RESEARCH ARTICLE

Comparative study of young and mature leaves of *Terminalia catappa* for evaluation of Physico-chemical, Pharmacognostical and Phytochemical analysis

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Abstract

In recent years there has been rapid increase in the standardization of selected medicinal plant of potential therapeutic significance. Despite the modern techniques, identification of plant drug by pharmacognostic study is more reliable. *Terminalia catappa* belongs to family Combretaceae, commonly known as Indian Almond, native to most regions of the India. By looking at the high traditional use of the young and mature leaves of plant *Terminalia catappa* Linn, the present investigation was undertaken for research with the purpose of drawing the pharmacopoeial standards for this species. The present study deals with pharmacognostical parameters for the young and mature leaves of *Terminalia catappa* which mainly consists of macroscopic and microscopic characters, powder characteristics, physio-chemical constants and phytochemical screening. The macroscopic, microscopic characteristics of Almond leaves were identified. In physico-chemical parameters, foreign matters, total moisture content, pH, different ash contents [total ash, acid insoluble ash, water soluble ash, sulphated ash] and extractive values [alcohol soluble and water soluble] were studied. In qualitative phytochemical analysis, it was observed that the mature leaves of *Terminalia catappa* contain more phytoconstituents as compared to young leaves. The study revealed specific characteristics for the particular crude drug which will be of significant use in identification, control to adulterations of raw drug and can serve as a reference for any further investigations.

Keywords: *Terminalia catappa*, Physicochemical study, Pharmacognostic study, Phytochemical screening.

INTRODUCTION

Medicinal plants are plants that have at least one of their parts (leaves, stem, barks or roots) used for therapeutic purposes (Bruneton, 1993). Recently, medicinal plants have become important for the treatment of different disease conditions such as diabetes, malaria, anaemia (Fola, 1993). The availability and relatively cheaper cost of medicinal plants in sub-Saharan Africa, makes them more attractive as therapeutic agents when compared to 'modern' medicines (Agbor *et al.*, 2005). The importance of medicinal plants, and the contribution of phytomedicines to the well-being of a significant number of the world's population, has attracted interest from a variety of disciplines. It is no wonder that the world's one-fourth population i.e. 1.42 billion people, are dependent on traditional medicines for the treatment of various ailments. However a key obstacle, which has hindered the acceptance of the alternative medicines in the developed countries, is the lack of documentation and stringent quality control (Jena *et al.*, 2011). There is a need for documentation of research work carried out on traditional medicines. With this backdrop, it becomes extremely important to make an effort towards standardization of the plant material to be used as a medicine. The process of standardization can be achieved by stepwise pharmacognostic and phytochemical studies. These studies help in identification and standardization of the plant material. Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy. *Terminalia catappa* Linn. (Indian almond) commonly known as Laal Badam (Red almond), belonging to Combretaceae family. It grows best in moist tropical climates. The species loses its leaves twice a year in most areas, with a brilliant red-and-yellow display of leaf-fall. Although Indian almond does grow when planted on uplands, the natural habitat of the species is in areas just inland from ocean beaches, near river mouths and on coastal plains. These areas are typically flat, but they may have dunes or rocky bluffs (Orwa *et al.*, 2009). In the traditional Indian system of medicine, the ayurveda and various folk system of medicine, *Terminalia catappa* possess several medicinal properties. It is very rich in phytochemicals and a good source of natural antioxidants (Punniya *et al.*, 2014). Parts of the tree, such as the leaves and fruit, are astringent. The leaves, crushed with *Dacrydium elatum* and rhizomes of *Cyperus rotundus*, are combined to treat dysentery. The red leaves act as a vermifuge, while the sap of young leaves, cooked with oil from the kernel, is used to treat leprosy. Leaves, bark and fruit are used to treat yaws. The bark and root bark are useful for bilious fever, diarrhoea, thrush, and as a remedy for sores and abscesses. The kernel of the fruit mixed with beeswax stops putrid exudation and bloody faeces. It is recommended as a mild laxative and a galactagogue for women, but too frequent use causes diarrhoea. The young leaves are used to cure headaches and colic (Arumugam *et al.*, 2015). In the present work pharmacogno- stical, physicochemical and phytochemical characteristics were studied on young and mature leaves of *Terminalia catappa*.

MATERIAL AND METHODS

***Material*:** Young (Green) and mature (red) leaves of *Terminalia catappa* (Indian almond) were collected in the month of September 2014 from Thakurli near Kalyan of Maharashtra, India. The plant was identified and authenticated from the Blatter Herbarium, St. Xavier's College, Mumbai. All chemicals used in assays were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and Merck Co. (Santa Ana, CA, USA).

Preparation of the extracts and fractions:

The air-dried powdered young (green) and mature (red) leaves of *T. catappa* (5 g) were extracted with n-butanol, chloroform, acetone, petroleum ether and water using a mechanical shaker for 12-18h. The resultant extracts were concentrated using sonicator. Then the crude n-

butanol, chloroform, acetone, petroleum ether and aqueous extracts were filtered and fractions were used for further analysis.

***Macroscopic Description*:** The *Terminalia catappa* was subjected to macroscopic studies which comprised of organoleptic characteristics *viz*. color, odour, taste, size, etc. of the drug. These parameters are considered as useful in quality control of the crude drug and were evaluated as per standard WHO guidelines (Khandelwal, 2006; Kokate, 1997; Wallis, 2005).

***Microscopic Description*:** Free hand transverse sections of the leaves were taken, stained with safranin and fast green. Then observed under compound microscope (Lawrence & Mayo–LM–52-1602) and Phase contrast microscope (Lawrence & Mayo–LM–52-1802) for their peculiar characters.

***Powder characteristics*:** Preliminary examination of the young and mature leaf powder with different chemical reagents and microscopical observation was carried out as per reported methods (Iyengar and Nayak, 2008; Iyengar 1997). The powder characteristics were observed using compound and Phase contrast microscope.

***Physicochemical Evaluation*:** Analysis of physicochemical constants of the young and mature leaf powder has been done to evaluate the quality and purity of the drug. Various physic-chemical parameters like moisture contents, foreign organic matters, pH, ash values and extractive values were calculated as per WHO guidelines. The information collected from these test will be useful for standardization and obtaining the quality standards (Indian Pharmacopoeia, 1996; World Health Organization 1998).

***Preliminary phytochemical analysis*:**
The qualitative chemical tests were carried out for the identification of the different phytoconstituents present in the powered crude drug. (Saxena *et al.*, 2012). Presence of Alkaloids, Flavonoids, Cardiac glycosides, Anthronal glycosides, Phenols, Saponins, Sterols, Triterpenoids, Tannins, Hydrolysable Tannins, Carbohydrate, Starch and Proteins were tested by using standard qualitative methods.

RESULTS AND DISCUSSION

Macroscopic description

Terminalia catappa is a tall deciduous and erect tree reaching 15-25 m, trunk 1-1.5 m in diameter, often buttressed at the base. Whorls of nearly horizontal, slightly ascending branches spaced 1-2 m apart in tiers or storeys are present up the trunk. The pagoda-like habit becomes less noticeable as the branches elongate and droop at the tips. Bark grey-brown, rough with age. The leaves of *Terminalia catappa are* alternate obovate with short petioles, spirally clustered at the branch tips, 15-36 cm long, 8-24 cm wide, dark green above, paler beneath, leathery and glossy. They turn bright scarlet, dark red, dark purplish-red, or yellow (Fig. 1 and Table 1).

The organoleptic evaluation of the young and mature leaves of *T. catappa* powder revealed that young leaves powder was grayish green in color with mild pleasant aromatic odour, agriable taste whereas mature leaves powder was brownish red colour, having mild earthy odour and was tasteless (Table 2).

Microscopic Description

The T.S. of midrib (Fig. 3a, 3b, 3c) showed dorsiventral structure and a distinct biconvex out line in the basal region where as in the apical region it is Plano convex. The T.S. showed single layered epidermis covered with thick cuticle. Epidermal cells of the ventral side and dorsal side were rectangular in shape with distinct thickening on radial walls. Some of the epidermal cells on the ventral sides elongated to form covering of pointed trichomes. Beneath the epidermal cells on either side the layers of collenchymatous cells were present however towards ventral side they are wider with 3–4

layers of cells. Followed by collenchyma cells, the 6–7 layers of parenchymatous cells with angular thickening were observed (Fig. 2a). Some of the parenchyma cells showed presence of rosette of calcium oxalate crystal (Fig. 4b). Arc-shaped/ Triangular vascular strand showed layer of xylem surrounded by phloem on either side of xylem. Xylem consisted of metaxylem and protoxylem. Metaxylem showed extended arms of protoxylem on both the sides (Fig. 4a). 3 Air cavities were observed in the ring of vascular strand with presence of giant crystal located in the pith region (Fig. 4c). Hairs were extended from the epidermal layer of cells (Fig. 4d). In mature leaves a prominent layer of lignified cells was observed in the apical region of the midrib surrounding the vascular tissue (Fig. 2, 3b).

Table 1: Characters of leaf of *Terminalia catappa*

Sr. No.	Characteristic features	Description
1	Arrangement	Alternate
2	Type	Simple
3	Margin	Entire
4	Shape	Obovate
5	Length	8-12 Inches
6	Colour	Green
7	Fall colour	Red
8	Fall characteristic	Showy

Table 2: Organoleptic characters of *Terminalia catappa.*

Sr. No.	Characters	Observation	
		Young leaves	Mature leaves
1	Colour	Grayish green	Brownish red
2	Odour	Mild pleasant aromatic	Mild earthy
3	Taste	Agriable	Tasteless
4	Size	Course	Medium
5	Miscellaneous	Cellulosic fibers	Fibers

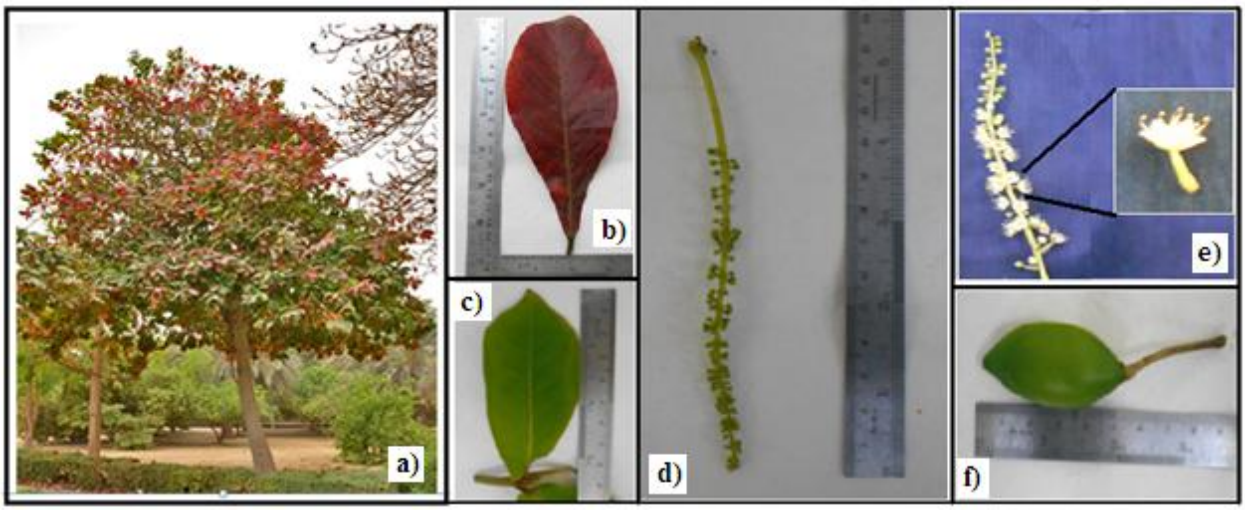

Fig. 1: Macroscopic description – a) *Terminalia catappa* Tree; b) Mature leaf of *T. catappa;* c) Young leaf of *T. catappa;* d) Influorensence; e) Flower of *T. catappa;* f) Fruit of *T. catappa.*

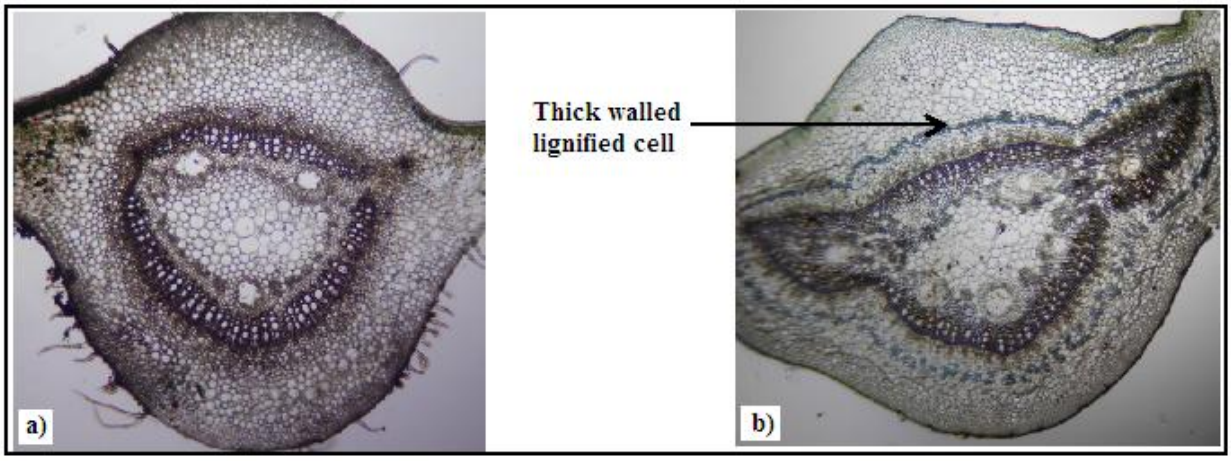

Fig. 2: (a) T.S. of young leaf *Terminalia catappa* through midrib at 4X (b) T.S. of mature leaf *Terminalia catappa* through midrib at 4X

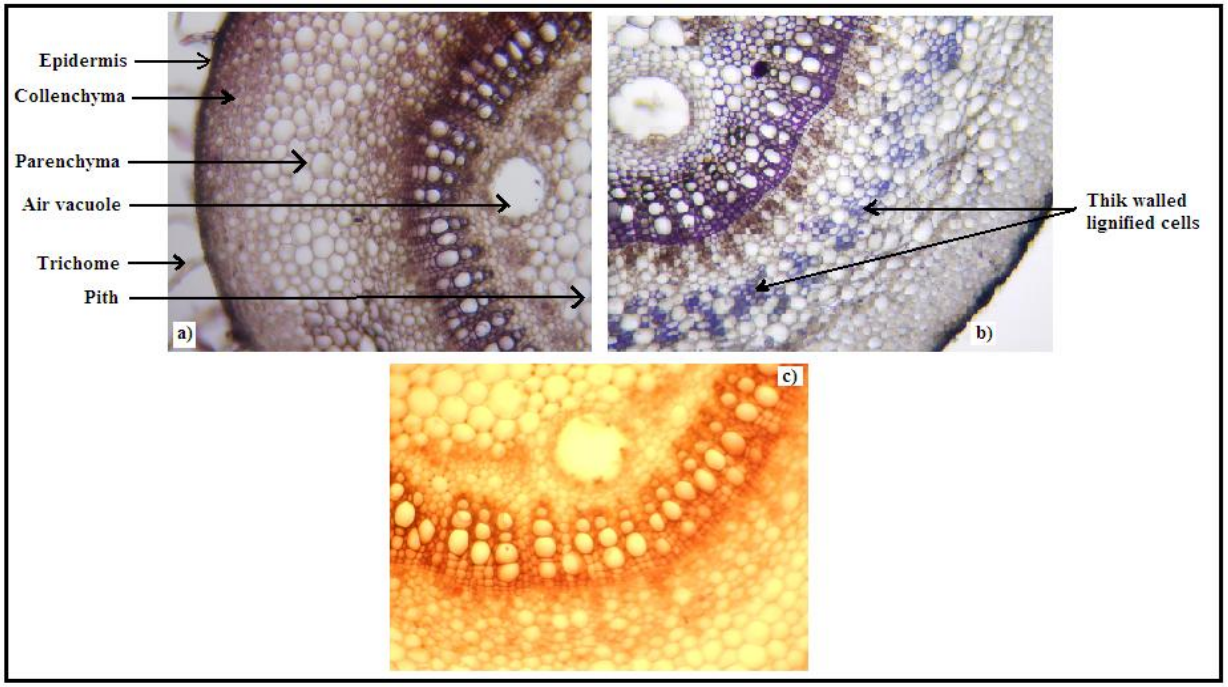

Fig. 3: (a) T.S. of leaf through midrib at 10X, (b) T.S. midrib of mature leaf showing thick walled lignified cells at 10X (c) T.S. of midrib under phase contrast microscope at 10X

Table 3: Ash values for young and mature leaves of *Terminalia catappa.*

Parameters	Leaves of *Terminalia catappa*	
	Young	Mature
Foreign matter (% w/w)	1.23% ± 0.001	1% ± 0.001
Total Moisture Content (% w/w)	5.71% ±0.002	4.32% ± 0.002
Total Ash (% w/w)	8.89% ±0.004	8.60% ± 0.004
Acid Insoluble Ash (% w/w)	4% ± 0.002	1.71% ± 0.001
Water soluble Ash (% w/w)	4.71% ± 0.002	2.51% ± 0.001
Sulfated Ash (% w/w)	9.80% ± 0.001	10.40% ± 0.003
pH (5%)	3.0 - 5.5	3.5 - 5.5

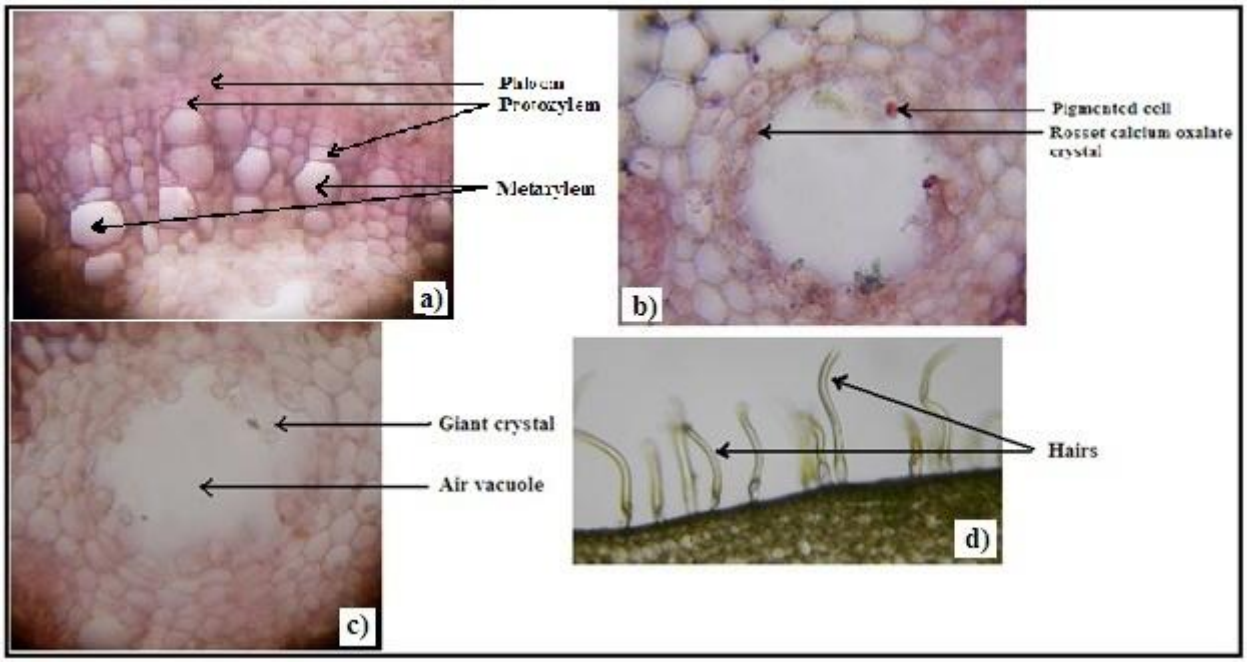

Fig. 4: (a) T.S. of leaf midrib at 40X showing xylem and phloem (b) T.S. of midrib showing pigmented cell and rosset calcium oxalate crystal at 40X (c) T.S. of midrib showing giant crystal (d) T.S of midrib showing hairs at 40X

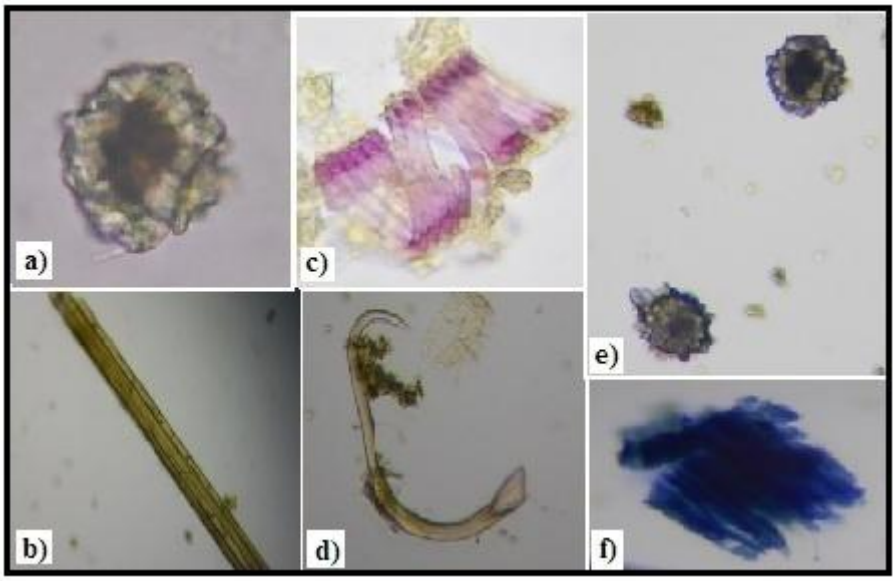

Fig. 5: Microscopy of leaf powder a) rosette calcium oxalate crystals (40X) b) phloem fibres (10X), c) xylem vessel (10X), d) unicellular trichome (10X) e) rosette calcium oxalate crystals (10X), f) cellulose (40X).

Table 4: Extractive values for young and mature leaves of *Terminalia catappa.*

Parameters	Leaves of *Terminalia catappa*	
	Young	**Mature**
Water Extractive Value (**% w/w**)	7.55% ± 0.002	10.45% ± 0.001
Alcohol Extractive Value (**% w/w**)	10.27% ± 0.002	11.97% ± 0.002

Table 5: Phytochemical screening of young and mature leaves of *Terminalia catappa.*

Solvents	n-butanol		Chloroform		Acetone		P. Ether		Aqueous	
Phytoconstituents	**TG**	**TR**	**TG**	**TR**	**TG**	**TR**	**TG**	**TR**	**TG**	**TR**
Alkaloids	-	-	-	-	+	+	-	+	-	+
Flavonoids	-	+	-	-	+	+	-	-	-	+
Cardiac glycosides	+	+	+	+	-	-	+	+	-	-
Anthronal glycosides	+	+	+	+	+	+	+	+	+	+
Phenols	+	+	-	-	-	+	-	-	+	+
Saponins	-	-	-	-	-	-	-	-	-	-
Sterols	+	+	+	-	+	-	+	-	-	-
Triterpenoids	+	+	-	+	-	+	-	+	+	+
Tannins	-	+	-	-	-	+	+	+	-	+
Hydrolysable tannins	-	+	-	-	+	+	-	-	-	+
Carbohydrates	+	+	+	+	+	+	+	+	+	+
Starch	-	-	-	-	-	-	-	-	-	-
Proteins	-	-	-	-	-	-	-	-	-	-

Key : TG: Young green leaves of *T. catappa*, **TR:** Mature red leaves of *T. catappa* **P. Ether**: Petroleum Ether ; " **+**" Detected ; "**–**" Not Detected.

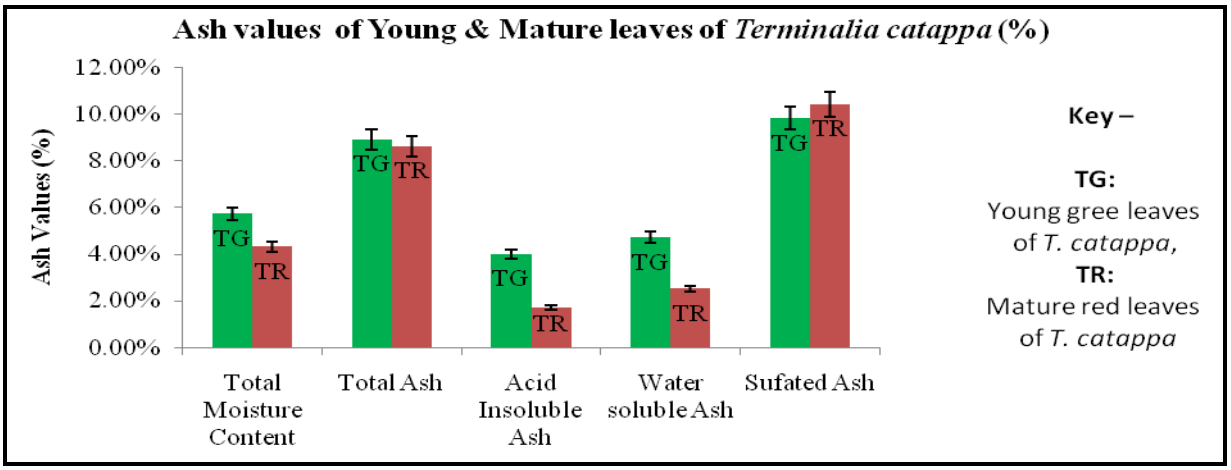

Fig. 6: Graphical representation of mean ash values of young and mature leaves of *Terminalia catappa*

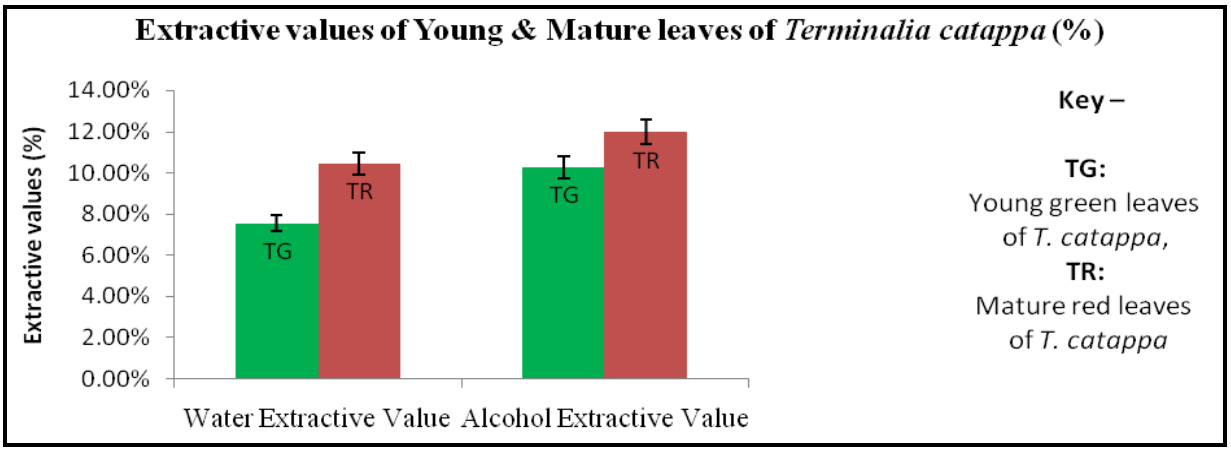

Fig.7: Graphical representation of mean extractive values of young and mature leaves of *Terminalia catappa.*

Powder Characteristics

The powder of young leaves of *Terminalia catappa* was grayish green in color with mild pleasant aromatic odour, agriable taste whereas mature leaf powder was brownish red in colour, having mild earthy odour and was tasteless. The microscopic examination of the powder of young and mature leaves showed similar characteristic features like presence of rosette calcium oxalate crystals, phloem fibres, xylem vessel, unicellular trichome and cellulose (Fig. 5).

Physicochemical Evaluation:

Ash values of a drug give an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. The foreign organic matter give the presence of any organism, part or product of an organism, other than that named in the specification and description of the herbal material concerned in Indian Pharmacopoeia, 1996 (Mukherjee, 2002). The foreign organic matter was found to be 1.23% ± 0.001 and 1% ± 0.001 for young and mature leaves respectively, it indicated that their may be presence of part or product of an organism in very less amount. The total ash value was higher than that of the acid insoluble and water soluble ash value for both young and mature leaves and a decrease in the acid insoluble ash value may be due to presence of smaller quantity of silicaceous matters. The extractive values are primarily useful for the determination of exhausted or adulterated drug. The water extractive value indicates the presence of sugar, acids and inorganic compound (Indian Pharmacopoeia, 1996, Mukherjee, 2002). The water extractive value was found to be 7.55% ± 0.002 and 10.45% ± 0.001 for young and mature leaves respectively (Table 5). The alcohol extractive value indicates the presence of polar constituents like phenols, alkaloids, steroids, glycosides, flavonoids. (Indian Pharmacopoeia, 1996, World Health Organization, 1998). The alcohol extractive value was found to be 10.27% ± 0.002 and 11.97% ± 0.002 for young and mature leaves respectively (Table 5). This signifies that the large amount of constituents of leaves were soluble in alcohol than water.

Preliminary Phytochemical Screening

The preliminary phytochemical investigations of n-butanol, chloroform, acetone, petroleum ether and aqueous extracts of young and mature leaves of *Terminalia catappa* were performed. The young leaves of *Terminalia catappa* showed the presence of Phenolic compound, Glycosides, Sterols, Saponins, Triterpenes and Carbohydrate type of major secondary metabolites whereas mature leaves of *Terminalia catappa* showed the presence of alkaloids, flavonoids, glycosides, phenolic compounds, triterpenes, tannin and carbohydrate as major secondary metabolites which revealed their potent therapeutic activity. In young leaves of *Terminalia catappa*, n-butanol and acetone extracts showed presence of higher phytochemicals whereas in mature leaves of *Terminalia catappa*, n-butanol extract showed presence of higher phytochemicals. The results showed that, the mature leaves of *T. catappa* contain more amounts of phytochemicals as compared to young leaves, so it can be concluded that with the increasing age of leaves, there is also increase in the amount of phytoconstituents.

CONCLUSION

Standardization is essential measure for quality, purity and sample identification. Macroscopy and microscopy is one of the simplest and cheapest methods to start with for establishing the correct identity of the source materials. Studies of physicochemical constants can serve as a valuable source of information and are usually used in judging the purity and quality of the drug. The extractive values give an idea about the maximum extraction of drug in particular solvent and from the study, for both young and mature leaves, the extractive value of alcohol was highest followed by water. The mature leaves of *Terminalia catappa* showed presence of higher

phytoconstituents than young leaves. The preparations made from the leaves of *Terminalia catappa* are currently being used in several traditional and folklore systems of medicine for the treatment of various diseases without standardization. These findings would help as a tool for characterization of *Terminalia catappa* with its pharmacognostic and physicochemical characteristics, discriminating it from its other species diversity and aid in further screening of young and mature leaves for anti-cancerous activity, fungicidal, anti-diabetic activity and other specific bioassays.

REFERENCES

Agbor AG, Oben JE, Ngogang JY (2005) Haematinic activity of *Hibiscus cannabinus*. *Afr J Biotechnol*.; 4(8):833–837.

Anonymous. Indian Pharmacopoeia (1996) Vol-II, Ministry of Health and Family welfare, Govt of India, New Delhi, Controller of Publications; pp., A - 53 – 54, A-95, A-97, A-109.

Arumugam V, Natarajan D and Pannerselvam PK (2015) An updated review of *Terminalia catappa*. *Pharmacognocy review*; 9(18): 93-98.

Bruneton J (1993) Local medecinal plants and the health of the consumers. A paper delivered at the PSN/CF PCON organization workshop: in Clinical Pharmacy and Herbal Medicine, Plantes Médicinales: *Phytochimie, Pharmacognosie. 2 éme.*New York: Lavoisier p. 914. Fola A. 1993; 9:28–31.

Fola A (1993) Local medicinal plants and the health of the consumers. A paper delivered at the PSN/CF PCON organization workshop: in *Clinical Pharmacy and Herbal Medicine*; 9: 28–31.

Iyengar MA (1997) Pharmacognosy of Powdered Crude Drugs. 5th ed., Manipal, Manipal Press Limited; pp.21-31.

Iyengar MA, Nayak SG (2008) Anatomy of Crude Drugs, 11th ed., Manipal, Manipal Press Limited; pp. 01-08.

Jena A, Saha D, Biswal B, Jana SB, Koley A, Sur D, Battacharya A (2011) *International Journal of Research in Pharmaceutical and Biomedical Science*. 2(1), 2229-3701.

Khandelwal KR (2006) Practical Pharmacognosy Techniques and Experiments. 15th ed., Pune, Nirali Prakashan; pp. 15–163.

Kokate CK (1997) Practical Pharmacognosy, 4th ed., Delhi, Vallabh Prakashan,; pp.107 -111.

Mukherjee PK (2002) Quality Control of Herbal Drugs, 1st ed., Business horizon publications; pp. 186.

Orwa C, Mutua A, Kindt R., Jamnadass R., S Anthony (2009) Agroforestree Database: a tree reference and selection guide version 4.0.

Punniya Kotti P and Vijaya A (2014) Phytochemical analysis and *in-vitro* antioxidant activity of *Terminalia catappa*; *World Journal of Pharmaceutical Science.*

Saxena M (2012) Phytochemical screening of *Acorus calamus* and *Lantana camara*; *International journal of pharmacy*: ISSN 2230 – 8407.

Wallis TE (2005). Text Book of Pharmacognosy. 5th ed., Delhi, CBS publishers and Distributors; pp. 104 – 158.

World Health Organization (1998). Quality control methods for medicinal plant materials, WHO/PHARM/92.559; pp. 4-46.