INVESTIGATION OF PHARMACOGNOSTIC, PHYSICOCHEMICAL AND PHYTOCHEMICAL STUDIES OF *TREVESIA PALMATA*.

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Abstract : The objective of the study is to investigate the Pharmacognostic, physicochemical and phytochemical studies of the leaves of *Trevesia palmata* (Araliaceae). It is also known as snowflake tree and is considered as medicinal plant used to cure various health ailments. They are remarkable for the unusual pseudo-compound leaves that occur in several of the species. The leaf extracts of *Trevesia palmata* is used as thrombolytic, antiarthritic, antihyperglycemic and analgesic (Mohammed and Mohammed, 2015). The standardization of medicinal plant of potential therapeutic significance has been increased rapidly in recent years. Pharmacognostic study is more reliable than the modern techniques regarding identification and authentication of the medicinal plant. The plant *Trevesia palmata* has been taken for Pharmacognostic study for checking the quality, purity and for the correct sample identification. This information will be of used for further pharmacological and therapeutical evaluation of the species in order to ensure the use of only genuine and uniform materials in preparation of herbal formulation.

IndexTerms **- Pharmacognostic, Physicochemical studies, Phytochemical, *Trevesia palmata***

I. INTRODUCTION

The plant *Trevesia palmata* (Araliaceae) is native to Asia, China, Bangladesh, Bhutan, India, Nepal, Cambodia, Laos, Myanmar, Thailand and Vietnam. They are evergreen tree to 15-20 feet tall with few or no side branches and topped with a crown of long stalked 1-2 foot wide leaves that are deeply lobed giving the leaf a lacy snow flake look. The genus name Trevesia was described by the Italian Botanist Roberto De Visiani in 1840 (Mathew and Jebb, 1998). This species is used in traditional system of medicines to treat venereal diseases and to treat bruising (Rahman *et al.*, 2007). Its potential for lowering of blood glucose and alleviating pain with methanolic extract of *Trevesia palmata* is also been reported (Rahman *et al*., 2014). In addition shoots are edible and the whole plants are used as ornamental plants. So far no proper pharmacognostical studies have been reported for this plant; hence our efforts were devoted in this direction.

II. MATERIALS AND METHODS

2.1. Plant materials

Trevesia palmata leaves were collected from Aizawl, Mizoram, India. The plant was identified by Botanical Survey of India, Shillong, India and voucher specimen of the plant No. BSI/ERC/Tech/Plant Iden./2016/321 was deposited in the Department of Pharmacy, RIPANS for future reference.

2. 2. METHODOLOGY

2.2.1. Macroscopic evaluations

The following macroscopic characters for the fresh leaves were noted: size and shape, colour, odour, taste, the apex, margin and base (Trease and Evans, 2002).

2.2.2. Microscopic evaluations

Microscopic evaluations were carried out for both qualitative and quantitative basis. All evaluations were performed using Projection Microscope.

2.2.2.1. Qualitative microscopy

For qualitative microscopy transverse sections of fresh leaves and was treated with Phloroglucinol hydrochloride solution, mounted with glycerine and was observed under Projection microscope at 10x first and then at 40x.

2.2.2.2. Quantitative investigation

Quantitative leaf microscopy was performed to determine the stomatal number, stomatal index, vein islet number and vein termination number on epidermal strips (Kokate, 1994).

Determination of Stomatal Number

Some piece of the leaf was boiled with chloral hydrate solution or alternatively with chlorinated soda. Upper and lower epidermis was peeled separately by means of forceps. It was kept on a slide and mounted in glycerine water. The slide was placed with cleared leaf on the stage and was observed under Projection microscope at 40x. The epidermal cell and stomata were counted in an area of 200 µm. The cell was included if at least half of its area lies within the square. The result was recorded for each of the four fields and its per sq.mm.was calculated.

Determination of Stomatal Index

Some piece of the leaves was boiled by using chloral hydrate solution. Upper and lower epidermis was peeled separately by means of forceps. It was kept on the slide and was mounted in glycerine water. The slide was placed with cleared leaf on the stage

of the projection microscope at 40x. The number of epidermal cells(E) in each field was counted in an area of 200 µm. The stomatal index(I) was calculated by using the formula: I = S X 100 / E+S

Determination of Vein-islet and Vein-termination number `

Few leaves were boiled in chloral hydrate solution in a beaker on water bath. The preparation was mounted in glycerine water. The vein-islets and vein terminations were subjected to Projection microscope at 40x. The number of vein-islets and vein-termination present were counted within the square of 300 µm. The observation from four squares were taken and the mean was calculated (Trease and Evans, 2002).

2.2.3. Physicochemical parameters: The various physicochemical parameters such as moisture content, extractive value- alcohol soluble extractive value and water soluble extractive value, total ash, acid insoluble ash and water soluble ash were determined (IP, 1996).

Determination of moisture content: A glass stoppered shallow weighing bottle had been dried and was used for weighing the sample. 5 grams of the drug was transferred to the bottle and was covered . The bottle along with the contents was weighed accurately. Then, the bottle was placed in the oven at 105°C for 5 hours. The sample was dried to constant weight. After the completion of drying, the drying chamber was opened and the bottle was closed promptly and was allowed to cool to room temperature in a desiccator before weighing. The bottle and the contents were weighed.

Extractive value

Methanol soluble extractive: 5 grams of the air dried drug which was coarsely powdered was macerated with 100 ml of methanol in a closed flask for 24 hours by shaking frequently during the first six hours and was allowed to stand for 18 hours. Then, it was rapidly filtered taking precautions against loss of ethanol, 25 ml of the filtrate was evaporated in a tarred flat bottomed shallow dish, which was dried at 105°C and was weighed. The percentage of methanol soluble extractive value was calculated with reference to the air-dried drug.

Water soluble extractive: 5 grams of the accurately weighed air dried drug, coarsely powdered were macerated with 100 ml of chloroform water in a closed flask for 24 hours, by shaking frequently during the first six hours and allowed to stand for 16 hours. The extracts was filtered rapidly and 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105°C and weighed. The percentage of water soluble extractive was calculated with reference to the air-dried drug.

Total Ash : 2g of the air-dried crude drug was weighed accurately in a silica dish and was incinerated at a temperature not exceeding 450°C until free from carbon, it was cooled and weighed. If a carbon-free ash cannot be obtained in this way, the charred mass was exhausted with hot water, the residue was collected on an ashless filter paper, the residue was incinerated on filter paper until the ash is white or nearly so, the filtrate was added, evaporated to dryness and was ignite at a temperature not exceeding 450°C. The percentage of ash was calculated with reference to the air-dried drug. Total ash content (%) = z – x X 100 /y where, z = weight of the crucible; x = weight of the crucible with ash; y = weight of the plant taken.

Determination of Acid insoluble Ash: The ash was boiled with 25 ml of 2M hydrochloric acid for 5 minutes, the insoluble matter was collected in an ashless filter paper, washed with hot water. It was ignited and cooled in a desiccators and weighed. The percentage of acid-insoluble ash was calculated with reference to the air-dried drug. Acid insoluble ash value of the sample (%) = 100 X a / y where , a = weight of the acid insoluble ash; y = weight of the air dried drug

Determination of Water soluble Ash: The ash was boiled for 5 minutes with 25 ml of water, the insoluble matter was collected in an ashless filter paper, it was washed with hot water, and was ignited for 15 minutes at a temperature not exceeding 450°C. The weight of the insoluble matter was subtracted from the weight of the ash; the difference in weight represents the water soluble ash. The percentage of the water soluble ash was calculated with reference to the air-dried drug.

Water insoluble ash value of the sample (%) = 100 X a / y

where, a= weight of the water insoluble ash; y= weight of the air-dried drug.

2.2.4. Phytochemical group test: (Kokate, 1994; Trease and Evan, 1972; Harborne, 1984)

Test for Flavonoids

Zinc hydrochloride test: To the extract solution, add mixture of zinc dust and concentrated hydrochloric acid. It gives red colour after few minutes. Alkaline reagent test: To the test solution add few drops of sodium hydroxide solution, intense yellow colour is formed which turns to colourless on addition of few drops of acids indicate the presence of flavonoids.

Test for Steroids

Libermann-Burchard Test: 1ml of concentrated sulphuric acid was added to 10mg of extract dissolved in 1 ml of chloroform. A reddish-blue color exhibited by chloroform layer and green fluorescence by the acid layer suggested the presence of steroids.

Salkowski Test: When Conc. Sulphuric acid was added to a chloroform solution of the extract (10 mg of extract in 1 ml of chloroform), formation of reddish-blue colour in the chloroform layer and green florescence in the acid layer suggested the presence of steroids.

Test for Alkaloids

1 to 2ml of extract was taken in a test tube. 0.2ml of dilute hydrochloric acid and 0.1ml of Mayer's reagent were added. Formation of yellowish buff colored precipitate gives positive test for alkaloid.

0.1ml of dilute hydrochloric acid and 0.1 ml of Dragendroff's reagent were added in 2ml solution of extract in a test tube. Development of orange brown colored precipitate suggested the presence of alkaloid.

2ml of extract solution was treated with dilute hydrochloric acid and 0.1 ml of Wagner's reagent. Formation of reddish brown precipitate indicated the positive response for alkaloid.

2ml of extract was allowed to react with 0.2ml of dilute hydrochloric acid and 0.1 ml of Hager's reagent. A yellowish precipitate suggested the presence of alkaloid.

Test for Amino acids

Millon's test: To the 2ml of extract solution add about 2ml of Millon's reagent, white precipitate indicate the presence of amino acids. Ninhydrine test: To the 2ml of extract solution add ninhydrine solution, boil, Violet colour indicate the presence of amino acids. 2.4.5. Test for reducing sugars

To 5ml of the extract solution, mixed with 5ml of Fehling's solution was boiled for 5minutes. Formation of brick red colored precipitate demonstrated the positive test for reducing sugars.

To 5ml of the extract solution, 5ml of Benedict's solution was added in a test tube and boiled for few minutes. Development of brick red precipitate confirmed the presence of reducing sugars.

Test for Deoxysugars

Keller-Kiliani test

Small amount of the sugar is dissolved in glacial acetic acid and 2 drops of 5% ferric chloride was added. The solution was transferred to the surface of 2ml concentrated sulphuric acid. Reddish brown color (which changed to bluish green to dark on standing) at the junction confirmed the presence of deoxysugars in the sample.

Test for Tannins

Ferric chloride test: 5ml of extract solution was allowed to react with 1 ml of 5% ferric chloride solution. Greenish black coloration indicated the presence of tannins.

Lead acetate test: 5ml of the extract was treated with 1 ml of 10% lead acetate solution in water. Yellow color precipitation gave the test for tannins.

Test for Saponins

Foam test: 0.5 gm of extracts was shaken with 2 ml of water. If foam produced persists for 10 minutes, it indicates the presence of saponins. Froth formation test: Extracts were diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicate the presence of saponins**.**

III. RESULTS AND DISCUSSIONS

Pharmacognostic, Physicochemical and phytochemical screening test

In this study the pharmacognostic standards for the leaves of *Trevesia palmata* was carried out for the first time. Morphological and anatomical studies of the leaf will enable to identify the crude drug. The information obtained from preliminary phytochemical screening will be useful in finding out the genuinity of the drug. Ash value, extractive values can be used as reliable aid for detecting adulteration. These simple but reliable standards will be useful to a lay person in using the drug as a home remedy. Also the manufacturers can utilize them for identification and selection of the raw material for drug production. On macroscopic investigation (Table -1) *Trevesia palmata* leaves are palmate, ovate lanceolate, dark green, evergreen, margin serrate, apex acuminate, 15-20 ft tall, 5-10 ft width.

The microscopic study revealed the presence of unicellular unlignified covering trichomes, palisade cells, vascular bundles, collechyma and parenchyma cells (Fig- 2). Paracytic stomatas were seen (Fig-3). Various physico-chemical parameter of powdered drug has been investigated and reported in (Table -2). Moisture content of drugs might be at minimum level to dispirit the reduction of bacteria, yeast or fungi through storage. Ash values used to find out quality and purity of unsophisticated drug. It indicates the existence of a mixture of impurities like carbonate, oxalate and silicate. The acid insoluble ash consist mainly silica and indicate contamination with earthy material. The water soluble ash is used to estimate the amount of inorganic elements present in drugs. The extractive values are valuable to estimate the chemical constituents present in the crude drug and furthermore assist in evaluation of definite constituents soluble in a particular solvent. The stomatal number, stomatal index, vein islet number, vein termination numbers are comparatively constant for plants and can be used to make out differences between closely related species and the results are depicted in Table -3. The preliminary phytochemical screening of the various leaf extracts of *Trevesia palmata* were performed in order to find out what type of chemical constituents are present and the results are depicted in Table-4.

Table No. 1 Macroscopical characters of the leaves of *Trevesia palmata*

Size	Height 15, 20ft, width 5
Colour	Dark green
Odour	Foul odour
Taste	Somewhat bitter
Shape	Palmate, ovate lanceolate
Margin	Serrate
Apex	Acuminate
Base	Rounded plate

Table- 2: Physicochemical standardization of the leaves of *Trevesia palmata*

Sl. No	Parameters	Leaves
1	Total ash value	10.8%
2	Acid insoluble ash	7.5%
3	Water soluble ash	3.2%
4	Moisture content	10.3%
5	Water soluble extractive	36%
6	Alcohol soluble extractive	20.8%

Table- 3: Quantitative estimation of the leaves of *Trevesia palmata*.

Parameters	Range
Palisade ratio	12-13
Stomatal number (lower epidermis	4-5
Stomatal index (lower epidermis	15-16
Vein islet number	1-2
Vein termination	3-4

Table- 4: Qualitative phytochemical screening of the leaves of *Trevesia palmata*

Sl. No	Plant constituent	Test/ Reagent	Petroleum ether extract	Chloroform extract	Methanol extract
1	Alkaloids	Dragendorff's reagent Mayer's reagent Hager's reagent Wagner's reagent	- - - -	+ + + +	+ + + +
2	Amino acids	Ninhydrine reagent Millon's reagent	- -	- -	- -
3	Glycoside		-	-	+
4	Flavonoid	Alkaline reagent test Zinc hydrochloric test	- -	- -	+ +
5	Steroids	Liebermann- Burchard test Salkowski test	+ +	+ +	+ +
6	Tannins	Lead acetate test Ferric chloride test	- -	- -	+ +
7	Saponin	Foam test Froth formation test	- -	+ +	+ +

+ indicates positive
- indicates negative

Figure 1.*Trevesia palmata*

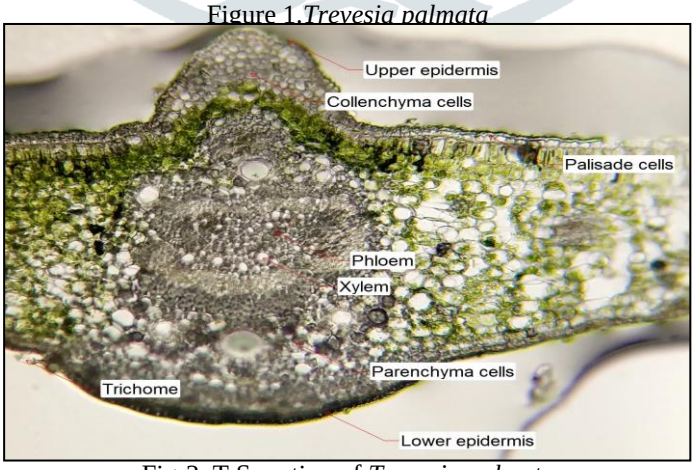

Fig-2. T.S section of *Trevesia palmata*

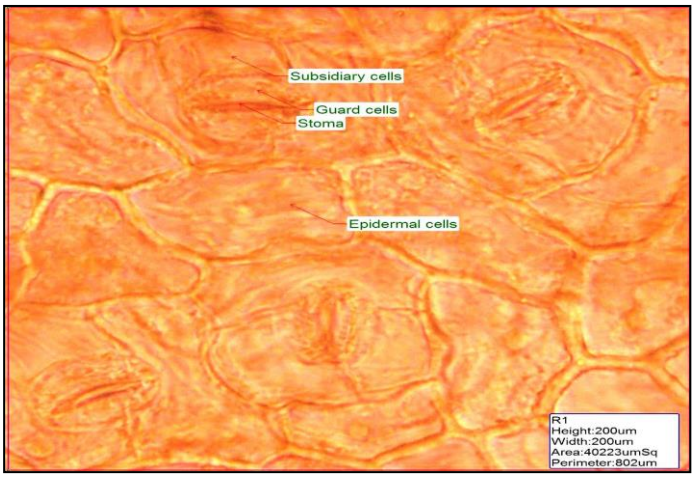

Fig. 3.Stomatal number and Stomatal index and observed from the leaves of *Trevesia palmata*

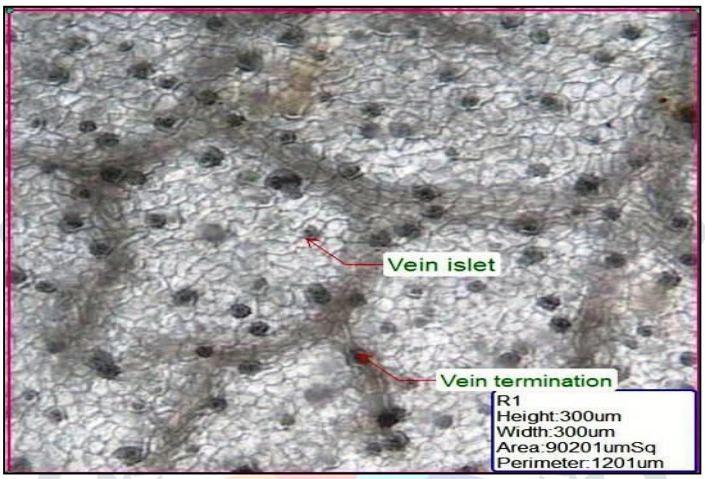

Fig. 4. Vein islet and Vein termination observed from the leaves of *Trevesia palmata*

IV. RESULTS AND DISCUSSIONS

Pharmacognostic, Physicochemical and phytochemical screening test:

In this study the pharmacognostic standards for the leaves of *Trevesia palmata* was carried out for the first time. Morphological and anatomical studies of the leaf will enable to identify the crude drug. The information obtained from preliminary phytochemical screening will be useful in finding out the genuinity of the drug (Modi *et al.* 2010). On macroscopic investigation (Table -1) *Trevesia palmata* leaves were found to be palmate, ovate lanceolate, dark green, evergreen, margin serrate, apex acuminate, 15-20 ft tall, 5-10 ft width.

The microscopic study revealed the presence of unicellular unlignified covering trichomes, palisade cells, vascular bundles, collenchymas and parenchyma cells (Fig- 2). Paracytic stomatas are seen (Fig-3). Various physico-chemical parameter of powdered drug has been investigated and reported in (Table -2). Total ash value of the plant was found to be 10.8%, Acid insoluble ash and water soluble ash were found to be 7.5 % and 3.2% respectively. Ash values and extractive values can be used as reliable aid for detecting adulteration. These simple but reliable standards will be useful to a lay person in using the drug as a home remedy. Also the manufacturers can utilize them for identification and selection of the raw material for drug production. Moisture content of drugs might be at minimum level to dispirit the reduction of bacteria, yeast or fungi through storage. Ash values used to find out quality and purity of unsophisticated drug. It indicates the existence of a mixture of impurities like carbonate, oxalate and silicate. The acid insoluble ash consist mainly silica and indicate contamination with earthy material. The water soluble ash is used to estimate the amount of inorganic elements present in drugs. The extractive values of the plant *Trevesia palmata* were found to be 36% and 20.8% for water soluble extractive and alcohol soluble extractive respectively. The extractive values are valuable to estimate the chemical constituents present in the crude drug and furthermore assist in evaluation of definite constituents soluble in a particular solvent. The stomatal number, stomatal index, vein islet number, vein termination numbers are comparatively constant for plants and can be used to make out differences between closely related species and the results are depicted in Table -3. The preliminary phytochemical screening of the various leaf extracts of *Trevesia palmata* were found to contained alkaloids, Flavonoids, Steroids, Glycoside,Tannins and Saponins and the results are depicted in Table-4. It is performed in order to find out what type of chemical constituents are present.

V. CONCLUSION

From the present study we can conclude that the majority of the information on the identity, purity and quality of the plant material can be obtained from its macroscopy, microscopy, physico-chemical, qualitative and quantitative parameters. As there is no record on pharmacognostical work on leaves of *Trevesia palmata*, the present work is undertaken to produce some pharmacognostical standards. In addition to this the preliminary phytochemical investigation shows the presence of Alkaloids, Flavonoids, Tannins, saponins, Glycosides and Steroids. The present investigation thus provides evidence for the total safety profile of the leaves of *Trevesia palmata*.

VI. ACKNOWLEDGEMENT

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