investigate their properties

and prove their pharmacological actions[1-4]. The *Syzygium cumini* (L) Skeels (*Syns. Syzygium jambolana DC, Eugenia cumini Druce, Eugenia jambolana Lam.*), commonly known as Jamun, belongs to the family Myrtaceae or Myrtle. The other common names of jamun are Indian blackberry, Java plum, Jambu, black plum and Jambul etc[2]. *Syzygium cumini* is an emerging fruit crop of the twenty-first century. The jamun fruit have high medicinal value as well as different plant parts possess varied uses to mankind. It gives authority of due to the presence of the various phytochemical constituents such as alkaloids, fatty acids, steroids and tannins. It is multipurpose tree cultivating for varied uses as a road bordered by trees for wind break, preserving fishing nets and tanning leather by brown dye which obtained from bark due to its have high value of tannin content. The wood also used for building, carpentry, fuel wood and preparation of different agricultural implements[5-8].

Taxonomy of the genus name Syzygium is derived from the Greek word Syzygos, meaning yoked together, possibly referring to the paired leaves. The taxonomy of the *Syzygium cumini* (L.). is as follows; Kingdom: Plantae - Plants, Subkingdom: Tracheobionta - Vascular plants, Super division : Spermatophyta - Seed plants, Division: Magnoliophyta - Flowering plants, Class: Magnoliopsida - Dicotyledons, Subclass: Rosidae, Order: Myrtales, Family: Myrtaceae - Myrtle family, Genus: Syzygium P. Br. ex Gaertn, Complete scientific name: *Syzygium cumini* (L.) Skeels.

Pharmacological potential of different parts of *Syzygium cumini* (L.). The *Syzygium cumini* (L). The jamun plant was used in the treatment of diabetes before the discovery of insulin. Its bark is acrid which is used in the treatment for sore throat, bronchitis, asthma, biliousness, dysentery, blood impurities and to cure ulcers. The decocsation of bark is used as lotion for removing ringworm of the head 4and also significantly decrease in blood glucose levels in mice. β-sitoterol present in the unsaponifiable matter of seed fat which is used in the treatment for antidiabetic, anti-inflammatory, hepatoprotective, anti-hyperlipidemic, diuretic and antibacterial activities have been reported in various extracts of *Syzygium cumini* seeds[9,10]. The different parts of jamun tree were make uses for cure of various diseases in the formation of powder, decoction, juice or paste. Fresh leaf juice is taken orally for stomach pain. In the Siddha system of medicine, the teeth and the gums are strengthening by the ash of the leaves. S*yzygium cumini* is considered to be a haematinic, semen promoting and to decrease excessive heat of the body. Several experimental and clinical studies have been confirmed antidiabetic activities of various parts of jamun. The various extracts of different parts of jamun possess a range of pharmacological properties such as antibacteria, antimicrobial, antifungal, antiviral, antioxidant and free radical scavenging activity, cardioprotective, anti-inflammatory, neuro-psychopharmacological, antiallergic, radioprotective, chemopreventive, larvicidal, gastroprotective and antiulcerogenic activities[11-14].

Uses of Antioxidants

Anti-oxidants used for treatments of stroke and neurodegenerative diseases. Such as Alzheimer's disease, Parkinson's diseases and it's prevent the cell-damaging by free radicals, It's help to lower risk of heart disease and some neurological diseases, widely used as ingredients in dietary supplements in the hope of maintaining health. Some evidence proven antioxidants source having vegetables and fruits who consume protect against a number of cancers, It's also used for preservatives in food and cosmetics material in industry, It is used to prevent oxidation of fuels and lubricants[15,16].

MATERIAL AND METHODS

Plant collection

The green leaf of *Syzygium Cumini* were collected from plants growing in the Cheran College of Pharmacy (Medicinal Garden), Coimbatore, Tamilnadu, India.

Materials

Petroleum ether, Hexane, Chloroform, Ethyl acetate, Methanol, Distilled water, Hydrogen peroxide, Griess reagent, DPPH (2, 2 -diphenyl-1-picrylhydrazyl), Hydrochloric acid, Sulphuric acid, Alpha-naphthol, Copper sulphate, Sodiumhydroxide, Barfoed's solution, Benedict's solution, Potassium

mercuric iodide, Potassium bismuth iodide, Iodine, Potassium iodide, Picric acid, Con.HNO3, NH4OH, Millon's reagent, Ninhydrin, Biuret reagent, Ammonia, 95% Ethanol, lead acetate, Potassium hydroxide, Phenolphthalein, Lead acetate, Ferric chloride, Agar-Agar, Potassium dihydrogen phosphate, Calcium carbonate, All the chemical purchased from Ranchem, Pure Chemicals in AR Grade. Rotary vaccum

Extraction

There are number of extraction techniques used to extract crude drugs, among these here we utilized some few methods like viz., Distillation Method, Maceration Method, Percolation Method, Soxhlet extraction Method.

Petroleum ether extraction

The Dried Leaf (1000gm) was extracted by macerating with 2.0 litter of Petroleum ether for 10 days at room temperature in a dark cabinet. After 10 days, the extracts were collected by filtration, the marc was separated. Solvent extract was evaporated to dryness using rotary vacuum evaporator and weighed.

Hexane extraction

The residue of petroleum ether extraction was dried at sun shade in dark place. After dried residue were extracted by macerating with 2.0 litter of hexane for 10days at room temperature in a dark cabinet. After 10 days, the extracts were collected by filtration, the marc was separated. Solvent extract was evaporated to dryness using rotary vacuum evaporator and weighed.

Chloroform extraction

The residue of hexane extraction was dried at sun shade in dark place. After dried residue were extracted by macerating with 2.0 litter of chloroform for 10 days at room temperature in a dark cabinet. After 10 days, the extracts were collected by filtration, the marc was separated. Solvent extract was evaporated to dryness using rotary vacuum evaporator and weighed.

Ethyl acetate extraction

The residue of chloroform extraction was dried at sun shade in dark place. After dried residue were extracted by macerating with 2.0 litter of ethyl acetate for 10days at room temperature in a dark cabinet. After 10 days, the extracts were collected by filtration, the marc was separated. Solvent extract was evaporated to dryness using rotary vacuum evaporator and weighed.

Methanol extraction

The residue of ethyl acetate extraction was dried at sun shade in dark place. After dried residue were extracted by macerating with 2.0 liter of methanol for 10 days at room temperature in a dark cabinet. After 10 days, the extracts were collected by filtration, the marc was separated. Solvent extract was evaporated to dryness using rotary vacuum evaporator and weighed.

Distilled water extraction

The residue of methanol extraction was dried at sun shade in dark place. After dried residue were extracted by macerating with 2.0 liter of distilled water for 10 days at room temperature in a dark cabinet. After 10 days, the extracts were collected by filtration, the marc was separated. Solvent extract was evaporated to dryness using rotary vacuum evaporator and weighed.

PHYTO CHEMICAL SCREENING PROCEDURE

Determination of total ASH

About 2g of air dried crude drug was weighed accurately in a tarred platinum or silica dish and was incinerated at a temperature not exceeding 4500^{o}C until free from carbon, it was then cooled and weighed. The percentage of ash was calculated with reference to air dried drug.

Determination of water soluble ASH

The total ash was boiled for 5.0 min, with 25.0ml of water. The insoluble matter was collected in a gooch crucible or an ash less filter paper. It was washed with hot water and ignited for 15min, at a temperature not exceeding 4500^{o}C. The weight of the insoluble matter was subtracted from the weight of the ash, the difference in the weight of the ash, represent the water soluble ash. The percentage of water soluble ash calculated with reference to the air dried drug.

Determination of acid in soluble ASH

The was boiled with 25ml of 2M HCL for 15min. the insoluble matter was collected in a Gooch crucible or

an ash less filter paper. It was washed with hot water and ignited; it was then cooled in a desiccators and weighed. The percentage of water soluble ash calculated with reference to the air dried drug.

Alcohol soluble extractive

5g of the powder was macerated with 100ml of alcohol of the specified strength in a closed flask for 24hrs, shaking frequently for 6hrs and allowing standing for 18hrs. It was filtered rapidly taking precautions against loss of alcohol and 25ml of the filtrate was evaporated to dryness at 105^{o}C and weighed. The percentage of water soluble extractive was collected with reference to the air dried drug.

Water soluble extractive

5g of the powder was macerated with 100ml of water in a closed flask for 24hrs, shaking frequently for 6hrs and allowing standing for 18hrs. It was filtered rapidly taking precautions against loss of alcohol and 25ml of the filtrate was evaporated to dryness at 105^{o}C and weighed. The percentage of water soluble extractive was collected with reference to the air dried drug.

Loss on drying

Loss on drying is the loss in weight in % w/v determined as per the following procedure. A glass stoppered weighing bottle that has been dried for 30min under the same condition to be employed in the determination was weighed. The sample was put in to the bottle, covered and the bottle and the contents were accurately weighed. The sample was distributed evenly to a depth not exceeding 10mm. the loaded bottle was placed in drying chamber (oven) and the stopper was removed. The sample was dried to a constant weight at a temperature of 110^{o}C in hot air.

PRELIMINARY CHEMICAL ANALYSIS

All the extracts were subjected to following chemical tests.

Test for alkaloids

Mayers test

A pinch of dried extract was taken and 2ml of dilute hydrochloric acid was added, mixed, filtered and to the filtrate 1 or 2 drops of Mayers reagent was added. Formation of white precipitate indicates the presence of Alkaloids.

Dragendroff"s test

A pinch of dried extract was taken and treated with 2ml of 2% acetic acid, mixed thoroughly and filtered. To the filtrate 1 or 2 drops of dragendroff's reagent was added. Formation of orange brown precipitate indicates the presence of Alkaloids.

Wagner's test

Small quantity of the extract was reacted with Wagner's reagent. Reddish brown colour precipitate was observed, indicating the presence of alkaloids

Hager's test

To small quantity of the extract, Hager's reagent was added. Yellow colour precipitate was observed, indicating the presence of alkaloids.

Test for carbohydrates

Molisch's test

The substance was treated with alpha-napthal and conc.sulphuric acid. Formation of violet colour indicates the presence of carbohydrates.

Borntrager's test

Few grams of substance was boiled with dil.sulphuric acid, it was filtered while hot and to the cold filtrate organic solvent like benzene or ether was added, it was shaken well and the organic layer was separated and equal volume of dil.ammonia was added. Formation of rose pink colour of ammonia layer indicates the presence of glycoside.

Test for cardiac glycosides

Legal's test

The substance was hydrolyzed for few hours in water bath; the hydrosylate was added with 2ml of pyridine sodium nitro prusside solution and was made alkaline with hydroxide solution. The change of colour from yellow to orange indicates the presence of cardiac glycoside.

Keller killiani test

About 1g of substance was boiled with 70% alcohol for 3min and filtered and to the filtrate 5ml of water, 0.5ml of strong solution of lead acetate is added, shake well and filter. The clear filtrate is treated with equal volume of chloroform and the chloroform layer is evaporated. The residue is dissolved in 3ml of glacial acetic acid and to this adds 2drops of ferric chloride. The contents are transferred to test tube containing 2ml of conc.sulphuric acid. Reddish

brown layer acquiring bluish green colour after standing indicates the presence of cardiac glycoside.

Test for sugars

Fehling's test

To the substance add equal quantity of Fehling's solution A and B were added and it was heated. Formation of brick red precipitate indicates the presence of sugar.

Benedict's test

To the substance was treated with Benedict's reagent and heated in a water bath. Formation of reddish brown precipitate indicates the presence of sugar.

Test for steroids

Liebermann's Burchard test

The extract was dissolved in 2ml of chloroform and 10 drops of acetic anhydride, 2drops of conc.sulphuric acid were added. The mixture turned violet, blue and finally emerald green indicates the presence of phytosterol's.

Salkowski test

The extract was dissolved in 2ml of chloroform and an equal volume of conc.sulphuric acid was added slowly through the sides of the test tube. A reddish violet colour was seen in the upper chloroform layer and the lower layer assumed a yellowish colour with a green fluorescence. This indicates the presence of phytosterol's.

Test for tannins

A Pinch of dried extract was dissolved in ethanol, mixed thoroughly and filtered. To the filtrate the following reagents were separately added lead acetate solution-formation of white precipitate shows the presence of tannins. Aqueous gelatin solution-formation of white colour precipitate shows the presence of tannins.

Test for proteins

Million's test

The extract was dissolved in 1ml of ethanol, filtered and filtrate was treated with millions reagent. Formation of red colour indicates the absencee of proteins.

Biuret test

The extract was dissolved in 1ml ethanol, filtered and filtrate was added with 40% sodium hydroxide and copper sulphate solution. Formation of violet colour indicates the absence of proteins

Ninhydrin test

Test extract was dissolved in 1ml of ethanol, filtered and filtrate was treated with Ninhydrin reagent, Formation of purple colour indicates the absence of protein.

Xanthoprotein test

The extract was dissolved in 1ml of water, filtered and filtrate was treated with 1ml of concentrated nitric acid. The solution was heated to boiling and allowed to boil for 1min. cool under tap water and the contents are divided into two parts. To one half of the yellow solution 40% sodium hydroxide was added till the solution was alkaline to litmus. Formation of the orange colour indicated the absence of protein.

Test for terpenoids

Noller's test

A pinch of dried extract was taken in a dried test tube. A bit of tin foil and 0.5ml of Thionyl chloride was added and heated gently. Formation of pink colour shows the absence of terpenoids.

Test for flavonoids

Shinoda test

A pinch of dried extract was dissolved in ethanol, mixed thoroughly and filtered. To the filtrate, pieces of magnesium metal and conc. Hydro chloric acid were added and heated. Appearance of magenta colour confirms the presence of flavonoids.

Test for anthocyanins

The aqueous or alcoholic extract is treated with sodium hydroxide solution. Formation of blue-violet colour indicates the presence of anthocyanins. The substance is treated with conc. Sulphuric acid. Formation of yellowish orange colour indicates the presence of anthocyanins.

Tests for saponins

Foam Test

To take a 1ml of the test solution was taken in a measuring cylinder. To this, 20ml of distilled water was added and shaked well which gives persistent foam shows presence of saponins.

RESULTS AND DISCUSSION

The *Syzygium cumini* (L) leaf extracted by using distillation, maceration, percolation and soxhlet extraction method. There are many organic solvents

used for extraction in polar solvents like, Methonol, Ethanol, Water, Acetone and non-polar solvents like, n-hexane, ether, choloroform). The present extracts allowed concentrating by rotary vacuum evaporator and the extraction were carried out by preliminary phytochemical studies. The results of the physiochemical evaluation and chemical constituents are given in Table No.1. There are many organic solvents used among these methanollic extract gives more productivity rather than ethnolic extract. From the Table No.2, water insoluble and acid insoluble materials are being about 7.8% and 16.0% respectively.

Moreover Hexane gives positive results of steroids and fats and oils. Purified water extract give positive results for proteins, glycosides, and alkaloids, Ethanol extract give positive results for steroids, alkaloids, tannins and flavonoids also. Ethyl acetate extract gives positive results for alkaloids and tannins, Methanol extract gives positive results for amino acid, flavonoids and tannins and the aqueous extract contains proteins, flavonoids and tannins.

Table No.1: Preliminary phytochemical screenings

S.No		Test	Result
1	Test for carbohydraes	Molish's test	Positive
		Fehling's test	Positive
		Benedict's test	Positive
		Barfoed's test	Positive
2	Test for starch	-	Positive
3	Tests for proteins and amino acids	Ninhydrin test	Positive
		Biuret test	Positive
		Xanthoprotein test	Positive
		Tannic acid test	Positive
4	Test for alkaloids	Mayer's test	Positive
		Dragendroff's test	Positive
		Wagner's test	Positive
		Hager's test	Positive
5	Test for flavonoids	Ferric chloride test	Positive
		Shinoda's test	Positive
		Fluorescence test	Positive
		Alkaline test	Positive
		Zinc HCL reduction test	Positive
		Lead acetate solution test	Positive
6	Test for tannins	Ferric chloride test	Positive
		Copper sulphate test	Positive
		Lead acetate test	Positive
		Potassium dichromate test	Positive
		Potassium ferric-cyanide test	Positive
7	Test for phytosterols	Liebermann's test	Positive
		Liebermann Burchard test	Positive
		Salkwoki's test	Positive
8	Test for saponins	Foam Test	Positive
9	Test for glycosides	Legal's test	Positive
		Baljet's test	Positive
		Borntrager's test	Positive

Table No.2: Physiochemical evaluation

S.No	Physiochemical Parameter	Experiments		
		I (% w/w)	II (% w/w)	Average (% w/w)
1	Total ash	22.0	23.0	22.5
2	Water insoluble ash	7.8	7.7	7.8
3	Acid insoluble Ash	15.4	16.0	16.0
4	Loss on drying	18.0	20.0	19.0

CONCLUSION

There are number of extraction techniques available among these maceration techniques gives more productivity and polar organic solvent (Methonol) and nonpolar solvent (Hexane) gives positive results of alkaloids, amino acid, proteins, flavonoids and tannins, steroids and fats and oils. Commonly *syzygium cumini* (L) leaf extract used in the treatment of diabetes.

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CONFLICT OF INTEREST

We declare that we have no conflict of Interest.

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