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Review article

Tamarindus indica (Imley bark): Ethnobotany, Phytochemistry and Pharmacology

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ABSTRACT

Traditional medicine has maintained its popularity in a number of Asian countries such as China, India, Japan and Pakistan. Medicinal plants are the oldest known health care products. Their importance is still growing. In most cases, biologically active compounds in Chinese medicine or herbal medicine have not been determined. Therefore, it is important to use the phytochemical methods to screen and analyze bioactive components, not only for the quality control of crude drugs, but also for the elucidation of their therapeutic mechanisms. Modern pharmacological studies indicate that binding to receptors or ion channels on cell membranes is the first step of some drug actions. So that this article contains the mathadology and there results of phytochemical screening of *Tamarindus indica.*

Keywords: Phytochemistry, refractive index, Alkaloids.

INTRODUCTION

The branch of chemistry concerned with plants, especially with secondary metabolites called Phytochemistry. Phytochemistry is in the strict sense of the word the study of phytochemicals. These are chemicals derived from plants. In a narrower sense the terms are often used to describe the large number of secondary metabolic compounds found in plants. Phytochemical technique mainly applies to the quality control of herbal medicine of various chemical components, such as saponins, alkaloids, volatile oils, flavonoids and anthraquinones. In the development of rapid and reproducible analytical techniques, the combination of HPLC with different detectors, such as diode array detector (DAD), refractive index detector (RID), evaporative light scattering detector (ELSD) and mass spectrometric detector (MSD), has been widely developed. Tamarindus indica is indigenous to tropical Africa, It is widely distributed throughout the tropical belt, The tamarind is a long-lived, medium-growth bushy tree which attains a maximum crown height of 12.1 to 18.3 metres (40 to 60 feet). Leaves are evergreen, bright green in colour, elliptical ovular, arrangement is alternate, of the pinnately compound type, with pinnate venation and less than 5 cm (2 inches) in length. The fruit is an indehiscent legume, sometimes called a pod, 12 to 15 cm (3 to 6 inches) in length, with a hard, brown shell. The fruit has a fleshy, juicy, acidulous pulp. Pulp contain tartaric acid 5.P.C, citric acid 4P.C, Malic Acid, Acetic acid, tataric acid, potassium 8 P.C., invert sugar 25 to 40 P.C., gum and pectin, seeds testa contain a fixed oil & insoluble matter seeds contain albuminoids, fat carbo-hydrates 63.22 P.C. fibre and ash contaning phosphorus & nitrogen. Fruit contain trace of oxalic acid. Anthelminthic, antimicrobial, antiviral, asthama, astringent, bacterial skin infections, boils, cholesterol metabolism disorders, first ripe fruits is useful in constipation [1, 2].

MATERIALS AND METHODS

Materials

The reagents such as Mayer's reagent, Dragendroff's reagent, Wagner's reagent, Hager's reagent, alpha naphthol, Conc. HCl, Sodium hydroxide, Chloroform were procured from Qualigens, India. Other reagents like Pyridine, Zinc dust was procured from Merck, India and that of magnesium ribbon from Loba Chemie Pvt. Ltd., India.

Preparation of Tamarindus indica powder The barks of

Tamarindus indica was dried in shade and then powder with a mechanical grinder. The powder was passing through sieve no. 40 and stored in a labelled air tight container for further studies.

Physicochemical studies Loss on drying

Accurately weighed quantity of sample was taken in a tarred loss on drying bottle and initial weight was noted. The sample was heated at 105°C in an oven and weighed. This procedure was repeated until a constant weight was obtained. The moisture content of the sample was calculated with reference to crude air dried drug.

Ash values Total ash value

Accurately weighed 2 g of air dried sample were taken in a tarred silica dish and incinerated at a temperature not exceeding 450°C until free from carbon. Then cooled and weighed. When a carbon free ash could not be obtained in this way, the charred mass was exhausted with hot water, residue was collected in an ash less filter paper, incinerated the residue along with the filter paper until the ash was white or nearly so, filtrate was added, evaporated to dryness and ignited at a temperature not exceeding 450°C. Percentage of ash value was calculated with reference to the crude air- dried drug.

Acid insoluble ash

Ash was boiled with 25 ml of 2 M

HCl for 5 min, insoluble matter was collected in a Gooch crucible in an ash less filter paper, washed with hot water, ignited, cooled in a desiccator and weighed. Percentage of acid insoluble ash was calculated with reference to the air dried drug [3, 4].

Water soluble ash

Ash was boiled for 5 min with 25 ml of water, insoluble matter was collected in a Gooch crucible in an ash less filter paper, washed with hot water and ignited for 15 min at a temperature not exceeding 450°C. Weight of insoluble matter was subtracted from the weight of the ash; the difference in weight represents the water-soluble ash. Percentage of water soluble ash was calculated with reference to the air dried drug.

Extractive Values

Water soluble extractives

5.0 g of air dried plant material was added to

50 ml of boiled water at 80°C in a glass stopper flask. It was shaken well and was allowed to stand for 10 min, cooled and filtered. The 5 ml of the filtrate was transferred to a tarred evaporating dish, 7.5 cm in diameter, the solvent was evaporated on a water bath, allowed to dry for 30 min, finally dried in an oven for 2 h at 100°C and residue was weighed. Percentage of water soluble extractives was calculated with reference to the crude air dried drug.

Alcohol soluble extractives

5.0 g of air dried plant material was macerated with 100 ml of ethanol in a closed flask, shaking frequently during the first 6 h and allowed to stand for 18 h. Thereafter it was filtered rapidly taking precaution against loss of ethanol. 25 ml of filtrate was evaporated to dryness in a tarred flat bottom shallow dish dried at 105°C and weighed. Percentage ethanol soluble extractive was calculated with reference to the crude air dried plant material.

Preliminary phytochemical screening Alkaloids

Mayer's test: Alkaloids give cream colour precipitate with Mayer's reagent (potassium mercuric iodide solution).

Dragandroff's test: Alkaloids give reddish brown precipitate with Dragandroff's reagent (potassium bismuth iodide solution).

Wagner's test: Alkaloids give a reddish brown precipitate with Wagner's reagent (Solution of iodine in potassium iodide).

Hager's test: Alkaloids give yellow colour precipitate with Hager's reagent (saturated solution of picric acid).

Glycosides

General test for the presence of glycosides

Part A: extracted 200 mg of the drug by warming in a test tube with 5 ml of dilute (10%) sulphuric acid on a water bath at 100°C for 2 min, centrifuge or filter, pipette off supernatant or filtrate. Neutralize the acid extract with 5% solution of Sodium hydroxide (noting the volume of NaOH added). Added 0.l ml of Fehling's solution A and then B until alkaline (test with pH paper) and heat on a water bath for 2 min. Noted the quantity of red precipitate formed and compare with that formed in Part -B.

Part B: extracted 200 mg of the drug using 5 ml of water instead of sulphuric acid. After boiling add volume of water equal to the volume of NaOH used in the above test. Add 0.l ml of Fehling's solution A and B until alkaline (test with pH paper) and heat on water bath for 2 min. noted the quantity of red precipitate formed.

Compare the quantity of precipitate formed in Part-B with that of formed in Part-A. If the precipitate in Part-A was greater than in Part- B then Glycoside may be present. Since Part- B represents the amount of free reducing sugar already present in the crude drug. Whereas Part-A represents free reducing sugar plus those related on acid hydrolysis of any sides in the crude drug.

Saponin glycosides

Froth test: Placed 1 ml solution of drug in water in a semi micro tube shake well and note the stable froth.

Anthraquinone glycosides

Borntrager's test: Boiled test material with 1.0 ml of dil. sulphuric acid in a test tube for 5 min (anthracene glycosides are hydrolyzed to aglycone and sugars by boiling with acids) centrifuge or filter while hot (if centrifuged hot, the plant material can be removed while anthracene aglycones are still sufficiently soluble in hot water, they are however insoluble in cold water), pipette out the supernatant or filtrate, cool and shake with an equal volume of dichloromethane (the aglycones will dissolve preferably in dichloromethane) separate the lower dichloromethane layer and shake with half its volume with dilute ammonia. A rose pink to red colour is produced in the ammonical layer (aglycones based on

anthroquinones give red colour in the presence of alkali).

Modified borntrager's test: Boiled 200 mg of the test material with 2 ml of dilute sulphuric acid, 2 ml of 5% aqueous ferric chloride solution for 5 min and continued the test as above. As some plant contain anthracene aglycone in a reduced form, if ferric chloride was used during the extraction, oxidation to anthroquinones took place, which showed response to the Borntrager's test [5, 6].

Cardiac glycosides

Kedde's test: Extracted the drug with chloroform, evaporated to dryness, added one drop of 90% alcohol and 2 drops of 2% 3, 5-dinitro benzoic acid (3, 5-dinitro benzene carboxylic acid Kedde's reagent) in 90% alcohol. Made alkaline with 20% sodium hydroxide solution. A purple colour was produced if cardiac glycosides are present. The colour reaction with 3, 5-diinitrobenzoic acids depends upon the presence of α, β- unsaturated-o- lactones in the aglycone.

Keller killiani test (Test for deoxy sugars): Extracted the drug with chloroform and evaporated it to dryness. Added 0.4 ml of glacial acetic acid containing a trace amount of ferric chloride. Transfered to a small test tube; added carefully 0.5 ml of concentrated sulphuric acid by the side of the test tube, blue colour appears in the acetic acid layer if cardiac glycoside was present.

Cyanogenetic glycosides

Placed 200 mg of drug in a conical flask and moisten with few drops of water, there should be no free liquid at the bottom of the flask (the test will not work if there is any liquid in the flask as the hydrogen cyanide produced will dissolve in the water rather than, come off as a gas to react with the paper). Moisten a piece of picric acid paper with sodium carbonate solution (5% aqueous) and suspended by means of cork in the neck of the flask, warm gently at about 37°C. Observe the change in colour. Hydrogen cyanide is liberated from cyanogenetic glycoside by the enzyme activity and reacts with sodium picrate to form the reddish purple sodium isopicrate.

Tannins and phenolic compounds

Gelatin test: Extract with 1% gelatin solution containing 10% sodium chloride gives white precipitate.

Ferric chloride test: Test solution gives blue green colour with ferric chloride.

Vanillin hydrochloride test: Test solution when treated with few drops of vanillin hydrochloride reagent gives purplish red colour.

Tannins get precipitated in the solution when treated with heavy metals.

Tannins yield bulky precipitate with phenazone especially in the presence of sodium and phosphate.

Alkaline reagent test: Test solution with sodium hydroxide solution gives yellow to red precipitate within short time.

Mitchell's test: With iron and ammonium citrate or iron and sodium tartarate. Tannins give a water soluble iron tannin complex, which is insoluble in solution of ammonium acetate.

Flavonoids

Shinoda test: (magnesium hydrochloride reduction test): To the test solution add few fragments of magnesium ribbon and add conc. hydrochloric acid drop wise, pink scarlet, crimson red or occasionally green to blue colour appears after few min.

Zinc hydrochloride reduction test: To the test solution add a mixture of zinc dust and conc. hydrochloric acid. It gives red colour after few minutes.

Alkaline reagent test: To the test solution add few drops of sodium hydroxide solution; formation of an intense yellow colour, which turns to colourless on addition of few drops of dil. acid, indicates presence of flavonoids.

Proteins and amino acids

Millons test: Test solution with 2 ml of Millons reagent (mercuric nitrate in nitric acid containing traces of nitrous acid), white precipitate appears, which turns red upon gentle heating.

Ninhydrin test: Amino acids and proteins when boiled with 0.2% solution of ninhydrin (Indane 1, 2, 3 trione hydrate), violet colour appears.

Steroids and triterpenoids

Libermann buchard test: Extract treated with few drops of acetic anhydride, boil and cool, cone. Sulphuric acid is added from the sides of the test tube, shows a brown ring at the junction of two layers and the upper layer turns green which shows the presence of steroids and formation of deep red colour indicates the presence of triterpenoids.

Salkowski test: Treat extract in chloroform with few drops of conc. sulphuric acid, shake well and allow to stand for some time, red colour appears at the lower layer indicates the presence of steroids and formation of yellow coloured lower layer indicates the presence of triterpenoids.

Pharmacognostical Study

Macroscopical Evaluation

Medicinal plant materials are categorized according to organoleptic, microscopical and macroscopical characteristics. Taking into consideration the variations in source of crude drug and their chemical nature, they are standardized by using different techniques including the methods of estimation of chief active constituents. Organoleptic evaluation of drugs refers to the evaluation of drugs by color, odour, size, shape, taste and special features including touch and texture etc. Organoleptic evaluations can be done by means of organs of special sense which includes the above parameters and thereby define some specific characteristics of the

material which can be considered as a first step towards establishment of identity and degree of purity.

The following organoleptic investigations were done.

Colour: the untreated samples was properly examined under diffused sunlight or artificial light source with wavelengths similar to that of daylight.

Shape and size: the length, breadth and thickness of the drugs are of great importance while evaluating a crude drugs. A graduated ruler with basic unit in millimeter is adequate for the measurement. Bark and leaves were measured by aligning ten of them on a sheet of a calibrated paper approx. 1mm apart between the lines and the result was divided by 10. Average length, breadth and thickness were determined [7, 8].

Odour and taste: the odour and taste of a crude drugs are extremely sensitive criteria based on individual's perception. Therefore the description of this feature may sometimes cause some differences of opinion. The sample was crushed in a mortar by applying pressure by pestle and the strength of the odour like weak, distinct, strong was first noted and then the odour sensation like rancid, aromatic etc was determined.

Surface characteristics, texture and fracture: the texture was best examined by taking a small quantity of material and rubbing it between the thumb and forefingers, it was usually described as smooth, rough and gritty. The physical evaluation of the bark by palpitation (touch) of the material determines the softness or hardness. The study of morphology of bark and leaves was done by taking ten samples and was observed for various qualitative and quantitative macroscopical characters.

Microscopical evaluation

Microscopical parameters observed were,

Arrangement of tissues in a transverse section.

Type of epidermal cells, stone cells, testa and endosperm.

Presence and type of crystalline structures eg. Calcium oxalate, starch etc.

Presence of oil globules, aleurone grains and trichomes.

All determination was carried out by using Almicro compound microscope (100x) attached with a camera.

Powder microscopy

The dried bark of Tamarindus indica was powdered and sieved to obtained fine powder it was taken up for powder microscopy evaluation as follows A small quantity of powder was kept on a slide and after mounting on glycerin, 10 min were provided as spread out time. Finally, it was observed for powder microscopical characters.

Another small quantity of powder was stained with phloroglucinol and HCl, ruthenium red, safranin, sudan red III, iodine and acetic acid respectively. Mounted with glycerin on microscopical slide and observed for powder microscopic characters.

Extraction

Alcohoic Extractction by soxhlet extractor of Tamarindus indica.

Procedure

In soxhlet extraction the dried leaf is placed in a porous filter paper and placed in an extraction chamber , which is suspended above a flask containing the solvent (Ethanol) and below a condenser. The flask is heated and the solvent evaporates and moves up into the condenser where it is converted into a liquid that trickles into the extraction chamber containing the sample. The extraction chamber is designed so that when the solvent surrounding the sample exceeds a certain level it overflows and trickles back down into the boiling flask. At the end of the extraction process, which lasts a few hours, the flask containing the solvent and seed extract is removed. Finally the solvent in the flask is evaporated and extract are remaining in flask.

RESULTS AND DISCUSSION

Macroscopical study

The bark and leaves of Tamarindus indica was selected on the basis of the tribal information and literature review, the plant Tamarindus indica was collected from Bhopal in the month of October 2010 and authenticated at Safia College of Science, Peer gate,

Microscopical study

Tamarindus indica

The bark is grey white, the surface is smooth with shallow irregular fissures at frequent places. The bark is hard and brittle, fibrous and flexible. The bark consists of periderm and secondary phloem and the entire bark is about 4 mm thick.

Periderm :- The periderm is superficial and undulate with deep folding and irregular thick ending. The periderm is 150-200 µm thick . The outer part of the periderm consist of thin walled, subsized phloem, the cells being tubular in shape and subsized walls.

Secondary phloem:- The secondary phloem is differentiated into outer wider zone of collapsed phloem and inner narrow zone of noncollapsed phloem.

Collapsed phloem:- The collapsed phloem is characterized by wide phloem-rays which gradually dilates into wide funnel shaped expansions. The rays are wavy and dialated, The rays cells are arranged in tangential bands.

Noncollapsed phloem:- This consist of intact phloem elements and narrow phloem rays, the sclerenchyma cylinder are reduced in thickness or totally absents. The phloem elements are polygonal in outline and have prominent companion cells.

Powder microscopy

The bark powder exhibit the following inclusions when examined under the microscope.

Fragments of phloem fibres: Broken pieces of fibres are abundant in the powder, the fibre- fragment have thick and lignified

walls and narrow lumen. The fibres are 10-12µm thick; they range from 40-150 µm.

Wide pieces of phloem tissues are visible in the powder. They consist of phloem ray cells which are horizontal in orientation and the ray cells are rectangular and thin walled.

Sieve tubes and parenchyma cells were observed in the powder. The sieve tubes are narrow, long tubes. The parenchyma cells are wide, short, vertically oblong cells, often found in bundles. Crystal strands are very common. They are scattered individually or in continuous vertical strand.

Physicochemical study

Loss on drying of barks of Tamarindus indica was found to be not less than 1.58% w/w.

Ash value of any crude drug give an idea about the earthy matter and/or inorganic composition and/or other impurities present along with the crude drug. In the present, study ash values (total ash, acid insoluble ash and water soluble ash value)

The water soluble extractive value and alcohol soluble extractive value for Tamarindus indica was found to be not less than 5% w/w and 15.0% w/w.

Preliminary Phytochemical Scrrening

The preliminary phytochemical screening of various extracts of Tamarindus indica barks were carried out and it was found that:

Petroleum ether shows positive result for tannins, steroids.

Chloroform shows positive result for alkaloids.

Ethyl acetate shows positive result for alkaloids, tannins, and glycoside.

Ethanol shows positive result for alkaloids, flavonoids, steroid, glycoside and Water shows positive result for flavonoid and Triterpenoids [9, 10].

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