



DETERMINATION OF NUTRITIONAL, ELEMENTAL AND PHYSICOCHEMICAL CONTENTS OF LEAF EXTRACTS OF *TERMINALIA CATAPPA* IN GEIDAM LOCAL GOVERNMENT AREA OF YOBE STATE, NIGERIA.

BY

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ABSTRACT

Medicinal plants have been identified and used throughout human history, plants have ability to synthesize a wide variety of chemical compounds. Many of which are effective and contain compounds that are potential drugs which require further examinations. Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids etc., which have been found to possess antioxidant properties *in vitro*. Antioxidants are molecules that inhibit or scavenge free radical reactions and delay or inhibit cellular damage. Although, almost all organisms possess antioxidant defence and repair mechanisms that have evolved to protect them against oxidative damage, these mechanisms are insufficient to prevent the damage entirely. However,

antioxidant supplements, or foods containing antioxidants, may be used to help human body reduced oxidative damage. Studies on the nutritional value and biological activity of the kernel of *Terminalia catappa* revealed that it has a good digestibility, exerts a strong antioxidant activity, possesses anti-HIV properties, anti-asthma properties, anti-inflammatory, anti-carcinogenic, antibacterial and hepatoprotective properties. The fresh leaf of *Terminalia catappa* was air dried under laboratory conditions, was ground into fine powder extracted with methanol using cold infusion method and partitioned using solvent of gradient polarities such as *n*-hexane, ethyl acetate and *n*-butanol. Methanol crude extract, *n*-hexane portion, ethyl acetate portion, *n*-butanol portion and aqueous portion revealed the presence of carbohydrate. The proximate analysis indicates that the dry matter content in *Terminalia catappa* leaf is 93.0%, moisture content is 7.0%, ether extract is 1.0%, crude fibre is 21.0%, ash is 10.0%, crude protein is 10.00%, carbohydrate is 51.00% and therefore this plant can be used as a good source of energy and nutrients. The leaf of *Terminalia catappa* indicates the presence of Calcium (19.68µg/ml), Cadmium (0.12µg/ml), Copper (6.84µg/ml), Iron (10.67µg/ml), Potassium (18.90µg/ml), Magnesium (10.27µg/ml), Manganese (1.27µg/ml), Sodium (15.30µg/ml) Nickel (1.00µg/ml), Zinc (4.17µg/ml), Chloride (0.72µg/ml), Nitrate (46.00µg/ml), Phosphate (70.00µg/ml) and Sulphate (227.33µg/ml).

Keywords: *Terminalia catappa*, Proximate analysis, Elemental analysis and Physicochemical

Introduction

Medicinal plants have been in used throughout human history to treat aliment and diseases. Plants have the ability to synthesize a wide variety of chemical compounds (Lai and Roy, 2004). Many of which are efficacious and contain substances that are potential drugs which require further examinations (Abdulrahman *et al.*, 2007). Plants have evolved with the ability to synthesize chemical compounds that can help them defend against attack from a wide variety of predators such as insects, fungi and herbivorous mammals (Neelavathi *et al.*, 2013). Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids etc. which have been found to possess to antimicrobial properties *in vitro* (Dahanukar, 2000). *Terminalia Catappa* is a large, deciduous tree with smooth grey bark and whorled branches that form a canopy and is found in tropical and subtropical regions, it is widely planted throughout the tropics as an ornamental tree for shade for the edible nuts. *Terminalia catappa* contains hydrolyzable tannins punicalagin (major tannin), punicalin, terflavins A and B, tergalagin, tercatatin, chebulagic acid, geraniin, granatin B, corilagin), flavonoids (isovitexin, vitexin, isoorientin, rutin) and triterpenoids (ursolic acid, 2 α , 3 β , 23-trihydroxyurs-12-en-28 oic acid and asiatic acid) (Kinoshita *et al.*, 2007). The leaves, bark and fruit of the tree *Terminalia catappa* L. (Combretaceae) have been commonly used as a folk medicine for antidiarrhea, antipyretic and haemostatic purposes (Lin, 1992). The leaves of *T. catappa* have been used for the prevention and treatment of hepatitis and liver-related diseases (Lin and Khan, 1990). Antioxidants are molecules that inhibit or quench free radical reactions and delay or inhibit cellular damage (Young *et al.*, 2001). Although almost all organisms possess antioxidant defence and repair systems that have evolved to protect them against oxidative damage, these systems are insufficient to entirely prevent the damage (Simic, 1988). The aim of this study is to find out chemical composition and scavenging effectiveness of the leaf of *Terminalia catappa*.

Methodology

Study Area

This study was conducted in Geidam Local Government Area of Yobe State, it lies between longitude 12° 53' 49" N and latitude 11° 55' 49" E. It has an area of 4,357km² and a population of 244,900 at the 2022 census.

Sample collection and Preparation

Fresh leaf of *Terminalia catappa* was collected from Geidam local government area of Yobe State, Nigeria, and identified by a Plant Taxonomist. The plant leaf material was air-dried in the laboratory at room temperature. The leaf of the plant was grounded to fine powder using wooden mortar and pestle and the sample was given a voucher number (TT99) and stored in the laboratory in department of science laboratory technology (S.L.T) Mai Idris Aloomo Polytechnic Geidam for further analysis.

Preparation and Elemental Content Analysis

Ashing of Sample

The air-dried plant leaf was ground and placed in a porcelain crucible and put into muffle furnace at a temperature 500°C for about 3 hours after the sample has been ashed, it was removed and cooled in a desiccator for further analysis.

Digestion of Sample

Zero point five grams (0.5g) of the ashed sample was weighed into 250ml beaker. 10ml of 6 mole of hydrochloric acid was added. The beaker was covered with watch glass and heated for 15 minutes, 1ml of concentrated nitric acid was added and the sample was heated to evaporate to dryness. 1ml of 6 mole of hydrochloric acid and 10ml of distilled water was added and heat again on a hot plate to complete dissociation. It was left to cool and then filtered with Whatman filter paper into 100ml volumetric flask and made up to the mark with distilled water and transfer into polythene bottle for analysis. Micro and macro elements were analysed using Atomic Absorption Spectroscopy (AAS) using standard procedure and anions was analysed using spectrophotometer following guided protocols (AOAC, 1990).

Sample extraction and Partitioning

The ground leaf material (800g) was extracted with 85% methanol using maceration (cold infusion) method for 72 hours. The crude extract was concentrated under reduced temperature. The crude extract was then stored in a desiccator. The methanol extract of *Terminalia catappa* was partitioned with n-hexane until exhaustion. The aqueous fraction was partitioned with ethyl acetate and also partitioned exhaustively with n-butanol. The n-butanol portion and the aqueous portion were then evaporated using rotatory oven.

Test for Carbohydrate Molish's Test

The extract (0.5g) was dissolved in 5ml distilled water in a test tube. To the mixture, few drops of the Molish's reagent was added and then followed by 1ml of conc. tetraoxosulphate (VI) acid by the side of the test tube. The mixture was allowed to stand for two minutes and was diluted with 5ml of distilled water. Formation of a red or dull violet colour at the inter phase of the two layers was a positive test (Trease and Evans, 2002).

Test for Monosaccharide (Barfoed's Test)

The extract (0.5g) was dissolved in 5ml distilled water and filtered. One ml (1ml) of the filtrate was mixed with 1ml of Barfoed's reagent in a test tube. This was heated on a water bath for two minutes. A red precipitates of cuprous oxide was considered as positive test (Brain and Turner, 1975).

Test for Combined Reducing Sugar

The extract (0.2g) was mixed with 5ml dilute hydrochloric acid and boiled. The mixture was neutralized with 3ml sodium hydroxide solution. Drops of Fehling's solution was added to it and then heated on a water bath for two minutes. Reddish brown precipitates of cuprous oxide was showing the presence of combined reducing sugars (Trease and Evans, 2002)

Test for Free Reducing Sugar (Fehling's Test)

The extract (0.2g) was dissolved in 5ml distilled water and filtered. To the filtrate, 5ml of equal volumes of Fehling's solution A and B was added. Formation of red precipitates of cuprous oxide was an indication for the presence of reducing sugar (Trease and Evans, 2002).

Test for Soluble Starch

The extract (0.5g) was boiled with 1ml of 5% potassium hydroxide and cooled. To the mixture 2ml of tetraoxosulphate (VI) acid was added. A yellow colouration indicates the presence of soluble starch (Vishnoi, 1979).

Test for Pentoses

The extract (0.5g), 1ml of hydrochloric acid was added and 0.2g of phoroglucinol. The mixture was heated with low flame. The appearance of red colour was indicating the presence of pentose (Vishnoi, 1979).

Salivanoff's Test (Standard Test for Ketones)

The extract (0.5g) few crystals of resorcinol and 2ml of hydrochloric acid was added and the mixture was boiled for 5 minutes. The appearance of red colouration was indicating the presence of ketones (Vishnoi, 1979).

Lead Ethanoate Test

The extract (0.5g) was boiled with 5ml of distilled water and then filtered. To 5ml of the filtrate, 3ml of lead ethanoate solution was added. The appearance of a buff coloured precipitate was indicating the presence of flavonoids (Brain and Turner, 1975).

Sodium Hydroxide Test

The extract (0.5g), 5ml of Water was added and filtered. To the filtrate, 2ml of 10% aqueous sodium hydroxide was added to produce a yellow colouration. A change in the colour from yellow to colourless on addition of dilute hydrochloric acid was an indication for the presence of flavonoids (Trease and Evans, 2002).

Results and Discussion

Table 4.1: The Weight, Percentage Yield, Colour and Texture of the Methanol Crude extract and Partitioned portions of *Terminalia catappa*.

S/NO.	Extract	Weight (g)	Percentage yield (%) ^{W/W}	Colour	Texture
1.	MCE	83.82	8.382	Dark green	Gummy
2.	NHP	16.38	1.638	Dark green	Oily
3.	EAP	0.75	0.075	Black	Gummy
4.	NBP	7.77	0.777	Brown	Oily
5.	AQP	29.97	2.997	Dark brown	Powdered

Key: MCE = Methanol Crude Extract, AQP = Aqueous Portion, NHP = n-Hexane Portion, NBP= n-Butanol Portion and EAP = Ethyl Acetate Portion.

The methanol crude extract yield 8.382% ^W/_W dark green in colour gummy in texture, n-hexane portion yield 1.638% ^W/_W, dark green in colour, oily in texture, ethyl acetate portion yield 0.075% ^W/_W, black in colour, gummy in texture, n-butanol portion yield 0.777% ^W/_W, brown in colour, oily in texture and aqueous portion yield 2.997% ^W/_W, dark brown in colour, powdered in texture.

Table 1: Proximate Content Analysis of the Leaf of *Terminalia catappa*

S/NO.	Proximate Content	Percentage Content (%)
1.	Dry Matter	93.00
2.	Moisture Content	7.00
3.	Crude Protein	10.00
4.	Ether Extract	1.00
5.	Crude Fibre	21.00
6.	Ash	10.00
7.	Carbohydrate	51.00

The proximate analysis indicates that the dry matter content in *Terminalia catappa* leaf is 93.0%, moisture content is 7.0%, ether extract is 1.0%, crude fibre is 21.0%, ash is 10.0%, crude protein is 10.00%, carbohydrate is 51.00% and therefore this plant can be used as a good source of energy and nutrients.

Table 2: Physicochemical Analysis of Macro Element, Micro Elements and Anions in *Terminalia catappa* Leaf

S/NO.	Elements	Concentration level (µg/ml)	WHO Standard (1996) (µg/ml)
1.	Ca	19.68	3600-80000
2.	Cd	0.12	10-35
3.	Cu	6.84	100-300
4.	Fe	10.67	50-5000
5.	K	18.90	10-100
6.	Mg	10.27	100-200
7.	Mn	1.27	100-20000
8.	Na	15.30	400-500
9.	Ni	1.00	10-50
10.	Zn	4.17	150-20000
11.	Cl ⁻	0.72	250
12.	NO ₃ ⁻	46.00	50
13.	PO ₄ ³⁻	70.00	0.40
14.	SO ₄ ²⁻	227.33	200

Key: Ca = Calcium, Cd = Cadmium, Cu = Copper, Fe = Iron, K = Potassium, Mg = Magnesium, Mn = Manganese, Na = Sodium, Ni = Nickel, Zn = Zinc, Cl⁻ = Chloride, NO₃⁻ = Nitrate, PO₄³⁻ = Phosphate, SO₄²⁻ = Sulphate

The leaf of *Terminalia catappa* indicates the presence of Calcium (19.68µg/ml), Cadmium (0.12µg/ml), Copper (6.84µg/ml), Iron (10.67µg/ml), Potassium (18.90µg/ml), Magnesium (10.27µg/ml), Manganese (1.27µg/ml), Sodium (15.30µg/ml) Nickel (1.00µg/ml), Zinc (4.17µg/ml), Chloride (0.72µg/ml), Nitrate (46.00µg/ml), Phosphate (70.00µg/ml) and Sulphate (227.33µg/ml). However, only phosphate and sulphate exceeded the permissible limit of World Health Organization (WHO) standard, although they are important nutrient in less concentration. Heavy metals such as Copper (Cu), Iron (Fe), Manganese (Mn), and Zinc (Zn) which are present in this study are important micronutrients for plants. Elements such as Cu, and Zn are integral part of Cu-ZnSOD (superoxide dismutase), Fe is an integral part of Catalase and Mn is an integral part of Mn-SOD so, the enzymes superoxide dismutase which can be found in various cell compartments and it catalyses the disproportionation of two O₂⁻ radicals to H₂O₂ and O₂ (Hegedus *et al.*, 2001; Scandalios, 1993) and catalase is

eliminates peroxide H_2O_2 (Hernandez *et al.*, 1999; Kono and Fridovich, 1983; Scandalios, 1993). Copper can act as both antioxidant and pro-oxidant, as antioxidant copper scavenges or neutralizes free radical and may reduce or help prevent some of the damage they cause (Araya *et al.*, 2006, Davis, 2003) Copper promotes free radical damage to the tissues when it acts as prooxidant (Christen, 2000). Mn is necessary for building chloroplasts and deficiency may result in chlorosis (Jagessar and Allen, 2012). Iron is necessary for photosynthesis and is present as an enzyme cofactor in plants. Iron deficiency results in interveinal chlorosis and necrosis it is not the structural part of chlorophyll but very much essential for its synthesis (Jagessar and Allen 2012). Zinc 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 dehydrogenases, proteinase 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 (Jagessar and Allen, 2012). Potassium (K) is an important macronutrient with concentration level of $18.90\mu\text{g/ml}$. It is an essential element which plays role in both physiological and biochemical process. It plays also a special role under environment stresses condition for plant survival and shortage of potassium levels and plants sensitive increase to environmental stresses (Cakmak, 2005). In stresses condition, reactive oxygen species (ROS) production in plants stimulates severely (Marschner and Cakmak, 1989) during stress period usually enzymes such as Superoxide Dimutase, Asurbate Peroxidase and Glutatin Reductase stimulate (Mishra *et al.*, 1995). Calcium was found with the highest concentration of $19.68\mu\text{g/ml}$ among micro/macro concentration level, it acts a role by activating large number of enzymes which plays an important role in scavenging activity (Williams, 1992). Hence, *Terminalia catappa* can serve as a source for calcium supplement. Anions determined were chloride, nitrate, phosphate and sulphate. Sulphate shows the highest concentration and will increase the activity of the enzymes Ascorbate peroxidase which is the most solute antioxidant in chloroplast and it can be used as good source of sulphate (Parvaneh *et al.*, 2013).

Conclusions

Terminalia catappa revealed the presence of calcium ($19.68\mu\text{g/ml}$), cadmium ($0.12\mu\text{g/ml}$), copper ($6.84\mu\text{g/ml}$), iron ($10.67\mu\text{g/ml}$), potassium ($18.90\mu\text{g/ml}$), magnesium ($10.27\mu\text{g/ml}$), manganese ($1.27\mu\text{g/ml}$), sodium ($15.30\mu\text{g/ml}$), nickel ($1.00\mu\text{g/ml}$), zinc ($4.17\mu\text{g/ml}$) and anions chloride ($0.72\mu\text{g/ml}$), nitrate ($46.00\mu\text{g/ml}$), phosphate ($70.00\mu\text{g/ml}$) and sulphate ($227.33\mu\text{g/ml}$). And nutrient such as moisture, has percentage 7%, crude protein, 10%, fat, 1%, crude fibre, 2%, ash, 10% and carbohydrate, 51%. This study has already shown that the leaf of *Terminalia catappa* contains many medicinal and important phytochemicals properties.

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