POLLEN MORPHOLOGY AND PHYTOCHEMICAL INVESTIGATION OF LEAVES EXTRACT OF *TERMINALIA CATAPPA* L. FROM THE YAN LAW TRACT, KYAING TONG TOWNSHIP

Nwe Ni Tin[1], Nang Moon Sar[2], Aye Thida Hlaing[3]

Abstract

In this study an attempt has been made to identify and to authenticate the source pant *Terminalia catappa* L. In identifying the taxon, both external morphological and palynological characters are used as the taxonomic tools. The systematic treatment is worked out following the classification scheme of Byng J.W & M.W Chasem, 2016. The detection of phytochemical constituents present in leaf extract of *Terminalia catappa* L. has been done mainly based on the pharmacognostic methods. Characterization of major groups of plant compounds was carried out employing University of Yangon, Botany Department Laboratory. The investigation revealed the presence of glycosides, phenols,α-amino acid, saponin, tannin, flavonoid, steroid, terpenoid, reducing sugar and starch.

Keyword: *Terminalia catappa* L. Pollen Morphology and Phytochemical characterization.

Introduction

Terminalia catappa L. belong to the family Combretaceae. It is also known as Tropical almond is a large tree growing upto the height of about 10m with an upright closely spiral and symmetrical tiers of horizontal branches, thus named “umbrella tree”. It is widely cultivated as ornamental and shade - tree.

This Combretaceous plant can be recognized by its simple, entire, stipulate leaves which are arranged alternate or opposite to each other. Bractate, sessile flowers are of spike inflorescence. Drupaceous fruits are often winged. To establish the validity and identity of *Terminalia catappa* L. the systematic treatment is carried out according to APG IV classification system (Byng J.W & M.W. Chase, 2016).

The significance of pollen attributes in taxonomy has been widely realized. Recent palynological data are finding increasing application in construction of diagnoses of unknown taxa. Diagnoses based on pollen features have been found in agreement with those prepared on the basis of anatomical characters and data from other displines of Botany (Pritishukla, 1997).

In identifying *Terminalia catappa* L. both macro - morphology (external characters) and pollen morphology have been taken into consideration. The characterization of the chemical constituents of leaf extract was carried out according to pharmacognostic basis.

Leaf extracts have potentials in treatment as anti-oxidant, anti - cancer, anti - diabetic, anti - septic, cardiotonic and anti- inflammatory effects. It is also notable in assisting in wound healing. It is obvious that the chemical principles isolated from plants are compounded as drugs, and health supplements.

Asian nation such as Ayurveda, Indian traditional medicine, and traditional Chinese medicine have been extensively researched, standardized and are properly regulated. In constract, some other herbal medicinal practices do not have much achieved high commercial value due to lack of standardization and poor regulation for plant products. To enhance its worldwide

[1] Dr, Associate professor, Department of Botany, Kyaing Tong University.
[2.] Lecturer, Department of Botany, Kyaing Tong University.
[3.] Demonstrator, Department of Botany, Kyaing Tong University.

acceptance, there is a need of identity and authenticity of the source plants concerned. The chemical characterization of the bioactive principles of leaf extract is also important.

This study is an attempt to ascertain the identity and the systematic of the source plant as *Terminalia catappa* L., to determine the phytochemical constituents, present in leaf extracts of *Terminalia catappa* L. and to provide a phytochemical characterization which may suggest a certain evidence for its ethno-medicinal use.

Materials and Methods

1. Plant material

The specimen upon which the present study is based were collected from plants mainly located at *Yan Law* tract, Kyaing Tong Township. The specimens were collected during the June to December in 2019 at the period of anthesis and fructification. Natural habit and vegetative and reproductive parts of the plants are documented by photography method. The plant materials were identified and specimens were mounted on herbarium sheets and were deposited in the Department of Botany, University of Kyaing Tong.

2. Collection of pollen samples

For pollen samples anthers were freshly collected from the mature flower buds or from partially opened flower to avoid contaminations. Collected pollen of each species was stored in small glass vials with 1 cc of glacial acetic acid and labeled.

3. Preliminary Phytochemical Examination of leaves of *Terminalia catappa* L.

Detection of the major Phytocomponents present in leaf extracts of *Terminalia catappa* L. employed the Department of Botany University of Yangon. The reagents and chemical used of this study are of analytical grade and are all provided by the Department of Botany (Yangon). The operational procedure for detection of leaf extracts were based on the methods described in British pharmacopoeia (1965) and those stated by Harbone (1973). Marini - Bettelo (1981) and Trease & Evans (2002).

The fresh leaves are thoroughly washed under tap water and finally will distilled water. Then they are air- dried for three weeks. Dried leaves were then milled to powder by grinding machine.

Using the corresponding solvent like that of 1% HCl, ethanol, methanol, hydro-chloric acid, pet-ether and water; the individual extracts was then subjected to qualitative analysis of Phyto-components.

In this study altogether thirteen major groups of Phytochemicals were characterized as shown in the Table.

1. Test for Alkaloid

The powdered sample 3g was boiled with 1% HCl 50 ml for about 20 minutes and filtered. The filtrate was divided into four portions and tested with modified Mayer's reagent, Wagner's reagent, Dragendroff's reagent and Hager's reagent. Treatment with the above-mentioned alkaloid reagents furnished turbid or brown precipitates, indicating the presence of alkaloid in the plant materials.

2. Test for Carbohydrates

The powdered sample 1g was boiled with 10 ml of distilled water and filtered. The filtrate was introduced into a test tube and a few drops of 10% α-napthol was added and shaken. The test tube was then inclined at an angle of 45° and concentrated sulphuric acid was added slowly along the side of test tube. A red ring was formed between the two layers, showing the presence of carbohydrates.

3. Test for Glycosides

The powdered sample 1g was boiled with 10 ml of distilled water for about 10 minutes, allowed to cool and filtered. The filtrate was treated with 10% lead acetate solution. White precipitate took place on addition of the reagent.

4. Test for Phenolic Compound

The powdered sample 1g was boiled with 10 ml of distilled water for about 20 minutes and filtered. The filtrate was treated with 5% ferric chloride solution. Greenish brown pracipitates appeared showing the present of phenols.

5. Test for α-Amino Acid

The powdered sample 1g was boiled with 10 ml of distilled water for about 10 minutes and then filtered. An aliquot portion of filtrate was transferred to a filter paper with the help of the micropipette and allowed to dry. Then this filter paper was sprayed with ninhydrin reagent and allowed to dry at 110°C in an oven for a few minutes. The filter paper turned violet color spot.

6. Test for Saponin

The powdered sample 1g was put into a test tube and 10 ml of distilled water was added. Then the mixture was vigorously shaken for a few minutes. Frothings or persistent foams took place.

7. Test for Tannin

The powdered sample 2g was boiled with 20 ml of distilled water about 20 minutes and then allowed to cool and filtered. The filtrate was treated with a few drops of 1% gelatin with introduced and 10% NaCl solution. Formation of green or yellowish green color precipitate indicates the presence of tannins.

8. Test for Flavonoid

The aqueous extract residue of powdered sample was dissolved 70% ethanol. The alcoholic solution was then treated with 0.5g of magnesium turning and few drops of concentrated hydrochloric acid. Red coloration developed within three minutes.

9. Test for Steroid

Three gm of dried powdered samples were refluxed with benzene and the solvent was removed by distillation under reduced pressure. Acetic anhydride 3 dropswas added and the mixture was shaken. Then a few drops of concentrated hydrochloric acid were carefully added and shaken. The solution turned to blue color.

10. Test for Terpenoid

A few gm of petroleum ether extract was dissolved in 15 ml of chloroform. The chloroform extract and 0.3 ml of acetic anhydride were noted after the addition of a few drops of concentrated sulphuric acid. Formation of pink color indicates the presence of terpenoids.

11. Test for Reducing Sugar

The powdered sample 1g was boiled with 10 ml of distilled water and filtered. When the resulting solution was treated with Fehling's solution, it furnished brick red precipitates; indication and presence of reducing sugars.

12. Test for Starch

The powdered sample 2g was boiled with 20 ml of distilled water for about 10 minutes and filtered, 2 drops of iodine solution added to filtrate. Bluish black precipitates were observed.

13. Test for Cyanogenic Glycoside

The powdered sample 1g was mixed with distilled water in a conical flask. About 5 drops of concentrated sulphuric acid was added and sodium picrate paper trapped in a neck by means of a cork. The resulting mixture was heated by means of spirit burner. Sodium pictrate paper turned brick red.

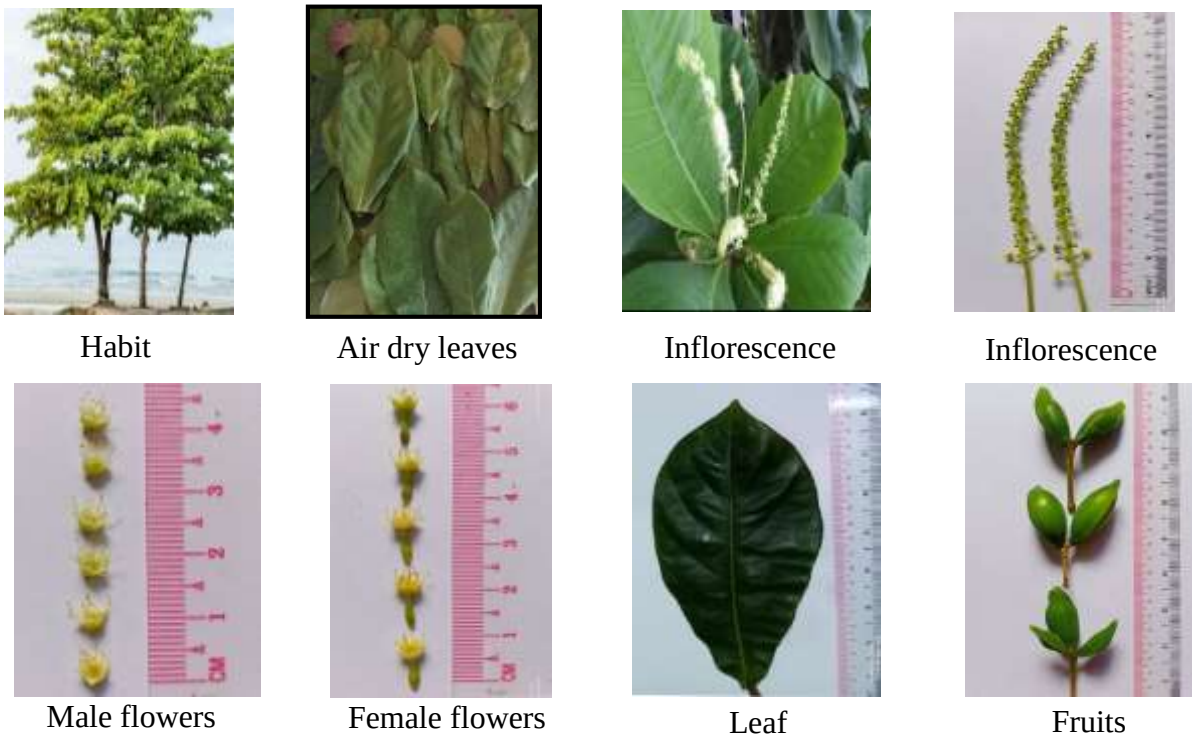
Results

1. Morphological characters of *Terminalia catappa* L. Syst, Na, 2: 674 (err.638). 1767, Clarke in Hook.f.,Fl.Br.Ind. 2: 444, 1878;Philcox Dassanayake, Fl. Cey.9:39.1995

Group	- Eudicots
Superorder	- Rosids
Order	- Myrtales
Family name	- Combretaceae
Vernacular name	- *Banda Pin*
Local name (Shan name)	-Twme – taung - tune
Flowering period	- August to October
English name	- India almond, Tropical almond, Singapore almond, Umbrella tree,
Locality	- *Yan –Law* Tract, Kyaing Tong

A large deciduous tree, up to 10 m high; stems and branches terete, densely yellow-brown sericeous- pubescent, glabrescent. Leaves simple, alternate, clustered at the ends of branches, exstipulate; petiole 5 – 12 mm long; blades obovate or elliptic- ovate, 13-22 cm by 7-12 cm subcordate at the base, entire along the margin, rounded or shortly acuminate apex, sessile gland on each side of the midrib, glossy when mature, finely verrucose on both surfaces. Inflorescence axillary, long spikes; peduncles 5-14 cm long. Flowers creamy- white, 5-6 mm in diameter at anthesis, bisexual in the lower portions, of upper ones many staminate, apetalous. Calyx 5- lobed, broadly deltoid, 2 mm by 1 mm, reflexed at maturity. Corolla (Petals) absent. Stamen 10 in 2 rows, adnate on the calyx tube, exserted; filaments filiform, 2-3 mm long, glabrous; anthers dithecous. Disk densely whitish- barbate. Ovary inferior, ellipsoid, 2 mm long, angular, glabrous, unilocular, with one ovule on the pendulous placenta; style filiform, 2 mm long, glabrous; stigma simple.

Fruits drupaceous, indehiscent, ellipsoid or ovoid, 3.5-5.5 cm by 2.5 cm, laterally more or less compressed, glabrous, ringed by a rigid wing or often without wing, green to yellow and red at maturity; stone surrounded by a thick layer of juicy flesh.



Habit | Air dry leaves | Inflorescence | Inflorescence

Male flowers | Female flowers | Leaf | Fruits

Figure 1 Morphology characters of *Terminalia catappa* L.

Pollen morphology

External morphology of pollen grains has been studied under the light microscope and was characterized as follows:

Tri- colporate with prominent psdeudo - colpi: isopolar; radiosymmetric; small size; equatorial view: prolate-spheroidal to sub- prolate; polar view: circular; colpi linear with acute ends and broad at middle; pseudo - colpi almost the size of colpi, fused at apocolpia; oralalongate; exine sculpture: microregulate.

Polar View | Equatorial view

Figure 2 Pollen morphology of *T. catappa* L.

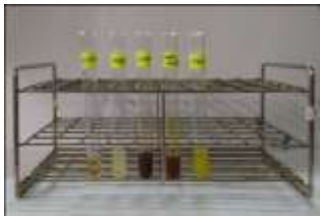
2. Preliminary Photochemical examination of leaves of *Terminalia catappa* L.

Preliminary phytochemical tests of the leaves of *Terminalia catappa* L. indicated the presence of alkaloids, carbohydrate, glycoside, phenolic compound, α-amino acid, saponin, tannin, flavonoid, steroid, terpenoid, reducing sugar and starch are present in the leaves and the test have shown that cyanogenic glycoside was absent found in *T. catappa* L. The experimental result was shown in table (1) and figure (3).

Table showing the detection of major phytochemical in leaf extracts of *T erminalia catappa* L.

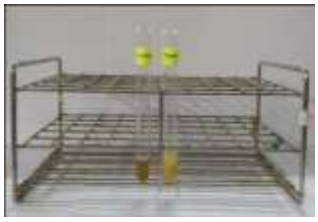
No.	Type of compound	Extract	Reagent used	Observation	Results
1.	Alkaloid	1%HCL	Mayer's reagent	Cream colour (turbid)	+
			Wagner's reagent	Brown ppt.	
			Dragendorff 's reagent	Brown ppt.	
			Hager's reagent	Yellow colour (turbid)	
2.	Carbohydrate	H_2O	10% α –naphthol & H_2SO_4(Conc:)	Red ring	+
3.	Glycoside	H_2O	10% Lead acetate solution	White ppt.	+
4.	Phenol	H_2O	5% $FeCl_3$ solution	Brownish green ppt.	+
5.	α-amino acid	H_2O	Ninhydrin reagent	Light purple colour	+
6.	Saponin	H_2O	H_2O	Persistent foam	+
7.	Tannin	H_2O	1% Gelatin & 10% NaCl solution	No ppt.	+
8.	Flavonoid	70%EtOH	Mg ribbon & Conc; HCl	Pink colour.	+
9.	Steroid	Petroleum ether	Acetic anhydrite & Conc; H_2SO_4	Bluish green	+
10.	Terpenoid	Petroleum ether	Acetic anhydrite & Conc; H_2SO_4	Pink colour.	+
11.	Reducing sugar	H_2O	Fehling's solution	Brick red ppt.	+
12.	Starch	H_2O	Iodine solution	Brown ppt.	+
13.	Cyanogenic Glycoside	Powder	H_2O,Conc; H_2SO_4, sodium picrate paper	No colour change	-

(+) = presence (-) = absence



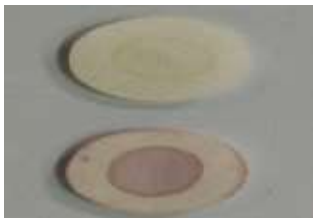
Test for Alkaloid

Test for Carbohydrate



Test for Glycoside

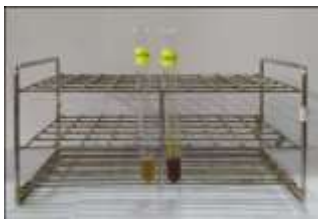
Test for Phenol



Test α- amino acid

Test for Saponin

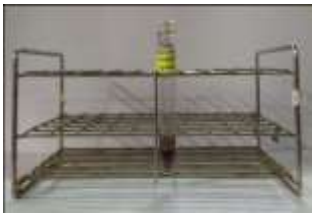
Test for Tannin



Test for Flavonoid

Test for Steroid &

Test for Reducing



Test for Starch

Test for Cyanogenic

Figure 3 Phytochemical test of leaves from the *Terminalia catappa* L.

Discussion and Conclusion

Terminalia catappa L. (Benda pin) collected from the Tract of Yan Law, Kyaing Tong District, Eastern of Shan State. In this study verification of nomenclature and systematic treatment of *Terminalia catappa* L. was done following the flora of British India (Hooker, 1881-87), Flora of Java (Backer, 1963), A Checklist of the Trees, Herbs and Shrubs of Myanmar, Kress.J. *etal*, 2003.

Distinguishing characters in delimiting *T.catappa* L. *are* trees up to 10m of height and large deciduous tree; Barks brownish black with longitudinal peelings without ridges; Young stems and leaves not hairy; Leaves simple, large, obavate, distichous turn red in senescence; inflorescence axillary, long spikes; flowers creamy - white, bisexual in the lower portions, of upper ones many staminate; petioles with 2 glands at the summit; stamen 10 in 2 rows, adnate on the calyx tube, exserted; filaments filiform; anthers dithecous; ovary inferior, ellipsoid, angular, glabrous, unilocular, with one ovule on the pendulous placenta; style filiform, glabrous; stigma simple; Fruits drupaceous, indehiscent, ellipsoid or oval, rounded to flattened, green red, two - ridged or two - winged.

Pollen morphological characters are used as a taxonomic device for identification of *T. catappa* L. Outstanding features of pollen grains are Tri- colporate with prominent psdeudo - colpi: isopolar; radiosymmetric; small size; equatorial view with prolate - spheroidal to sub - prolate; polar view with circular; colpi linear with acute ends and broad at middle; pseudo - colpi almost the size of colpi, fused at apocolpia; ora lalongate; exine sculpture with microregulate.

In this study isolation of leaf extracts showed the presence of twelve major components, viz: Alkaloid, Carbohydrate, Glycoside, Phenol, α-amino acid, Saponin, Tannin, Flavonoid, Steroid, Terpenoid, Reducing sugar, starch and Cyanogenic glycoside.

Four different kinds of solvents are used to isolate plant constituents from leaf extract as shown in the table. Water soluble extracts have the ability to isolate Carbohydrate, Glycoside, Phenol, α-amino acid, Saponin, Tannin, Reducing sugar and starch. Pet -ether extract revealed Steroid and Terpenoid; whereas 1% HCl extract shows the present of Alkaloids, while in 70% Ethenol flavonoids are present.

For global recognition of traditional medicinal practice extensive research standardization of plant product is required. While the knowledge of the therapeutic properties of plant extract was orally transmitted from mouth to mouth, identity and authenticity of source plants concerned are needed. The chemical characterization of the bioactive principles of plant extracts is also important.

Further investigation is needed for quantitative analysis of active phytocomponents of leaf extracts to determine their molecular formular, molecular weight, and relative amount in percentage in association with their usage in ethno- medicine.

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