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PHYTOCHEMICAL SCREENING AND ATOMIC ABSORPTION SPECTROSCOPIC STUDIES OF SOLVENT TYPE EXTRACT FROM LEAVES OF *TERMINALIA CATAPPA*, (ALMOND)

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ABSTRACT

Leaves of Terminalia catappa (almond) were phytochemically screened for Natural products using solvents of varying polarity: n-C_6H_{14}, CH_2Cl_2, EtOAc, CH_3CH_2OH and H_2O. Phytochemical screening of the leaves revealed selective presence of sterols, triterpenes, carotenoids, flavones aglycone, emodols, coumarins, tannins, reducing compounds, anthraquinones, steroid glycosides, alkaloids, cardenolides, saponins, flavanosides, cholesterol, flavanoids, amino acids, phlobatinins, cholesterol and cardiac glycosides in the various solvent type extracts. These phytochemicals are localized and vary in concentration and type in different parts of the plant, suggesting that the biosynthesis of natural products are selective. The number of phytochemicals tested for vary according to the sequence: EtOAc > n-C_6H_{14} > CH_2Cl_2 > CH_3CH_2OH > Aqueous. Atomic absorption analyses indicates selective presence of metals in the solvent type extract with the CH_3CH_2OH extract showing a high concentration of Ca and Mg. The non-detection of nitrogen and Mg in the various solvent type extract is also evident.

Keywords: Natural Products; Phytochemicals; Phytochemical screening; Atomic absorption Analyses; Solvent type extract.

INTRODUCTION

Natural products, secondary metabolites of plants and animals are of restricted occurrence and are classified into major groups such as sterols, triterpenes, flavones aglycones, emodols (anthracenosides aglycones), coumarins, coumarins lactone derivatives, tannins (gallic), reducing compounds, sterols glycosides, cardenolides, saponins and sapogenins etc. They vary widely in both type and concentrations in different parts of the plant and are found as minor components of plant tissues (Mann, 1986; Mann, *et al.,* 1994). They are either novel or known and are isolated from crude plant extracts whose whose medicinal activity can be compared with that of the isolates (Aponte *et al.,* 2008; Manga *et al.,* 2006; Wafo *et al.,* 2010).

Medicinally, isolated natural products after been subjected to clinical trials can be used as drugs for the treatment of cancer Crow (2008), antimicrobial agents Woldemichael *et al.* (2003), Jagessar (2011) Prasad (Prasad *et al.,* 2008) antitumor (Flores *et al.,* 2010), antioxidant agents (Manga *et al.,* 2006), Kukic (Kukic *et al.,* 2008), Nile (Nile *et al.,* 2010). Also, isolated natural products have been the impetus for the design and synthesis of many pharmaceutical drugs to date. For example, *Cyanthiwigin F*, a complex *bis* molecule active against tumours was first isolated from the Jamaica sea sponge *Myrmekioderma styx* (Enquist *et al.,* 2008). Phytochemical screening provides first hand knowledge of the chemical constituents of the plant (Mohammed *et al.,* 2007; Jagessar *et al.,* 2010).

Terminalia catappa, a flowering plant of the Combretaceae, is a large deciduous tree reaching an height of 20-25 metres. The branches are horizontally arranged. Leaves are shiny and are about 10-25 cm long, forming a narrow heart shaped base with an expanded round apex. A scientific classification of the plant is shown in Table 1.0.

Table 1. Scientific Classification of *Terminalia catappa*

Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Lamiales
Family	Combretaceae
Genus	*Terminalia*
Species	*Terminalia catappa*

Leaves of *Terminalia catappa* have been investigated for its medicinal activities. These include the *in vitro* and *in vivo* antimetastic effects (Chen *et al.,* 2007), antidiabetic activities (Rao *et al.,* 2006; Nagappa *et al.,* 2003), actinociceptive activity, antiparasitic (Elizabeth, 2005) antibacterial (Elizabeth, 2005; Rajarajan *et al.,* 2010; Kinoshita *et al.,* 2007), antifungal (Elizabeth, 2005), antioxidant (Kinoshita *et al.,* 2007; Mau *et al.,* 2007; Mety *et al.,* 2011; Cheng *et al.,* 2003; Lin *et al.,* 1997; Lin *et al.,* 1999) and anticancer properties (Chen *et al.,* 2006; Chu *et al.,* 2007), □-glucosidase activity (Anam *et al.,* 2009). The leaves when mixed with oil and rubbed onto the breast can relieve mammary pain. The sap of young leaves mixed with the kernel oil has been used for the treatment of leprosy, rheumatic joint pain, scabies and other cutaneous diseases, headaches colic, diuretic and cardiotonic (Parrota, 2000). It has also been used for tonsillitis. The bark is used for gastric ailments, bilious, diarrhea and dysentery. Other species such as *Terminalia chebula* is used in the treatment of fevers, cough, asthma, urinary diseases, piles and worms (Parrota, 2000). *Terminalia bellerica* is used for the treatment of fever, cough, asthma, urinary diseases, piles, chronic diarrhea, dysentery, flatulence, vomiting, colic and enlarged spleen and liver (Elizabeth, 2005).

Natural products have also been isolated from the plant. *T. catappa* contains hydrolysed tannins such as punicalagan as a major component, punicalin, terflavins A and B, tergallagin, tercatain, chebulagic acid, geranin, granato B, corilagin, flavanoids (isovitexin, vitexin, rutin, triterpenoids (ursolic acid, 2□, 3□, 23-trihydroxyurs, 12-en-28 oic acid, Asiatic acid), squalene but no caeffeine. Both punicalagan and punicalin showed anti-inflammatory activity on carrageenan-induced hind paw edema in rats [26]

However, little work has been done on the Phytochemically screening of *Terminalia catappa* even though related species of *Terminalia* have been phytochemically screened for their natural products and atomic absorption analyses (AAS) of the various extracts have never been investigated.

EXPERIMENTAL

Materials and Equipment: n-C_6H_{14}, CH_2Cl_2, EtOAc, CH_3CH_2OH were purchased from Sigma Aldrich Company (USA) and were redistilled prior to use. Na_2SO_4 was used to dry each solvent type extract. Solvents were removed in *vacuo* from the dried extracts using a Buchii rotavapor, R-124 with laboport vacuum pump. Filter paper of size 150mm and 240mm diameter were used for filtration. Analytical TLC analyses were done on precoated Kiesegel 60 F254 (Merck) plates and were 0.25 mm thick. TLC plates were viewed under a UV lamp, (Spectroline Longlife Filter) and developed chromatograms

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were visualized via initial spraying with iodine. Extracts were weighed using a Metler Citizen type. Elemental analyses were determined using Atomic Absorption Spectrophotometry (AAS) on a Varian AAS 50 spectrophotometer.

Plant Material: *Terminalia catappa* leaves were collected from the University of Guyana in 2010. Leaves were freed of visible fungal and bacterial infection. As products of microbial synthesis might result in added chemical composition of the plant material inspection was important. It was identified by a Taxonomist at the Bio Diversity Centre of the University. A voucher specimen (# 031992) is deposited in the herbarium of the Bio Diversity centre. Leaves were then subjected to aerial drying for a week and were later ground into a fine powder (250g) using a grinding mill, Arthur H. Thomas Co. model (# 750611).

Plant extracts: The extraction was done in extraction jars at ambient temperature using sequentially freshly distilled n-C_6H_{12}, CH_2Cl_2, EtOAc and CH_3CH_2OH. For each solvent type, three extractions were done and each extract was filtered and dried over anhydrous Na_2SO_4 and solvents were removed in *vacuo* to yield viscous oils and semi solids.The physical nature of the C_6H_{14}, CH_2Cl_2, EtOAc and CH_3CH_2OH extract was greenish brown, black, brown and gummy black respectively.

Elemental analyses**:** 1.0g of the dried solvent type extracts was dissolved in 10ml of the requisite solvent. This was followed with the addition of 1ml of 10% HNO3. It was shaken to ensure a uniform mixture and then made up to 100ml mark. Appropriate working standard solutions were prepared for each element. The levels of requisite metal elements (K, Cu, Fe, Mn, Co and Zn) were determined using Atomic Absorption Spectrophotometry (AAS) method on a Varian AAS 50 spectrophotometer. First the sample solution was aspirated by a pneumatic nebulizer, transformed into an aerosol, which was introduced into a spray chamber, where it was mixed with the flame gases and conditioned in a way that only the finest aerosol droplets (< 10µm) entered the flame. On top of the spray chamber, a burner head produces a flame that is laterally long (usually 5–10cm) and only a few mm deep. The radiation beam passed through this flame at its longest axis, and the flame gas flowrates was adjusted to produce the highest concentration of free atoms. The burner height was adjusted, so that the radiation beam passed through the zone of highest atom cloud density in the flame, resulting in the highest sensitivity. The concentration (C) of each analyte in the solutions aspirated was recorded.

Phytochemical screening for secondary metabolites: On each solvent type extract, test for alkaloids, Saponin glycosides, Cardenolides, Bufadionolides, Flavonoids, Tannins, Polyphenolic compounds, Anthraquinones, Cyanogenic glycosides, Carbohydrates, Fixed oils, Fats, and Volatile oils were carried out using standard Phytochemical methods.

Cholesterol: To 2 ml of the extract, 2ml of $CHCl_3$ was added in a dry test tube. This was followed with the addition of 10 drops of acetic anhydride and 2 to 3 drops of conc. H_2SO_4. It is anticipated for a positive test that a red-rose colour should be evident.

Cardiac Glycosides: 5ml of each extract was treated with 2ml of glacial acetic acid, containing one drop of ferric chloride solution. This was underlayered with 1ml of conc. H_2SO_4. A brown ring of the interface indicated a deoxy sugar characteristic of cardenolides. A violet ring might appear below the brown ring, whereas the acetic acid layer, a greenish ring might form just gradually throughout the thin layers.

Glycosides**:** A small amount of alcoholic extract was dissolved in 1ml of water and a few drops of aqueous sodium hydroxide solution were added. A yellow colour was taken to signify the presence of glycosides.

Tannins: About 0.5g of extract was dissolved in 5 to 10ml of distilled water and was filtered. A few drops of a 5% $FeCl_3$ solution were added to the filtrate. A blue, blueblack, green, or blue-green colour or a precipitate was taken as an indication of the presence of tannins.

Flavonoids**:** A few drops of concentrated hydrochloric acid were added to a small amount of an alcoholic extract of the plant material. Immediate development of a red colour was taken as an indication of the presence of flavonoids.

Sterols and Triterpenes

***Liebermann-Burchard Reaction*:** 10ml of the extract was placed in a test tube and evaporated to dryness on a water bath. The residue was dissolved in 1ml of acetic anhydride and 1ml of chloroform. The solution was then transferred to two clean dry test tubes; one served as the reference tube. 1-2ml of conc. H2SO4 was added to the other tube using a teat pipette. A violet ring was formed at the two liquids, with the supernatant becoming violet this indicates the presence of sterols and triterpenoids.

***Carotenoids:* (*Carr-Price Reaction*):** 10ml of the extract was added to a test tube and was evaporated to dryness on a water bath. This was followed with the addition of 2-3 drops of saturated $SbCl_3$ in $CHCl_3$ to the residue. A blue-green colour eventually changing to red indicates the presence of carotenoids.

***Flavone Aglycones: Shibata's Reaction or Cyanidin test*:** 3ml of the extract was evaporated to dryness in a water bath. The residue was then dissolved in 1-2ml of 50% CH_3CH_2OH while heating. A piece of magnesium ribbon and 4-5 drops of concentrated HCl were added. A red or orange colour indicates the presence of Flavone Aglycones.

Emodols (Anthracenoside and Anthracenoside Aglycone): Borntrager's Reaction: 1ml of 25% NH3 was added to 3ml of the extract in a test tube. The mixture was then shaken well. A red colour indicates the presence of Emodols. To 2ml of the extract in a test tube, 1-2ml of 25% NH_3 was added while it was been shaken. A cherry-red colour indicates the presence of anthracenosides.

Test for Coumarin and derivatives: 5ml of ether extract was evaporated to dryness. The residue was dissolved in 1-2ml of water by heating. It was then divided in two equal portions. To the non-reference tube, 0.5ml of 10% NH3 was added and then the tubes were viewed under UV light. The presence of blue-green fluorescence indicated the presence of coumarin. 4-5 drops of hydroxylamine hydrochloride solution and alcoholic KOH were added to the non-reference test tube until the pH was approximately 8-9. The resulting solution was evaporated to dryness. The residue was dissolved and the pH was adjusted to 3-4 by adding 10% HCl add 1-2 drops 3% FeCl3 while being observed. A fast disappearing violet colour indicates the presence of coumarin derivatives.

Alkaloids: 10ml of the extract was evaporated to dryness. The residue was then dissolved in 1.5ml of 2% HCl. The solution was divided into two equal portions, one served as a reference. 2-3 drops of Mayer's reagent was added to the non-reference tube. The development of opalescence or a yellowish white precipitate indicates the presence of alkaloids.

Mayer's reagent: 1.35g of mercuric chloride was dissolved in 60 ml of water, 5g of potassium iodide was added to 10ml of water and diluted to 100 ml. 0.85g of basic bismuth nitrate was dissolved in a mixture of 40 ml of water and 10 ml of acetic acid. 8g of potassium iodide dissolved in 20 ml of water and homogenize was added. The solution obtained was kept in a dark bottle for 2-3 months.

Phlobatinins: Each plant extract is boiled with 1% aqueous HCl. A red precipitate is expected to be deposited and is taken as evidence for the presence of phlobatinins.

Proteins: To 2 ml of plant extract, 1ml of 40% NaOH solution and 1 to 2 drops of 1% $CuSO_4$ solution was added. A violet colour indicates the presence of peptide linkage of the molecule.

Non-Hydrolysed CH_3CH_2OH Extract

Tannins: 1-2 ml of water was added to 0.5-1.0ml of the extract and then 2-3 drops diluted (3% FeCl3) solution was added to the mixture. A blackish-blue colour indicated the presence of Gallic tannins, while a greenish-black colour indicates the presence of catechol tannins.

Reducing compounds: 1-2ml of water was added to 0.5ml of the extract the 0.5-1.0ml of Fehling solution (1 and 2) were added. The mixture was then heated in a water bath. A brick red precipitate indicates the presence of reducing compound.

Alkaloids salt: 20 ml of the extract was evaporated to dryness and 5-10ml of 10% HCl was added to the residue. 10% NH_3 was then added until pH of 8-9 was achieved.

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The solution was placed into a separatory funnel and extracted with a polar solvent. The resulting extract was evaporated to dryness. The residue was then dissolved in 1.5ml of 2% HCl and divided into two portions; one was a reference. 2-3 drops of Mayer's reagent was added to the non-reference tube. Development of opalescence or a yellow-white precipitate indicates the presence of alkaloid salts.

Hydrolysed CH_3CH_2OH extract: 2ml of 10% HCl was added to the 25ml extract to hydrolyze the alcohol extract. The solution was refluxed for 30 minutes and then allowed to cool. 10-12ml of ether was extracted three times and the extracts were combined. A spatula full of anhydrous Na_2SO_4, filter was added and left for the following test.

***Cardenolides (Cardiac Glycosides and Aglycones: The Kedee's test*:** 4ml of the ether extract was evaporated to dryness. The residue was dissolved in 1-2ml methanol. 1- 2ml of alcoholic KOH was added to the mixture. 3-4 drops of 1% alcoholic 3, 5- dinitrobenzene was added and the solution was heated. A disappearing violet colour indicates the presence of Cardenolides.

Saponins: 2ml of the ether extract was evaporated to dryness. The residue was dissolved in 1ml water and shaken vigorously. The presence of saponins was indicated by persistent foam (1cm in test tube).

Flavanosides: (Shibata's Reaction): 5ml of ether extract was evaporated to dryness. The residue was dissolved by heating in 1-2ml of 50% methanol. Metallic magnesium was added to the mixture followed with 5-6 drops conc. HCl. The development of an orange colour indicates the presence of flavanones. (Flavanols gave a characteristic red colour).

RESULTS

Table 2. Phytochemical Screening analyses for *Terminalia catappa*

Extract	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
n-C_6H_{14}	+	+	-	-	+	-	-	-	+	-	+	+	+	+	+	-	-	-	-	+
CH_2Cl_2	+	-	-	-	+	+	-	+	-	+	+	+	-	+	+	-	-	-	-	+
EtOAc	+	-	+	-	+	-	+	-	-	+	+	+	-	+	+	-	+	+	-	+
CH_3CH_2OH (non-hydrolysed)	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	+	+	+	-	+
CH_3CH_2OH (hydrolysed)	+	+	-	-	-	+	-	-	-	-	+	-	-	-	-	-	+	-	-	-
Aqueous	-	-	+	-	-	-	+	-	-	+	-	-	-	+	-	+	-	-	-	+

A : Test for Steroids and Triterpenes B : Test for Carotenoids; C : Test for Flavone Aglycone; D : Test for Emodols; E : Test for Coumarin; F: Test for Alkaloids; G : Test for Tannins s; H : Test for Alkaloid salts; I : Test for Coumarin Derivatives; J : Test forAnthracenosides; K : Test for Steroid glycosides; L: Test for Cardenolides M: ; Saponins, N: Flavanosides, O: Cholesterol; P: Flavanoids Q: Amino acids R: Phlobatinins S: Proteins T: cardiac glycosides u: reducing compounds.

Table 3. TLC analyses of extract for *Terminalia catappa*

Extract	Eluent	R_f value(s)
CH_2Cl_2	CH_2Cl_2/n-C_6H_{14} 9:1	Sp 1: (0.82,0.32, 0.25, 0.11) Sp 2: (0.85,0.36, 0.23, 0.13) Sp 3: (0.85,0.35, 0.23, 0.14) Sp 4: (0.86,0.33, 0.22, 0.11)
CH_3CH_2OH	CH_3CH_2OH/ n-C_6H_{14}, 6:4	0.8, 0.733, 0.88, 0.89 Spots 1-4 respectively
EtOAc	EtOAc/n-C_6H_{14}, 9:1	Sp 1: (1,0.79) Sp 2: (0.97, 0.80) Sp 3: (1, 0.80) Sp 4: (1, 0.82)
n-C_6H_{14}	n-C_6H_{14}/CH_2Cl_2, 9:1	Sp 1: (0.96, 0.53), Sp 2: (0.96, 0.51), Sp 3: (0.91, 0.48), Sp 4: (0.94, 0.48), Sp 5 (0.93, 0.53)

Table 4. Atomic absorption spectroscopic analyses of the extract of *Terminalia catappa*

Sample Description	Parameter								
	Total N(%)	P (%)	K (%)	Ca (mg/kg)	Mg (mg/kg)	Fe (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	Mn (mg/kg)
CH_2Cl_2	--	--	3.32	nd	1.26	Nd	2.60	nd	nd
CH_3CH_2OH extract	--	0.89	1.31	2087	2706	29.3	7.11	11.05	nd
n-C_6H_{14}	--	--	3.35	26.2	nd	0.15	nd	4.87	nd
H_2O extract	--	0.15	0.12	319	1603	Nd	20.09	nd	nd

- A: Nitrogen (N); B: Phosphorus (P); C: Potassium (K); D: Calcium (Ca); E: Magnesium (Mg); F: Iron (Fe); G: Copper (Cu); H: Zinc (Zn); I: Manganese (Mn).
- ND: Not detected

DISCUSSION

The ground plant material of *Terminalia catappa* was phytochemically screened using solvents of varying polarity: n-C_6H_{14}, CH_2Cl_2, EtOAc and CH_3CH_2OH. Standard phytochemical tests were carried out on the selected extracts. Phytochemicals tested for were sterols, triterpenes, carotenoids, flavones aglycone, emodols, coumarins, coumarins derivatives, alkaloids, anthracenosides, steroid glycosides, cardenolides, saponoins, flavanosides, cholesterol, flavanoids, amino acids, phlobatinins, protein, cardiac glycosides and reducing compounds.

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Phytochemical screening revealed selective presence of phytochemicals in the solvent type extract. For example, the n-C_6H_{10} extract showed the presence of carotenoids, steroids, triterpenes, coumarin and coumarin derivatives, steroid glycosides, cardenolides, saponins, flavanosides, cholesterol and cardiac glycosides. However, these compounds with the exception of cardiac glycosides are absent in the CH_3CH_2OH extract.

The CH_2Cl_2 extract showed the presence of sterols and triterpenes, coumarins, alkaloids, alkaloid salts, steroid glycosides, cardenolides, saponins, cholesterol and cardiac glycosides compared with the CH_3CH_2OH extract. These compounds with the exception of cardiac glycosides are not present in the ethanol extract.

The EtOAc extract showed preference for sterols, triterpenes, flavones aglycones, coumarins, tannins, anthracenosides, steroid glycosides, cardenolides, flavonoids, cholesterol and cardiac glycosides. These compounds with the exception of tannins, phlobatinins and cardiac glycosides are not present in the CH_3CH_2OH extract.

The aqueous extract showed the presence of few phytochemicals. These include flavones aglycones, tannins, anthracenosides, flavanosides, flavanoids and cardiac glycosides. The hydrolysed CH_3CH_2OH extract revealed the presence of steroids and triterpenoids, Carotenoids, alkaloids, steroid glycosides and Amino acids. Negative tests were revealed for the presence of the other class of natural products.

The number of phytochemicals tested for vary according to the sequence: EtOAc > n-C_6H_{14} > CH_2Cl_2 > CH_3CH_2OH > H_2O. One would expect that the more polar solvent type extract would contain the more polar natural product.

TLC analyses were done on all five solvent type extracts with the exception of water, using appropriate solvent systems. Several spots were noticeable for each extract. These are shown in Table 3.0. For example, the n-C_6H_{14} and CH_2Cl_2 extract showed the presence of four and five components respectively. EtOAc and CH_3CH_2OH extract showed the presence of five components also. Each spot is presumambly due to a pure phytochemical/natural product and provides the basis for separation via silica gel column chromatography

Atomic Absorption Spectroscopic analyses (AAS) were carried out on all of the extracts for the presence of the elements: N, P, K, Ca, Mg, Fe, Cu, Zn and Mn. Spectroscopic analyses revealed selective presence of the metals in the solvent type extract. Atomic spectroscopic analyses of the CH_2Cl_2, CH_3CH_2OH, n-C_6H_{14} and H_2O extract were investigated. Spectroscopic analyses revealed the selective presence of metals. For example, the ethanolic and H_2O extract showed the presence of phosphorus, whereas this is lacking for the CH_2Cl_2 and hexane extract. CH_3CH_2OH, n-C_6H_{14} and CH_2Cl_2 extract showed the presence of Ca. However, this is lacking in the CH_2Cl_2 extract. Copper is detected in the CH_2Cl_2 (2.60 mg/k), CH_3CH_2OH (7.11 mg/k) and aqueous extract (20.09 mg/K) but is lacking in the n-C_6H_{14} extract. Zn is present in the CH_3CH_2OH (11.05mg/kg) and n-C_6H_{14} (4.87 mg/kg) extract but is not detected in the CH_2Cl_2 and H_2O extract. Nitrogen is not present in any of the extract nor Mn (mg/kg) detected in any of the extract. Of further significance is the high concentration of Ca and Mg in the CH_3CH_2OH and aqueous extract. These been 2087, 2706 mg/K) for the CH_3CH_2OH extract and 319 and 1603 mg/K for the H_2O extrac, respectively. The CH_3CH_2OH extract seems to have most of the metals present, whereas the CH_2Cl_2 and n-C_6H_{14} extract seem to have few. Moderate amount of Fe and Cu were present in the CH_3CH_2OH and aqueous extract. The order of presence of elements seem to follow the trend: CH_3CH_2OH > H_2O > n-C_6H_{14} > CH_2Cl_2

Heavy metals such as Cu, Fe, Mn, Co and Zn are important micronutrients of plants, whereas K is an important macronutrient. These elements play important roles in the plant biochemistry and affect the surrounding environment. 9% of eukaryotic proteins bind various metals and 40% of all enzyme catalysed reactions involve metals such as Mg, Zn, Fe, Mn, Ca, Co, Cu, Ni, Mo, W, Na, K and V [32]. Cu is a prerequisite for many enzyme processes, proper photosynthesis, manufacture of lignin (cell walls) and in grain production. Mn is necessary for building chloroplasts. Manganese deficiency may result in chlorosis. Iron is necessary for photosynthesis and is present as an enzyme cofactor in plants. Iron deficiency results in interveinal chlorosis and necrosis. Iron is not the structural part of chlorophyll but very much essential for its synthesis. Potassium as macronutrients regulates the

opening and closing of the stomata via a potassium ion pump. Potassium deficiency may cause necrosis or interveinal chlorosis. K is highly mobile and can aid in balancing the anionic charges within the plant. It serves as an activator of enzymes used in photosynthesis and respiration. Potassium deficiency results in higher risk of pathogens, wilting, chlorosis, brown spotting, and higher chances of damage from frost and heat. Zinc, a micronutrients is essential for optimum crop growth. Its deficiency causes various adverse effects on growth and yield of crops. It is also involved in formation of chlorophyll, carbohydrates, in several dehydrogenises, proteinese and peptidase enzymes. It promotes growth hormones (auxin) and starch formation. It also responsible for the biosynthesis of cytochrome: a pigment and maintain plasma membrane integrity and synthesis of leaf cuticle. Cobalt is beneficial is essential in legumes where it is required for nitrogen fixation for the symbiotic relationship it has with nitrogen-fixing bacteria. Protein synthesis of *Rhizobium* is impaired due to Cobalt deficiency.

CONCLUSION

Leaves of *Terminalia catappa* were phytochemically screened and shown selective presence of various phytochemicals/natural products: Isoprenoids, Triterpenes, Carotenoids, Steroids. Tannins. Phenyl Propanoids, Coumarins, Flavanoids, Alkaloids, Alkaloid salts, Amino acids, Cholesterol, Cardiac glycosides, Cardenolides, Anthraquinone, Saponins and Coumarins, *phlobatinins, cholesterol*. Atomic absorption analyses also show selective presence of heavy metals in the various solvent type extracts.

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