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Ambikadevi D. Sharma
Department of Botany, R. J. College, Ghatkopar (west), Mumbai - 400086.

Usha Mukundan
Department of Biological Sciences, Ramniranjan Jhunjhunwala College, Ghatkopar, Mumbai.
Email: umukundan@hotmail.com

Correspondence:
Usha Mukundan
Department of Biological Sciences,
Ramniranjan Jhunjhunwala College,
Ghatkopar, Mumbai.
Email: umukundan@hotmail.com
Tel: +91- 9869003389

Pharmacognostic evaluation and metal analysis of *Terminalia Catappa* Linn. leaves

Ambikadevi D. Sharma and Usha Mukundan

ABSTRACT

Objective: The safety and quality of herbal medicines have become increasingly important for health authorities, the scientific community and the public alike. Natural products have provided important therapeutic use in several areas of medicine. Leaves of *Terminalia catappa* Linn. were reported to possess good anti-diabetic activity. This study deals with the detection of metals present in *Terminalia catappa* Linn. by Inductively Coupled Plasma Atomic Emission Spectroscopy instrument. The leaves were examined for macroscopic, microscopic, proximate and heavy metal analysis. Metals like Mercury, Arsenic, Lead, Iron, magnesium and Zinc were analyzed using Inductively Coupled Plasma Atomic Emission Spectroscopy. Presence of rosettes of calcium oxalate crystal, unicellular trichomes and anomocytic stomata and spiral xylem vessels confirmed through microscopic analysis of leaves were the discerning anatomical features and can be used as anatomical markers. The essential metals like Iron, Magnesium and Zinc were found to be predominant while the heavy metals like Mercury, Arsenic and Lead were found to be within the permissible limit. It has been clinically proved that the essential metals e.g. Magnesium and zinc were used in the treatment of type II diabetes. The present study supports anti-diabetic activity of this species. Also, the pharmacognostic constants presented in this paper may help to establish the authenticity of the drug, differentiate the drug from other species and drawing the pharmacopoeial standards for this species.

Keywords: *Terminalia catappa* Linn., Microscopical, Preliminary phytochemical analysis, Metal analysis

1. Introduction

Terminalia catappa Linn. (Combretaceae) is found in the warmer parts of India. The leaves, trunk bark and fruits have been used in folk medicine for the treatment of dermatitis, and for antipyretic and homeostatic purposes. *Terminalia catappa* Linn leaves have the ability to reduce blood sugar level in alloxan induced diabetic rats. Researchers have carefully studied the use of nutritional supplements in the treatment of diabetes such as vitamins C and E, dietary minerals and herbs can safely lower the blood sugar and help to prevent the diabetic complications [2]. Also, type 2 diabetes is closely associated with obesity [3, 4]. The various extracts of leaves and bark of the plant have been reported to be anticancer, antioxidant, hepatoprotective, anti-inflammatory and aphrodisiac. *Terminalia catappa* Linn. leaves has shown the presence of phenols, triterpenoids, flavonoids, alkaloids and saponins [5].

Proximate analysis in plants gives valuable information and help to access the quality of the sample. It provide information on moisture content, ash content, volatile matter content, ash, fixed carbon etc. Ash is the inorganic residue remaining after water and organic matter have been removed by heating, which provides a measure of total amount of minerals within the drug. Minerals are not destroyed by heating and they have a low volatility as compared to other food components. Total ash may vary with in wide limits for specimen of genuine drugs due to variable natural or physiological ash. Ashes give us an idea of the mineral matter contained in a plant. Measuring it is important, because mineral matter may be the cause of a pharmacological effect [6, 7]. So the present studies were done on leaves of *Terminalia catappa* Linn.

The Microscopy and proximate analysis was conducted for the drug respectively. Heavy metal uptake by the plants is a main pathway of metal transfer from sediments and water to the food web. The metal uptake by the plant is determined by metal mobility and bioavailability. Most of the procedures adopted involve dry ashing or a wet digestion using nitric acid alone, or in combination with perchloric acid or hydrogen peroxide. This study adopts the procedure of overnight wet digestion with nitric acid alone for the determination of heavy metals. Plants can accumulate metal in their parts and transfers it from soils into the food chain.

This accumulation is one of the most serious environmental concerns because of the potential harmful effects that toxic metals could have on animals and human health Some metals like zinc, iron, copper, chromium and cobalt are toxic only at higher concentrations, while others like lead, mercury and cadmium are exclusively toxic. In most plants, contents of Lead, Zinc Iron and Magnesium are observed to decrease in particular parts of a plant in the following order: root–leaves–shoots–fruits and seeds, since metals absorbed by a plant from soil first face a root barrier that impairs their penetration into aerial parts of the plant. As, medicinal herbs are easily contaminated during growth, development and processing while using such herbs in medicinal treatment of various illnesses, one should be aware that apart from the pharmacological effect they could turn out to be toxic because of the presence of heavy metals and other impurities beyond the permissible limits [8, 12]. WHO prescribed limit for Arsenic, Mercury and Lead in herbal medicine is 1mg/kg, 0.5 μgg^{-1} and 10 μgg^{-1}/10ppm respectively so as to control level of heavy metal contamination in herbal drug. In case of essential metals like Iron and Zinc the dietary limit in the food is 100 ppm and 10-60 mg/day respectively [13, 14]. It has been clinically proved that the essential metals e.g. Magnesium and Zinc were used in the treatment of type II diabetes [2].

A relationship between Zinc and obesity was first found in obese patients and obese mice (genetically and dietary obese) to that the dietary zinc treatment increased body fat deposition in obese mice. Obese mice carried markedly low zinc levels in most of the peripheral tissues, but retained a great amount of zinc in liver and adipose tissues compared with lean mice. However, the therapeutic effect of zinc on obesity is still a controversial subject [15, 16]. From the literature review, the present work has been designed to detect the metals present in leaves of *Terminalia catappa* Linn. In spite of the numerous medicinal uses attributed to this plant, however, there is no pharmacognostical and metal analysis report on the leaf of the plant to determine the quality control of the crude drug. Hence, the present investigation includes morphological, anatomical, physiochemical analysis and metal (essential and non-essential) analysis of *Terminalia catappa* Linn. leaf.

2. Materials and Methods

2.1. Plant material:

Terminalia catappa Linn. leaves was collected in the month of June from a full grown tree in Mulund region of Maharashtra state, India and The plant was authenticated from Blatter Herbarium, St. Xavier's College, Mumbai under the specimen no. 16063. The matured leaves of *Terminalia catappa* Linn. were identified, collected, dried and crushed into powder by a mixer. This powder was taken for further analysis.

2.2. Chemicals and reagents: All the chemicals and reagents used were of laboratory grade. Microscopic sections were cut by free hand sectioning method. The sections of leaves were cleared with chloral hydrate solution & then stained with phloroglucinol & HCl & mounted in glycerine [17]. Numerous mounts of the microscopical sections of the leaf specimens were made and examined microscopically. Photomicrographs of the microscopical sections were taken with the help of MOTIC photomicroscope provide with MOTIC IMAGE PLUS 2.0 software.

2.3. Physiochemical analysis: Physicochemical properties such as the percentage of total ash, acid insoluble ash, water soluble ash, alcohol soluble extractive & water soluble extractive values were determined as per the standard procedure. Percentage of ash value is indicative of the purity of the drug and extractive values represent the presence of polar and non-polar compounds in the extract [18-20].

2.4. Nitric acid digestion method

One gram of leaf powder was placed in a 250 ml digestion tube and 10 ml of concentrated nitric acid was added. The sample was heated for 15 min at 90 ºC. Till it dissolves. After cooling solution, 5ml of perchloric acid was added and then the temperature was increased to 150 °C. At which the sample was boiled until a clear solution was obtained (if the solution evaporated before becoming colourless, cool the solution and add 5 ml nitric acid and 2.5 ml of per chloric acid). Heat, the solution again and allowed it to cool. Concentrated nitric acid was added to the sample (5 ml was added in three regular intervals of times). Digestion process was continued until the volume was reduced to about 1 ml and clear solution was obtained. The interior walls of the tube were washed down with a little amount of deionised water and the tube was swirled throughout the digestion to keep the wall clean and prevent the loss of the samples. The solution was filtered with Whatman No. 42 filter paper. It was then quantitatively made up to 100 mL with distilled water in a volumetric flask. This filtrate was used for the detection of metals by ICP OES metal analyser [2, 21].

3. Results

3.1. Leaf Macroscopic characteristics: The macroscopy of leaf revealed that leaf is alternate, simple, entire, obovate, pinnate, deciduous, 15-30 cm long, 9-24 cm wide, dark green, leathery and glossy above, paler beneath with short petioles 0.1-0.6 cm long spirally clustered at the branch tips,. They turn reddish-pink, yellowish brown or purplish pink before dropping.

3.2. Leaf microscopic characters: Midrib: The T.S. of midrib (Figure 1a) shows dorsiventral structure and a distinct biconvex out line in the basal regions where as in the apical region becomes Plano convex.

The T.S. show single layered epidermis covered with thick cuticle. Epidermal cells of the ventral side and dorsal side are rectangular in shape and distinct thinking on radial walls. Some of the epidermal cells on the ventral sides elongate to form covering trichomes show mostly pointed end. Beneath the epidermal cells on both the sides the layers of collenchymatous cells is wider towards the ventral side which is 3-4 layers. Followed by collenchyma cells is the 6-7 layers of parenchymatous cells with angular thickening. Some of the parenchyma cells contain dark reddish brown matter in cells and rosette of calcium oxalate crystal (Figure 1c). Next to parenchymatous layer is 1-2 layer thick walled sclerenchyma cells and groups of stone cells scattered in between. Arc-shaped vascular strands where xylem surrounded by upper and lower side by phloem. Xylem consist of metaxylem and protoxylem with protoxylem facing towards the upper epidermis. Air cavity is present in parenchymatous pith. Few thick wall cells are scattered in pith.

3.3. Lamina: TS of lamina (Figure 2A) shows single layered epidermis composed of radially elongated cells. Palisade is single layered cylindrical cells. Mesophlyll composed of loosely arranged spongy parenchyma cells (Figure 2B). Some of the cells in the mesophyll region show the presence of calcium oxalate crystals (Figure 2C). Vascular tissue consists of phloem at the centre and xylem around.

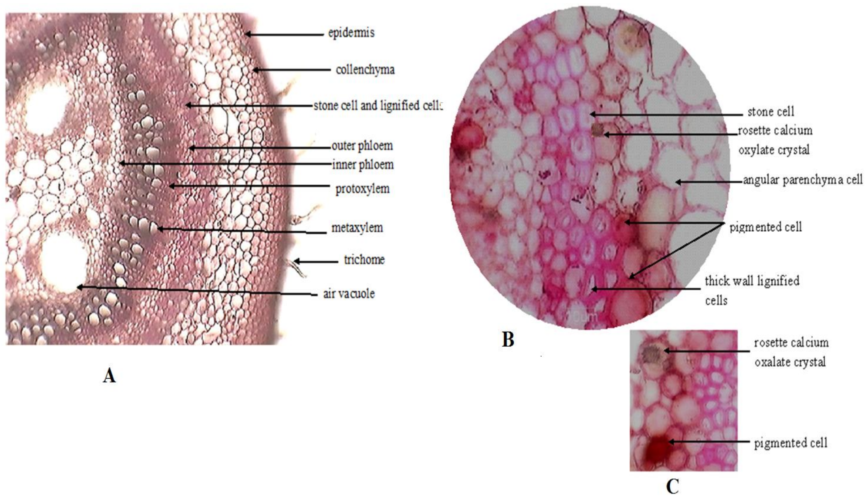

Fig 1: (A) T.S. leaf through midrib at 10X; (B) TS midrib showing parenchyma cells with angular thickening and rosette calcium oxalate crystal in parenchymatous cell and reddish brown matter in cell 40 X; (C) T.S. of midrib showing calcium oxalate crystal and pigment cell at 40 X

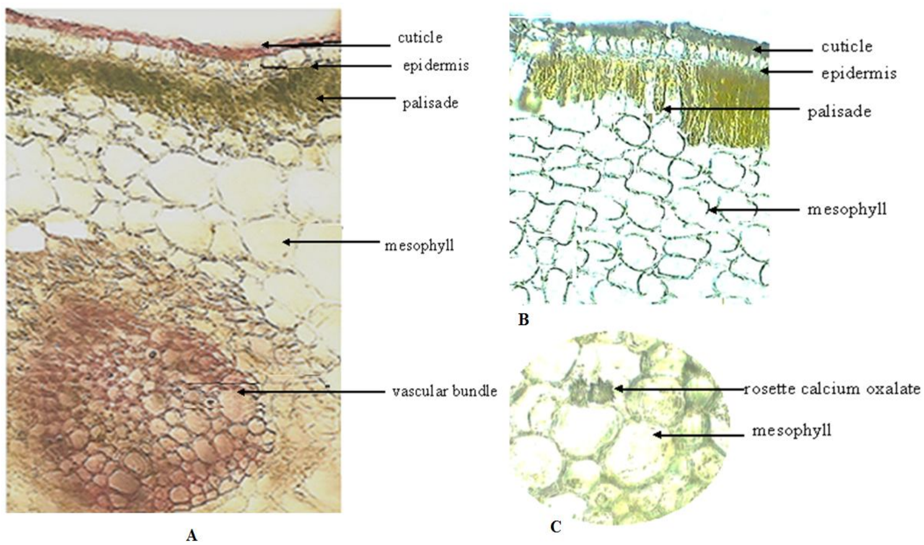

Fig 2: (A) T.S. of lamina at 4X; (B) T.S. of lamina showing epidermis, palisade cells, mesophyll and vascular bundle at 10X; (C) T.S. lamina showing rosette calcium oxalate crystal embedded in mesophyll cells at 40X

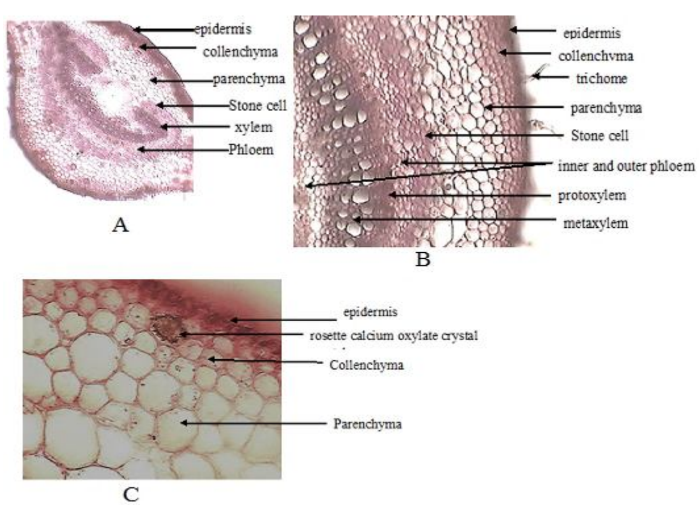

Fig 3: (A) T.S. of petiole at 4X; (B) T.S. of petiole showing epidermis, palisade cells, mesophyll and vascular bundle at 10X; (C) T.S. Petiole showing rosette calcium oxalate crystal embedded in collenchyma cell and angular parenchyma cell at 40X

3.4. Petiole: TS of petiole shows single layered epidermis (Figure 3B). Epidermal cells on both sides a strong thickening on their radial wall. Epidermal cells are followed by 3-4 layers of collenchyma cells. Collenchyma cells are bigger towards the plane region compared to the dorsal side. Followed by collenchymatous layer is the continuous layer of parenchyma composed of 5-6 layers. Some of the collenchymas cells shows presence of rosette calcium oxalate crystals (Figure 3C). Central region of petiole is occupied by arc shaped vascular strands showing xylem surrounded by phloem on upper and lower side. Air cavity is absent in pith region of petiole.

3.5. Characteristic of leaf powder: Powder characteristic of leaf revealed the presence of vessels with spiral thickening, rosette of calcium oxalate crystals, fragments of epidermal cells, anomocytic stomata and group of stone cells (Figure 4).

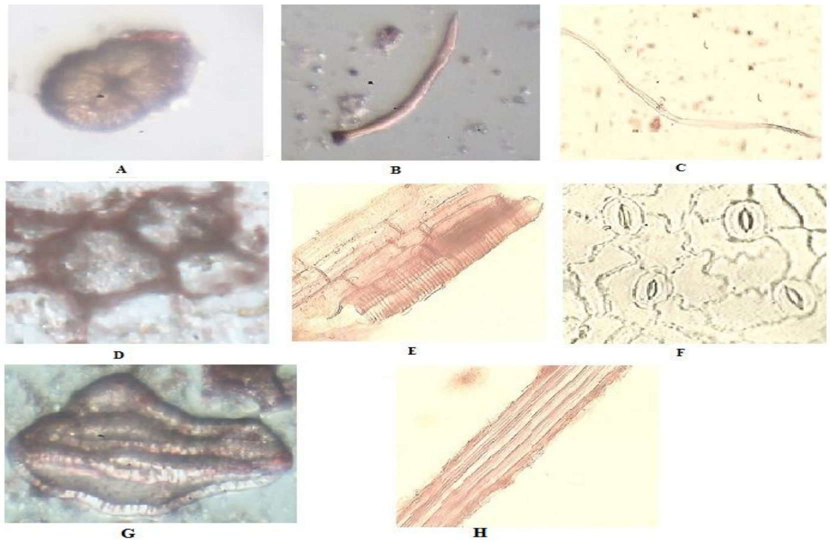

Fig 4: Microscopy of leaf powder at 4X(A) rosette calcium oxalate crystal; (B) unicellular trichome with pointed tip and swollen base; (C); phloem fibres; (D) epidermal cells in surface view; (E) xylem vessel with spiral thickening; (F) anomocytic stomata; (G) group of stone cells; (H) phloem fibre.

3.6. Physicochemical Parameters: 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 ash values (Table 1) of the powdered *Terminalia catappa* Linn. leaf. The total ash value was higher than that of the acid insoluble and water soluble ash value and a decrease in the acid insoluble ash value may be due to presence of smaller quantity of siliceous matters. The extractive values are primarily useful for the determination of exhausted or adulterated drug.

3.7. Quantitative Analysis: The fresh leaf samples were subjected to quantitative analysis for various leaf constants like stomatal number, stomatal frequency, vien islet number, vein termination number and palisade ratio (Table 2)

Table 1: The average percentage Physiochemical analysis of leaf powder of *Terminalia catappa* Linn.

Parameters	Average percentage (% w/w)
Total Ash	8.114
Acid insoluble ash	1.74
Water soluble ash	0.48
Alcohol soluble extractive	24.56
Water soluble extractive	29.66

Table 2: Quantitative microscopy

Parameters	Average	Mean
Palisade ratio	7-11	8.6
Stomatal frequency	19-25	21.1
Stomatal index	20-28	23.3
Vein islet number	19-27	22.5
Vein termination number	17-25	21.7

Table 3: Quantitative estimation of metals in leaves of *Terminalia catappa* Linn.

Metals	Concentration (mgL^{-1})
Arsenic	0.5
Lead	0.06
Mercury	0.2
Iron	20
Zinc	22
Magnesium	44

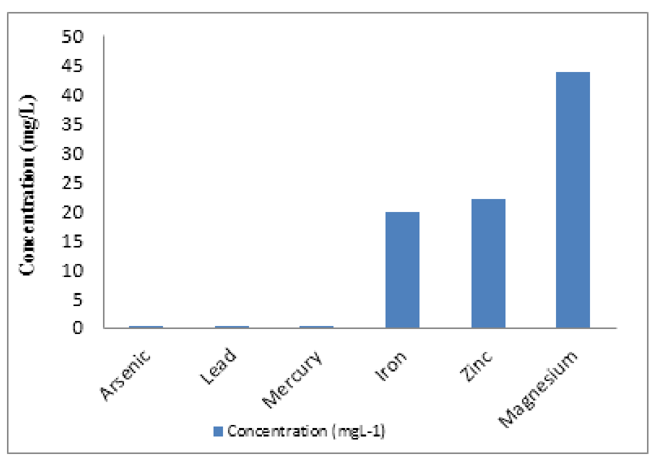

Fig 5: Graphical representation of metal concentration of *Terminalia catappa* Linn. leaves

Concentration of essential and non-essential heavy metals in medicinal plants beyond permissible limit is a matter of great concern to public safety all over the world. The problem is rather more serious in populated countries, because medicinal plants which form the raw materials for the finished products are neither

controlled nor properly regulated by quality assurance parameters.
An examination of the data from Table 3 shows distribution of elements like, Arsenic, Lead, Mercury, Iron, Zinc and Magnesium in various proportions. Figure 5 shows the graphical distribution of essential as well as toxic metals in leaves of *Terminalia catappa* Linn. Iron is an essential element for human beings and animals and is an essential component of hemoglobin. It facilitates the oxidation of carbohydrates, protein and fat to control body weight, which is very important factor in diabetes. Zinc plays an important role in various cell processes including normal growth, brain development, behavioural response, bone formation and wound healing. Zinc deficient diabetics fail to improve their power of perception and also causes loss of sense of touch and smell. Among the essential metals analysed Magnesium was found to be maximum (44mg/L) followed by Iron (20 mg/L) and zinc (22 mg/L). The dietary limit of Iron and Zn in the food is 100 ppm and 10-60 mg/day respectively. The heavy metals like Arsenic, Lead and Mercury are extremely toxic even in very small amounts and considered as non-essential for human consumption. The results obtained revealed that heavy metal Arsenic (0.5 mg/L), Lead (0.06 mg/L), Mercury (0.2 mg/L) levels in *Terminalia catappa* Linn. leaves were within the permissible limit and hence seem to be a safe herbal medicine to health. Thus among the essential elements analysed Magnesium was found to be maximum compared to Iron and Zinc in leaves of *Terminalia catappa* Linn. Thus, microscopical evaluation and heavy metals analysis of this medicinal plant represents one of the factors for the evaluation of their quality.

4. Discussion

The evaluation of a crude drug is an integral part of establishing its correct identity. Before any crude drug can be included in herbal pharmacopoeia, pharmacognostical parameters and standards must be established. Some diagnostic features have been evolved to identify and to differentiate the *Terminalia catappa* Linn. leaf from the other crude drugs and adulterants.
In this regard the important microscopic features of the leaf has been documented such as transverse section of midrib shows single layered epidermis, followed by 3-4 layers collenchymatous cells next to this is 6-7 layer parenchymatous cells, arch shaped vascular strands with parenchymatous pith. Some of the cells of collenchyma and parenchyma cells shows rosette of calcium oxalate crystals. Some of the parenchyma cells towards the ventral side show dark reddish brown pigmented cells. Transverse section of leaf is dorsiventral with distinct convex outline in the basal region where as apical region is plano convex. It shows single layered upper epidermis having one layered compact and radially elongated palisade cells, unicellular trichomes and anomocytic stomatas are present.
TS of petiole show single layered epidermis with strong thickening on their radial wall. Next is 3-4 layers of collenchyma cells which are bigger towards the plane region compared to the dorsal side. Followed by collenchymatous layer is the continuous layer of parenchyma composed of 5-6 layers. Central region of petiole is occupied by arc shaped vascular strands showing xylem surrounded by phloem on upper and lower side. Air cavity is absent in pith region of petiole.
Leaf revealed the presence of vessels with spiral thickening, rosette of calcium oxalate crystals, stone cells and pigmented cells, fragments of epidermal cells with anomocytic stomata, air cavity in mibrib and absence of air cavity in petiole serve as tool identification of leaf of *Terminalia catappa* Linn. The leaf constant parameters determined in the quantitative microscopy are relatively constant for plants and can be used to differentiate closely related species. In quantitative microscopy, the stomatal indexes were found to be 23.3 Vein islet number and vein-let termination number are 22.5 and 21.7 respectively. The total ash and acid insoluble ash of leaf was found to be 8.11% and 1.74% respectively. The extractive values were found to be 9.2%, 13.2% and 15.3% for chloroform, alcohol and water respectively. This shows that constituents of drug are more extracted and soluble in water as compared to alcohol thereby revealing presence of larger amount of water soluble constituents in the leaves such as plant acids, carbohydrates and phenolic compounds. Studies on physico-chemical constants can serve as a valuable source of information and provide suitable standards to determine the quality of this plant.
An examination of the data from Table 3 shows distribution of elements like, Arsenic, Lead, Mercury, Iron, Zinc and Magnesium in various proportions. Iron is an essential element for human beings and animals and is an essential component of hemoglobin. It facilitates the oxidation of carbohydrates, protein and fat to control body weight, which is very important factor in diabetes. Zinc plays an important role in various cell processes including normal growth, brain development, behavioural response, bone formation and wound healing. Zinc deficient diabetics fail to improve their power of perception and also causes loss of sense of touch and smell. Among the essential metals analysed Magnesium was found to be maximum (44 mg/L) followed by Iron (20 mg/L) and zinc (22 mg/L). The dietary limit of Iron and Zinc in the food is 100 ppm and 10-60 mg/day respectively.
The heavy metals like Arsenic, Lead and Mercury are extremely toxic even in very small amounts. The results obtained revealed that heavy metal Arsenic (0.5 mg/L), Lead (0.06 mg/L), Mercury (0.2 mg/L) levels in *Terminalia catappa* Linn. leaves were within the permissible limit and hence seem to be a safe herbal medicine to health. Thus among the essential elements analysed Magnesium was found to be maximum compared to Iron and Zinc in leaves of *Terminalia catappa* Linn.

5. Conclusion

As there is no pharmacognostic/anatomical work record of this much valued traditional drug, the present work was taken up with a view to lay down standards which could be useful to detect the authenticity of this medicinally useful plant. In other words, the pharmacognostic features examined in the present study may serve as tool for identification of the plant for validation of the raw material and for standardization of its formulations. Pharmacognostical studies can serve as a basis for proper identification, collection and investigation of the plant. These parameters, which are being reported, could be useful in the preparation of the herbal monograph for its proper identification.
The analysed plant species contained safe levels of the heavy metals (Arsenic, Mercury and Lead) and hence may have no adverse effects normally associated with heavy metal toxicity on people who patronize herbal products for their health needs. Although heavy metals in herbal plants do not pose any immediate risk to human health so far, a yearly monitoring program for heavy metals in food and other herbal products is a necessity. Everyone involved in traditional herbal medicine should be sensitized to heavy metal contamination risk to improve the quality, safety, and efficacy of herbal drugs.
From this study confirms that plant *Terminalia catappa* Linn. having the essential metals like Iron, Zinc and Magnesium, which supports for the treatment of diabetes, also this information provides the key role for the preparation of the formulation, which

would serve a better role in diabetic patients. Hope the modern system of medicine & manufacturer of drug-product will deals with this plant in future.

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