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Pharmacognostic And Preliminary Phytochemical Evaluation Of The Leaves Of Triadica Sebifera

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ABSTRACT :

Various traditional system of medicine enlightened the importance of the leaves of Triadica sebifera (Euphorbiaceae) to have a great medicinal value. Triadica sebifera is a tree native to eastern Asia. It is commonly called as Chinese tallow tree, Florida aspen, Chicken tree, gray popcorn tree, or candleberry tree which belongs to the family Euphorbiaceae. The leaves of Chinese tallow tree are used to treat boils, skin disorders, inflammation and also used as antibacterial, antimicrobial, antioxidant etc. The present study provides Pharmacognostic, Phytochemical details of leaves of Triadica sebifera. The dried leaves were subjected to maceration using methanol. These solvent extracts were subjected to a preliminary phytochemical screening to detect the different chemical principles present viz., carbohydrates, proteins, amino acids, steroids, glycosides, alkaloids, tannins and phenolic compounds.

Keywords: *Triadica sebifera*, Euphorbiaceae, Pharmacognostic, Phytochemical.

INTRODUCTION :

Herbs contain many biologically active substances that may be of interest for the food, pharmaceutical and cosmetic industries. Herbal medicines may include whole parts of plant or mostly prepared from leaves, roots, bark, seed and flowers of plants. Usage of herbal medicine includes many advantages like fewer side effects, cost-effective, long-lasting benefits for overall wellness and Herbal medicine focuses on maintaining balance in the body. The medicinal value of these plants lies in bioactive phytochemical constituents that produce definite physiological action on the human body. Over 50% of all modern clinical drugs are of natural origin and natural products play a vital role in modern drug development in the pharmaceutical industry [1].

Triadica sebifera is a tree native to eastern Asia which is commonly called as Chinese tallow tree, Florida aspen, chicken tree, gray popcorn tree, or candleberry tree and it belongs to the family Euphorbiaceae. Different parts of plant are used in the treatment of indigenous system of medicine for the treatment of various human ailments such as boils, skin disorders, inflammation. Its seeds are used to heal wounds, as purgatives, and the kernels of the seeds produce Stillingia oil which is used in paints, varnishes, machine oils, crude lamp oil. The flowers of this plant produce a light-colored, high-quality honey and it is often planted for its ornamental value [2].

The present investigation is an attempt to evaluate the Pharmacognostic and phytochemical characteristics of leaves of *Triadica sebifera*. The leaves were evaluated to observe their organoleptic, microscopic and physical parameters.

MATERIALS AND METHODS :

Chemicals: All the chemicals were of highest available purity and were proceed from E. Merck, Mumbai, India, HiMedia Laboratories, Mumbai, India and SD Fine Chemicals, Mumbai, India.

Procurement of plant material:

For the present investigation, *Triadica sebifera* leaves were collected from Vaageswari college, Karimnagar district. The leaves were separated, shade dried and powdered with mixer and sieved. The Pharmacognostic studies were conducted with fresh leaves and leaf powder. The plant was identified and authenticated by Dr. E. Narasimha Murthy, Specimen Accession No: ENM-100134. The leaves were dried in shade and stored at room temperature.

Pharmacognostic evaluation:

Organoleptic evaluation:

In organoleptic evaluation, various sensory parameters of the plant material, such as size, shape, color, odor, and taste of dried leaves were recorded.

Microscopic evaluation:

Microscopic evaluation is important to identify herbs and crude drugs, to determine purity, adulterant. It is mostly used for qualitative evaluation of organized crude drugs in entire and powdered forms. For the effective results, various reagents or stains can be used to distinguish cellular structure. Various diagnostic characters of leaves and leaf powder of *Triadica sebifera* were studied by microscopic analysis with or without staining [3].

1.Powder analysis of leaf:

Take a small quantity of powder onto a microscopic slide, 1-2 drops of 0.1% phloroglucinol solution and a drop of concentrated hydrochloric acid were added, covered with a cover slip and then observed under the microscope. The presence of starch grains was detected by the formation of blue color on addition of 2-3 drops of iodine solution.

2.Determination of stomatal index:

Leaf fragments were taken in a test tube containing 5ml of chloral hydrate solution and boiled on water bath until fragments became clear (~15min). These fragments were transferred onto microscopic slide, mounted in glycerol and observed under microscope for the presence and quantification of epidermal cells, stomata (type and distribution). The slide was examined to which camera lucida was attached and recorded the epidermal cells and stomata lying within a selected area. Stomatal index was calculated as the percentage of number of stomata present per number of epidermal cells and each stoma was counted as one cell [4].

Physical evaluation:

In physical evaluation, moisture content, ash values viz., total ash, acid insoluble ash and water-soluble ash, and extractive values viz., alcohol soluble extractive value, chloroform soluble extractive value were determined. The ash value represent the inorganic salts present in the drug. Extracts obtained by exhausting crude drugs are indicative of approximate measures of certain chemical compounds they contain, the diversity in chemical nature and properties of contents of drug.

1.Moisture content (loss on drying):

Accurately weighed one gram of fresh leaves of *Triadica sebifera* was placed in a China dish and dried at 100℃ for 1hr and weighed. This was continued until two successive weighing are equal [5].

2.Determination of total ash:

Two grams of powder of *T.sebifera* was taken in a China dish and incinerated at a temperature not exceeding 450℃ until free from carbon. The resultant ash was cooled and weighed. The percentage of ash was calculated with reference to the air-dried drug [6].

3.Acid insoluble ash:

The total ash obtained from 2g of leaf powder was boiled for 5min with 25ml of dilute hydrochloric acid and the insoluble matter was collected on ashless filter paper. It was washed with hot water, ignited and weighed. The percentage of acid insoluble ash was calculated with reference to the air-dried drug.

4.Water soluble ash:

The total ash obtained from 2g of leaf powder was boiled for 5min with 25ml of water and the insoluble matter was collected on ashless filter paper. It was washed with hot water, ignited, and weighed. The percentage of water-soluble ash was calculated with reference to the air-dried drug.

5.Determination of alcohol soluble extractive:

Accurately weighed powder (5g) of leaves was taken separately and macerated with 100ml of 95% alcohol for 24h. The contents were frequently shaken during the first 6h and allowed to remain for 18h. After 24h, the extract was filtered and filtrate was evaporated. The extract was dried to a constant weight.

6.Determination of chloroform soluble extractive:

Accurately weighed powder (5g) of leaves was taken separately and macerated with 100ml of chloroform for 24h. The contents were frequently shaken during the first 6h and allowed to remain for 18h. After 24h, the extract was filtered and filtrate was evaporated. The extract was dried to a constant weight [7].

Preliminary Phytochemical Screening:

The leaf powder was subjected to successive extraction in a Soxhlet apparatus using methanol and chloroform for 6hours and the extracts were evaporated to dryness. The dried extracts were weighed, and percentage yields were calculated. The extracts were used for preliminary phytochemical screening using chemical tests viz., Molisch's, Fehling's, Benedict's and Barfoed's tests for carbohydrates; Biuret and Millon's tests for proteins; Ninhydrin's test for amino acids; Salkowski and Liebermann-Burchard's reaction for steroids; Borntrager's test for anthraquinone glycosides; foam test for saponin glycosides; Shinoda and alkaline tests for flavonoid glycosides; Dragendorff's, Mayer's, Hager's and Wagner's tests for alkaloids; and ferric chloride, lead acetate, potassium dichromate and dilute iodine tests for tannins and phenolics [8,9].

Fluorescence analysis:

The dried leaves powder was placed on slide and treating with several drops of specified reagent like picric acid, Sodium hydroxide, glacial acetic acid, Hydrochloric acid, iodine, Nitric acid, Ferric chloride, Sulfuric acid and methanol. The slides were observed under Visible light and UV 254nm and 365nm and the emitted fluorescence was observed. This fluorescence analysis is used to identify and characterize substances by analyzing the intensity and characteristics of their fluorescence [10].

RESULTS AND DISCUSSION :

Pharmacognostic Evaluation

Organoleptic and microscopical evaluation:

In organoleptic evaluation, appropriate parameters of leaf were studied. Macroscopically the leaf is simple in composition, leaves are arranged alternately on the branches in which veins are arranged in parallel manner. The leaves have acuminate tip and entire margins, with broadly ovate leaf blades and rounded bases. The leaves are bright green in color and slightly paler underneath.

Micromorphological features revealed that the leaf powder contains numerous shaped starch grains, xylem fibers, trichomes.
The quantitative microscopic evaluation of fresh leaves and leaf powder was performed and the results obtained were shown in Table 1 and Figures 1-6.

TABLE.1: RESULTS OF MICROSCOPY OF FRESH LEAVES.

Parameter	Upper epidermis	Lower epidermis
Number of epidermal cells	35	36
Type of stomata	Anomocytic	Anomocytic
Stomatal number	5	5
Stomatal index	12.5 per sq.mm	12.1 per sq.mm

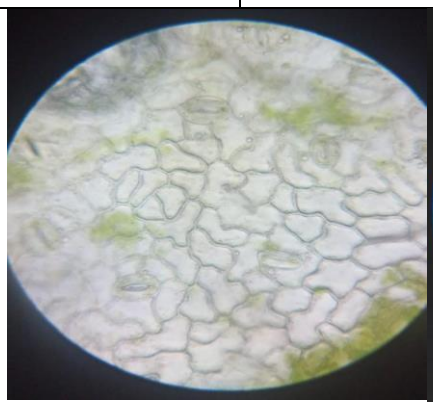

Fig.1: Stomata in lower epidermis of leaf

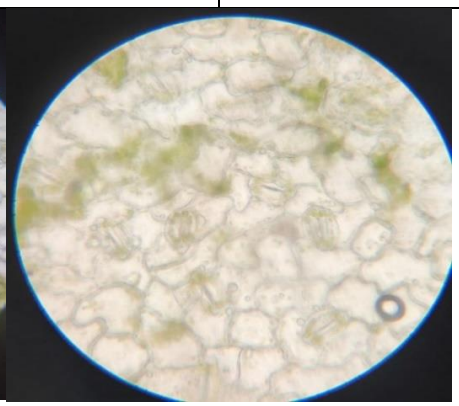

Fig.2: Stomata in upper epidermis of leaf

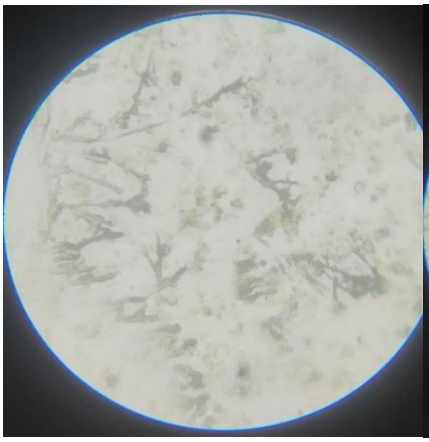

Fig.3: Trichomes

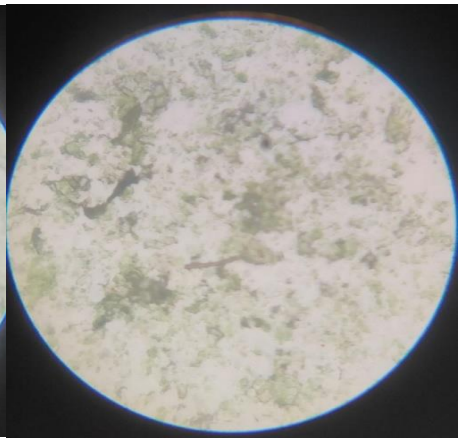

Fig.4: Phloem

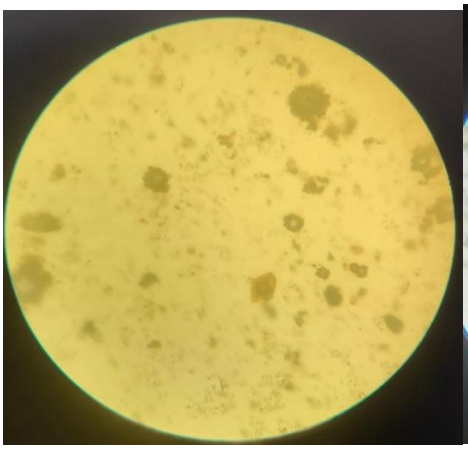

Fig.5: Starch grains

Fig.6: Xylem

Physical Evaluation:

The various physical parameters of leaves and leaf powder viz., moisture content, ash values viz., Total ash, acid insoluble ash, water-soluble ash, and extractive value viz., alcohol soluble extractive value, chloroform soluble extractive values were determined. The results were shown in Table 2.

The total ash is particularly important in the evaluation of purity of drug. Acid insoluble ash is used to measure the amount of inorganic residue in sample. Water soluble ash is used to determine the amount of inorganic material in plant ash and to help determine the extractive values of ash. Extractive values are used to determine the quality and purity of crude drugs, and to identify potential impurities.

TABLE.2: RESULTS OF PHYSICAL EVALUATION.

Physical parameter	Value
Moisture content	0.95
Total ash	0.38
Acid insoluble ash	0.13
Water soluble ash	0.17
Alcohol soluble extractive value	12.8
Chloroform soluble extractive value	9.73
Water soluble extractive value	6.4

Fluorescence characteristics:

It is a fast method for the design study of crude drug of unsure specimen, when other methods produce inappropriate results. This is a key technique which helps to identify the authentic plant samples and detect adulterants. Results are were shown in table 3.

TABLE.3: RESULTS OF FLUORESCENCE ANALYSIS.

	Visible light	**Ultraviolet light (254nm)**	**Ultraviolet light (365nm)**
Powder alone	Light green	Green	Bluish green
Powder + picric acid	Light green	Dark green	Black
Powder + NaOH	Green	Green	Bluish green
Powder + glacial acetic acid	Dark green	Bluish green	Dark brown
Powder + HCl	Brownish green	Dark green	Black
Powder + Iodine	Brownish	Green	Black
Powder + HNO3	Orange	Dark green	Black
Powder + FeCl3	Brown	Brownish green	Black
Powder + H2SO4	Blackish brown	Dark green	Black
Powder + Methanol	Green	Dark green	Orange

Preliminary phytochemical evaluation:

The leaf powder of *T. sebifera* was extracted with methanol and the nature and yield of the extracts were observed. The methanolic and chloroform extract of powdered leaves of *T. sebifera* showed the presence of carbohydrates, proteins, glycosides, steroids, Tannins, Flavonoids etc.

These secondary plant metabolites are known to possess various pharmacological effects and might be responsible for the various actions exerted by *T. sebifera*. It is used in the treatment of various disease conditions. The standardization of a crude drug is an integral part of establishing is correct identity. The results of present investigations could serve as a basis proper identification, collection and investigations of the plant.

The macro and micro morphological features of the leaf described, distinguishes it from other members of the genera. Numerical data and quantitative leaf microscopy are unique to the plant and are required in its standardization. Phytochemical evaluation revealed the presence of various secondary plant metabolites which have been claimed to be responsible for various pharmacological activities.

Phytochemicals	Test	Methanolic extract	Chloroform extract
Carbohydrates	Molisch test	+	+
	Fehling test	+	+
	Benedict test	+	+
	Tollen test	-	-
Proteins	Biuret test	+	+
	Millon's test	+	+
	Test for proteins containing sulfur	-	-
	Xanthoprotein test	+	+
Amino acids	Test for cysteine	-	-
	Ninhydrin test	+	+
	Test for tyrosine	+	+
Glycosides	Keller-killiani	+	+
	Legal test	-	-
	Borntrager test	+	+
	Foam test	+	+
Flavonoids	Shinoda test	+	+
Tannins	5% FeCl3 solution	+	+
	Acetic acid solution	+	+
	Dil.HNO3 solution	+	+
Steroid	Salkowski test	+	+

CONCLUSION :

As there is no pharmacognostical work on record of this traditionally much valued drug, the present work was taken up a view to lay down standards, which could be useful to establish the authenticity of this medicinally useful plant. The medicinal properties of *T. sebifera* leaves may be due to the presence of above-mentioned phytochemicals. The data produced in the present investigation is also helpful in the preparation of the crude drug's monograph and inclusion in various pharmacopoeias.

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