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PHYTOCHEMICAL, HEAVY METALS AND N.P.K ANALYSIS OF CRUDE LEAF EXTRACT OF ALMOND (TERMINALIA CATAPPA L.)

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Abstract

Terminalia catappa is a common tree found in Guyana but it is much underutilized. The objective was therefore set to investigate phytochemicals, heavy metals and N.P.K analysis. The medicinal and nutritive potential of two common varieties of the plant; the red and yellow varieties. Phytochemical screening was also determined using standard screening methods. The leaves of T. catappa L were found to contain saponins, general glycosides, flavonoids, alkaloids, anthraquinones, anthroquinone, glycosides, terpenoid, steroids and alkaloids present. In addition Qualitative estimation of phytochemicals was performed in different solvent extracts, namely ethyl acetate, ethanol, methanol and aqueous extracts. The results were represented as '+' for the presence and '–'for the absence of phytochemicals. N.P analyses were done by total Kjeldahl digests by UV-VIS spectrophotometry, and K analysis was done in Kjeldal digests by Flame photometry method.

Key words: Terminalia catappa, heavy metals, phytochemicals, ethanol, methanol, aqueous extract

I. INTRODUCTION

Terminalia catappa is among the most common trees found in Guyana. It grows in the wild but is sometimes cultivated for ornamental purposes. It is found in almost all the regions of the country as it thrives well in the tropics, hence its name Tropical almond. It has a single stem which grows to a height of about 10 m and then branches horizontally with leaves at the end of the branches that form a rosette [1,2] The leaves change colour from green to red, yellow or gold and copper brown during the dry season and then are shed. T. catappa Linn belongs to the family Combretaceae (Combretum family) and is locally called 'Abrofo nkate" (Whiteman's peanut). Studies on the plant have shown that it could serve a lot of useful purposes. Extracts of the flesh of the fruit have been found to be a good acid-base indicator [3]. The fruit is said to contain agents for chemo-prevention of cancer because it contains antioxidant phytochemicals that can break up the chromosomes of insects that feed on it [4]. The aqueous extract of T. catappa L. exhibits superoxide radical scavenging activity preventing lipid peroxidation [5]. It has also been found to possess anti-HIV reverse transcriptase [6], hepatoprotective, anti-inflammatory, aphrodisiac anti-diabetic [7]. The sun-dried kernel showed the potential of the nut to serve as dietary supplement. However, studies on the extracts of defatted T. catappa seed meal on the performance and carcass of rats led to the deduction that the defatted seed meal of T. catappa could cause depression in growth rate and enlargement of rat tissues [8]. They attributed their observation to the presence of anti-nutrients such as phytate, oxalate and tannin which are present in high amounts in the seed. The anti-nutritional nature of a fruit is likely to be due to the presence of phytochemicals. In Guyana, the flesh and kernel of the fruits is eaten raw. The kernel is also sun dried or roasted. The leaves, roots and bark are however used for treating diseases such as anaemia, hypertension, malaria,

fever and asthma [9]. There is very little information on the phytochemical components, heavy metals and N.P.K analysis studies in T. catappa leaves in modern scientific literature. As such, this paper aims to provide an extensive analyses studies of phytochemicals, heavy metals and N.P.K on the leaves.

II. MATERIALS AND METHODS

Plant materials:

Terminalia catappa (Almond) leave were collected from Region 4 and 6 in Guyana. The fresh leaves washed with tam water for serval times and dried in shadow conditions. Air dried leaves is grounded into powder using Thomas-Wiley Laboratory Mill Model 4 at Central Chemistry Laboratory, Department of Chemistry, Faculty of Natural Science, University of Guyana, Turkeyen Campus. The grounded powder were extracted with methanol, ethyl acetate, ethanol extract incubated for 72 hours in shaker, whereas the aqueous extract was prepared by incubating for overnight in shaker and it was boiled for 15 to 30 minutes till the volume was reduced to half its original. The solvents was then removed by filtration. The extracts were condensed using rotary vacuum evaporator and stored at 4^{o} C. the aqueous extracts was dissolved in water and used for further purpose whereas concentrated extract of ethanol and methanol was suspended in 0.25% dimethyl sulphoxide (DMSO) to the concentration of 100mg/ml and was used for analysis.

Phytochemicals analysis:

The aqueous, ethanol, methanol, ethyl acetate extracts of T. catappa were analysis for the presence of secondary metabolites using the stranded procedure. Three milliliter (ml) of each extract was measured into a test tube foe each test. Test were carried out for alkaloids, flavonoids, saponins, phenols, steroids, tannins, terpenoids, glycosides, carbohydrates, phlobatannins, thiols, anthroquinone, protein and amino acids, resins, fixed oils & fats, and phytosterols components according to standard methods [10.11].

Steroid (Liebermann Burchard reaction): 300µl of extract was added with 1ml of chloroform and few drops of Concentrated Sulphuric acid along the sides of the test tubes, Reddish brown color precipitate was observed at the bottom of the test tubes indicates the presence of steroid.

Cardiac Glycoside (Keller Kiliani Test) 300µl of extract was added with 1ml of Acetic acid followed by the addition of 300ul of 10 % Ferric Chloride and few drops of Concentrated Sulphuric acid along the sides of the test tubes, Brownish ring and green blue precipitate at the bottom of the test tube indicates the presence of Cardiac glycoside.

Phenol and Tannin (Ferric Chloride Test): Few drops of Ferric chloride 10% was added with 300µl of extract gives Blue or Green color precipitate due to the presence of Phenol Tannin.

Terpenoids (Salkowski Test): To 300µl of extract 1ml of chloroform and few drops of Concentrated Sulphuric acid was carefully added along the sides of test tubes, a reddish brown color precipitate indicates the presence of Terpenoid.

Alkaloids (Mayer's test): 300µl of Mayer's reagent was added with 300µl of fruit extract Pale precipitate indicates the presence of Alkaloids.

Resins: To 300µl of extracts 1ml of acetic anhydrite was added and dissolved by gentle heating after cooling, few drops of concentrated Sulphuric acid were added Bright purple color indicates the presence of Resin.

Carbohydrate (Molisch's Test): Few drops of Sulphuric acid and 300µl of Molisch's reagent were added with 300µl of extract, reddish color at the bottom of the test tubes indicates the presence of Carbohydrates.

Flavonoids: 300µl of extract was first added with 1ml of 10% ammonia and 1ml of Concentrated Sulphuric acid disappearance of yellow color indicates the presence of Flavonoids

Anthraquinones (Borntrager's Test): 1ml of benzene and 1ml of 10% ammonia was added with 300µl of extract presence of anthroquinone was observed by the formation of pink, red or violet color in the lower phase of ammonia.
Reducing sugar: Few drops of Molisch's reagent were added with dilute extracts and heated for 30 minutes and observed for the formation of brick red color precipitate.
Saponins (Froth Test): 300µl of extract was added with 2ml of distilled water in a test tube the solution was vigorously shaken and observed for the stable froth persistence.
Protein (Nin hydrin Test): 1ml of distilled water was added with 300µl of extract and 300µl of Nin hydrin the solution was boiled for 5 to 10 minutes Dark purple color indicates the presence of Amino acids.
Lipids and Fat: A small quantity of powered drug was rubbed on a clean and neat filter paper and observed for a permanent translucent strain.
Acidic compounds: one pinch of sodium bicarbonate was added with 300ul of extract. Effervescence indicates the presence of acidic compounds two duplicates are maintained for each test.
Heavy metals and N.P.K analysis:
Heavy metals analyses was done by using Gas Chromatography/Mass Spectrometry (GC/MS), and N.P analyses was done by total Kjeldahl digests by UV-VIS spectrophotometry, and K analysis was done in Kjeldal digests by Flame photometry method in Central Agriculture Laboratory, Guyana Sugar Corporation Inc. LBI, Guyana .

III. RESULTS AND DISCUSSION

Qualitative estimation of phytochemicals was performed in leaves of T. catappa. The results were tabled and represented as '+' for presence and '–'for absence of the phytochemicals in Table 1. Quantitative analysis was done to determine in the presence of heavy metals in leaves of T. catappa by using Gas Chromatography/Mass Spectrometry (GC/MS). Heavy metals estimated in mg per kg in Table 2, and analysis N.P. K in % Table in 3.

Table 1. Phytochemical analysis in the leaves of T. catappa

Plant Name	Phytochemicals Names	Aqueous	Ethyl acetate	Methanol	Ethanol
Terminalia catappa (Almond)	Alkaloids	+	+	+	+
	Flavonoids	+	+	+	+
	Tannins	+	+	+	+
	Thiols	-	-	-	-
	Amino acids	+	+	+	+
	Carbohydrates	+	+	+	+
	Phenols	+	+	+	+
	Phytosterols	+	+	+	+
	Glycosides	-	-	-	-
	Triterpenoids	+	+	+	+
	Fixed oils, fats	-	-	-	-
	Proteins	+	+	+	+
	Saponins	-	+	+	+
	Steroids	-	-	-	-
	Phlobatannins	-	-	-	-
	Anthroquinone	-	-	-	-
	Resins	-	-	-	-

+ = Presence - = Absence

Qualitative estimation of phytochemicals in leaves of T. catappa show that Alkaloids, Flavonoids, Tannins, Amino acids, Carbohydrates, Phenols, proteins, triterpenoids and Phytosterols are present in all extracts, whereas Thiols, Glycosides, Fixed oils, fats, Steroids, Phlobatannins, Resins and Anthroquinone phytochemicals are absent.

Table 2. Heavy metal analysis in Tamarind (T. catappa) leaves in mg/ kg

Name of plant	Used part	Parameter	Mg/kg
Terminalia catappa (Almond)	Leaves	Zn	66.63
		Cu	9.73
		Ni	Nd
		Mn	60.70
		Fe	136.71
		Ca	167.59
		Mg	297.80

Nd = Not detected

Heavy metal analysis was done in leaves of T. catappa by using Gas Chromatography/Mass Spectrometry (GC/MS). It shows the highest amounts was that of Mg (297.80mg/kg), followed by Ca (167.59mg/kg**),** whereas the least amount was that of Cu (9.73mg/kg). GC/MS did not detect Ni.

Table 3. N.P. K. analysis in Tamarind (T. catappa) leaves in percentage (%)

Name of plant	Used part	Parameter	(%)
Terminalia catappa (Almond)	Leaves		
		N	2.14
		P	0.18
		K	0.90

N.P.K. analysis shows the highest percentage was that of nitrogen in T. Indica leaves, followed by potassium; the least percentage of Phosphorus.

IV. CONCLUSION

The antioxidant and antimicrobial activity of T. catappa leaves has been reported worldwide, but it has not been possible to establish a relationship with the chemical composition due to the scanty information availed. In this study, we detected seven components of heavy metals not previously reported, and confirmed the high Fe and Ca presence in T. catappa leaves. In addition high percentage of nitrogen and some useful phytochemicals are available in T. catappa leaves. This information give light to the present intention to find chemical proof that supports the pharmacological activities of T. catappa leaves.

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