DETERMINATION OF CHEMICAL COMPOSITION AND SCAVENGING EFFECTIVENESS OF LEAF EXTRACTS OF TERMINALIA CATAPPA IN GEIDAM LOCAL GOVERNMENT AREA OF YOBE STATE, NIGERIA

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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 efficacious 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 antimicrobial properties in vitro. Antioxidants are molecules that inhibit or quench free radical reactions and delay or inhibit cellular damage. Although, almost all organisms possess antioxidant defence and repair systems that have evolved to protect them against oxidative damage, these systems are insufficient to prevent the damage entirely. However, antioxidant supplements, or foods containing antioxidants, may be used to help human body reduce 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 condition, 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, cardiac glycoside, flavonoid, terpenoids, saponins, tannins and alkaloid. However, soluble starch, phlabotannins and glycosides were not found in the extracts. The methanol crude extract showed the percentage inhibition of 98.25 at 10µg/ml, 97.40 at 20µg/ml, 96.94 at 30µg/ml, 96.63 at 40µg/ml and 97.10 at 50µg/ml; n-butanol portion showed the percentage inhibition of 95.75 at 10µg/ml, 96.40 at 20µg/ml, 96.15 at 30µg/ml, 96.40 at 40µg/ml and 96.15 at 50µg/ml; n-hexane portion showed the percentage inhibition of 95.50 at 10µg/ml, 95.65 at 20µg/ml, 95.80 at 30µg/ml, 95.75 at 40µg/ml and 95.75 at 50µg/ml; ethyl acetate portion showed the percentage inhibition of 78.35 at 10µg/ml, 87.65 at 20µg/ml, 95.00 at 30µg/ml, 94.75 at 40µg/ml and 94.70 at 50µg/ml and the aqueous portion showed the percentage inhibition of 94.40 at 10µg/ml, 95.10 at 20µg/ml, 96.00 at 30µg/ml, 95.50 at 40µg/ml and 96.05 at 50µg/ml.

Keywords: Terminalia catappa, Phytochemicals, Proximate analysis, Elemental analysis, Secondary metabolites
Introduction

Medicinal plants have been in used throughout human history to treat ailments 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, tergallagin, tercatain, chebulagic acid, geraniin, granatin B, corilagin), flavonoids (isovitexin, vitexin, isoorientin, rutin) and triterpenoids (ursolic acid, 2α, 3β, 23-trihydroxyurs-12-en-28 oic acid and asiatic acid) (Liu et al., 1996 and 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 12º 53’ 49”N and latitude 11º 55’ 49”E. It has area of 4,357km² and a population of 157,295 at the 2006 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 Alooma Polytechnic Geidam for further analysis.

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.

Phytochemical Screening of *Terminalia catappa*

Phytochemical screening was carried out on the methanol crude extract and the partitioned portions of the plant *Terminalia catappa* using standard protocols as described by (Trease and Evans, 2002, Silver et al., 1998, Markham, 1987, Brain and Turner, 1975).
Antioxidant Studies

The free radical scavenging capacity of the extracts was determined using 1,1-Diphenyl 1,2- Picrylhydrazyl (DPPH) (Li et al., 2001).

Results and Discussion

Table 1: Phytochemical Screening of *Terminalia catappa* Methanol Crude extract, n-Hexane Portion, Ethyl acetate Portion, n-Butanol Portion, and Aqueous Portion.

<table>
<thead>
<tr>
<th>S/NO</th>
<th>Test</th>
<th>MCE</th>
<th>NHP</th>
<th>EAP</th>
<th>NBP</th>
<th>AQP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Carbohydrate</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Soluble starch</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>Phlabotannins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td>Glycosides</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>Cardiac glycoside</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>Flavonoid</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td>Terpenoid</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>8.</td>
<td>Saponins</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>9.</td>
<td>Alkaloid</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>10.</td>
<td>Tannins</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>


(GC7890B-MSD-5977A-AGILENT TECH USA)
Mr. Umar Dan’Azumi (Fraction T<sub>CA</sub>)

Chromatogram

Fig. 4.1: Chromatogram of Fraction T<sub>CA</sub>
Methanol crude extract, n-hexane portion, ethyl acetate portion, n-butanol portion and aqueous portion revealed the presence of carbohydrate, cardiac glycoside, flavonoid, terpenoids, saponins, tannins and alkaloid. However, soluble starch, phlabotannins and glycosides were not found in the extracts. The presence of secondary metabolites in the *Terminalia catappa* such as tannins, cardiac glycoside, flavonoid, saponins and phenolic compound, indicates or implicate the medicinal value of it. These compounds have been reported to have antioxidants property and exhibit a wide range spectrum of medicinal properties such as anti-cancer, anti-inflammatory and anti-diabetes (Odukoya et al., 2005, Abdulrahman et al., 2010).
Table 2: Percentage Inhibition of Standard, Methanol extract, n-Butanol Portion, n-Hexane Portion, Ethyl acetate Portion, and Aqueous Portion of *Terminalia catappa*

<table>
<thead>
<tr>
<th>S/NO.</th>
<th>Extract</th>
<th>10µg/ml</th>
<th>20µg/ml</th>
<th>30µg/ml</th>
<th>40µg/ml</th>
<th>50µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>VIT C</td>
<td>99.25±0.05000(^a)</td>
<td>98.95±0.05000(^a)</td>
<td>98.85±0.1500(^a)</td>
<td>96.65±1.850(^a)</td>
<td>98.25±0.250(^a)</td>
</tr>
<tr>
<td>2</td>
<td>ME</td>
<td>98.25±0.1500(^a)</td>
<td>97.40±0.2000(^a)</td>
<td>96.94±0.0350(^a)</td>
<td>96.63±0.035(^a)</td>
<td>97.10±0.1000(^a)</td>
</tr>
<tr>
<td>3</td>
<td>NBP</td>
<td>95.75±0.3500(^a)</td>
<td>96.40±0.3000(^a)</td>
<td>96.15±0.7500(^a)</td>
<td>96.40±1.000(^a)</td>
<td>96.15±1.050(^a)</td>
</tr>
<tr>
<td>4</td>
<td>NHP</td>
<td>95.50±0.9000(^a)</td>
<td>95.65±1.5500(^a)</td>
<td>95.80±1.7000(^a)</td>
<td>95.75±1.950(^a)</td>
<td>95.75±2.150(^a)</td>
</tr>
<tr>
<td>5</td>
<td>EAP</td>
<td>78.35±0.95(^b)</td>
<td>87.65±0.7500(^a)</td>
<td>95.00±1.2000(^a)</td>
<td>94.75±1.450(^a)</td>
<td>94.70±0.9000(^a)</td>
</tr>
<tr>
<td>6</td>
<td>AQP</td>
<td>94.40±0.00(^a)</td>
<td>95.10±0.3000(^a)</td>
<td>96.00±0.1000(^a)</td>
<td>95.50±0.1000(^a)</td>
<td>96.05±0.150(^a)</td>
</tr>
</tbody>
</table>

**Key:** VIT C = Vitamin C (Ascorbic acid), ME = Methanol extract, NBP = n-Butanol Portion, NHP = n-Hexane Portion, EAP = Ethyl Acetate Portion, and AQP = Aqueous Portion.

\(^a\) = \(P \leq 0.05\) significance across the column

\(^b\) = \(P \leq 0.05\) insignificance across the column

**Chromatogram**

The chromatogram revealed the presence of forty-two (42) compounds, but nineteen (19) compounds were having the comparison from the National Institute Standard and Technology (NIST) library such as Octadecanoic acid, 4-hydroxybutyl ester. It is important for fetal growth and development particularly for the central nervous system, affecting visual activity as well as cognitive function (12) and Phen-1-4 diol, 2,3-dimethyl-5-trifloromethyl which can be used as an antioxidant, antithrombotic and anti-tuberculosis [13, 14, 15], other peaks could not be identified from the library.

**peak 1, rt: 8.179, base peak 73.1, Mz 355.1**

**peak 1, mass spectrum comparison**
peaks

peak 2, rt: 8.608, base peak 58.1 Mz 355.2

peak 3, rt: 10.050 mins, base peak 133.1 Mz 355.1

peak 4, rt: 10.250, base peak Mz

peak 5, rt: 10.851, base peak 207.1 Mz 355.0
peak 6, rt: 13.048, base peak 207.1 Mz 355.1

Hit 1. (mainlib) d-Mannitol, 1-decylsulfonyl-

peak 7, rt: 14.891, base peak 73.1 Mz 429.1

Hit 2. (mainlib) Octadecanoic acid, 4-hydroxybutyl ester

peak 8, rt: 15.720, base peak 73.1 Mz 429.1

Hit 1. (mainlib) Stearic acid, 3-(octadecyloxy)propyl ester
α-D-Glucopyranosyl, O-α-D-glucopyranosyl-(1→3)-β-D-fructofuranosyl

peak 8 Ms comparison

peak 9, rt: 17.488, base peak 71.1 Mz 355.0
4-Methyldocosane

peak 10, rt: 18.312, base peak Mz

peak 11, rt: 19.050, base peak 71.1 Mz 429.1

peak 12, rt: 19.594, base peak 71.1 Mz 429.1
peak 13, rt: 20.595, base peak 71.1 Mz 327.1

peak 14, rt: 21.190, base peak 71.1 Mz 405.1

peak 15, rt: 22.592, base peak 84.1 Mz 405.1

peak 16, rt: 23.479, base peak 73.1 Mz 503.1
peak 17, rt: 23.622, base peak 73.1 Mz 503.1

peak 18, rt: 23.834, base peak 57.1 Mz 405.1

peak 19, rt: 24.629, base peak 73.1 Mz 415.0
peak 20, rt: 24.732, base peak 121.0 Mz 405.1

peak 21, rt: 25.448, base peak 121.0 Mz 405.1

peak 22, rt: 26.043, base peak 253.0 Mz 405.1

peak 23, rt: 26.140 mins, base peak 71.1 Mz 405.0
peak 24, rt: 26.392 mins, base peak 71.1 Mz 405.0

peak 25, rt: 27.290 mins, base peak 253.0 Mz 405.1

peak 26, rt: 28.400, base peak 105.0 Mz 429.1

peak 27, rt: 29.991, base peak 405.1 Mz 405.1
Hit 1. (mainlib) 1,3-Dioxane, 5-(hexadecyloxy)-2-pentadecyl-, trans-

peak 28, rt: 30.906, base peak 73.1 Mz 503.1

peak 29, rt: 31.639, base peak 135.1 Mz 479.1

peak 30, rt: 32.091mins, base peak 149.0 Mz 429.0
peak 31, rt: 32.377, base peak 73.1 Mz 429.0

peak 32, rt: 32.675, base peak Mz

peak 33, rt: 33.739, base peak 135.1 Mz 403.0

peak 34, rt: 34.071, base peak 135.1 Mz 355.1

peak 35, rt: 35.141, base peak 73.1 Mz 429.0
peak 36, rt: 35.530, base peak 55.1 Mz 355.1

peak 37, rt: 36.531, base peak 207.0 Mz 429.1
peak 38, rt: 37.389, base peak Mz

peak 39, rt: 38.723, base peak 207.1 Mz 429.0

peak 40, rt: 39.117, base peak 207.1 Mz 479.0
Conclusions

The results of antioxidant evaluation showed that the methanol crude extract as well as the partitioned portions indicates promising antioxidant properties of leaf of *Terminalia catappa*, the reducing power of the extracts compared with the reference standard antioxidant drug (ascorbic acid). The DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants. This study has already shown that the leaf of *Terminalia catappa* contains many medicinal and important phytochemical properties.

Reference


antimetastatic effects of *Terminalia catappa* L. leaves on lung cancer cells. *Food and chemical tox.*, 45(7), 1194-1201.


