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Proximate Analysis and Phytochemical Profile of *Brachystegia eurycoma* Leaves

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Authors' contributions

This work was carried out in collaboration among all authors. Authors UOU, MOM and LCC designed the study. Author UOU wrote the protocol, performed the statistical analysis and wrote the first draft of the manuscript. Authors MOM and LCC managed the analyses of the study. Author LCC managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study investigated the proximate and phytochemical composition of *Brachystegia eurycoma* leaves.
Methods: Crude ethanol extract of *B. eurycoma* leaves was obtained by cold extraction method. AOAC method was used for proximate analysis. Phytochemical profiling was done with qualitative phytochemical evaluation and gas chromatography-mass spectrometry (GC/MS) analytical method. Matching and interpretation of the spectral was done with the National Institute standard and Technology (NIST05) library.
Results: The proximate analysis result showed *B. eurycoma* leaves to be abundant in parameters evaluated in the order of 31.47±0.43% Carbohydrate > 15.15±0.04% Ash > 14.45±0.15 crude fibre > 13.83±0.32 protein > 13.14±0.22 moisture > 1.97±0.01 fat. Qualitative phytochemical analysis detected alkaloid, saponin, tannin, diterpenes, phenol, quinine, flavonoid, protein, xanthoprotein and cardiac glycoside in the leaves of *B. eurycoma*. GC/MS data showed that the prevailing volatile bioactive compounds in ethanol leaf extract of *B. eurycoma* were 3-O-Methyl-d-glucose (13.23%), cis-9-Hexadecenal (10.40%), Desulphosinigrin (10.34%), Phytol (7.58%), Hydroquinone (7.23%), n-Hexadecanoic acid (6.61%), Oleoyl chloride (6.10%), 9,12-Octadecadienoic acid (Z,Z)- (5.89%),

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Hexadecanoic acid, (2.97%), Benzofuran, 2,3-dihydro-(1.94%), Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) (1.92%).
Conclusion: The result of this preliminary investigation reveals the potentials of *B. eurycoma* leaves as candidate for food, pharmaceutical, cosmetic and nutraceutical industries.

Keywords: Gas chromatography mass spectrometry (GC-MS); vegetable; volatile; nutraceutical; Desulphosinigrin; drug discovery.

1. INTRODUCTION

Plants contain a wide range of bioactive chemical substances (flavonoids, alkaloids, steroids, trepenoids, phenolic acids, tannins, saponins among others) that exhibits therapeutic, physiological and biochemical effects in human body [1-4]. Scientific research resulting in phytocomponent profiling, isolation, purification and characterization of phytochemicals has led to the discovery of drug candidates, production of active drugs, supplements and food additives used in the treatment and management of different ailments all over the world [5-7].

Proximate analysis is an important procedure to determine the overall composition, nutritional status, quality and energy value of any ingredient intended for use as food [8]. Preliminary phytochemical screening is used to identify the classes of bioactive constituents present in plant [9]. Gas Chromatography Mass Spectroscopy is a technique which is used to separate and identify volatile compounds based on retention time and fragmentation pattern. The fragmentation pattern for a compound is unique and is therefore an identifying characteristic of that compound [10,11]. GC-MS studies have been increasingly applied for the analysis of medicinal plants in recent year as this technique has proved to be a valuable method for the analysis of non-polar components and volatile essential oil, fatty acid, lipids and alkaloids [12].

Brachystegia eurycoma is a dicotyledonous leguminous tress belonging to the family *Fabaceae*. It is found in the swamps and rainforest of south, east and western Nigeria. It is called “Achi” by Igbos, “Ekalado” by the yorubas, “Taura” by the hausas, “Okweri” by the Edos and “Apaupan” by the Ijaw. In the eastern part of Nigeria, its seeds are used as thickener in preparation of local soups particularly egusi and ogbono soup, while its wound healing and inflammatory properties have been mentioned in literature [13].

The leaf, bark and root of the plant are used in ethno medicines for the treatment of various diseases including malaria, diabetes, rheumatism, hypertension, kidney problem, asthma, tuberculosis, bronchitis, catarrh and sore throat [14-16].

Research has shown the seed, stem bark and stem gum as well as phytocomponents isolated from them to be anti-inflammatory, antibacterial, analgesic, antioxidant, antimicrobial, anti-cancer and anti-diarrhea [17-21]. Several researches have been done on the seeds, stem bark and stem gum of *B. eurycoma* with a little attention on the leaves which despite its medicinal use with other parts of the plant is not eaten as food. Hence this research to evaluate the nutrition and phytochemical components of *B. eurycoma* leaves with the aim of unveiling it possible nutritional and nutraceutical potentials.

2. MATERIALS AND METHODS

2.1 Plant Material

B. eurycoma leaves were collected from Abakiliki, Ebonyi State, Nigeria, authenticated by Dr. N. L. Edwin-Nwosu of the Department of Plant Science and Biotechnology, University of Port Harcourt, River State, Nigeria and assigned voucher number UPH.NO.V.1209. The leaves were rinsed in distilled water, air-dried and ground into powder.

2.2 Preparation of Ethanol Extract

The leaf powder was soaked in ethanol (70%) for 48 hours with occasional shaking. It was filtered and the filtrate concentrated after eliminating the ethanol using rotary evaporator.

2.3 Determination of Proximate Composition

The amount (%) of moisture, ash, lipid, protein, fiber, carbohydrates as well as energy level in air dried *B. eurycoma leaf* was determined in triplicate using the method of AOAC [22].

2.4 Preliminary Phytochemical Determination

The concentrated leaves extract of *B. eurycoma* were analyzed for the presence of saponins, flavonoids, tanins, alkaloids, phenols, quinines, protein, xanthoprotein, cardiac glucoside, Coumarin, steroids, diterpene and Anthraquinone using standard methods as described by Fafowora [23], Trease and Evans [24] and Harborne [25].

2.5 Gas Chromatograph-Mass Spectroscopy (GC-MS) Analysis

GC-MS was performed on a system consisting of GC2010 gas chromatograph and a Shimadzu QP2010 ultra quadrupole mass spectrometer equipped with a DB-Wax fused silica capillary column (30m x 0.25mm ID x 0.25µm df, composed of silphenylene polymer). Initial temperature was programmed at 50°C, held for two minutes. It was increased to 300°C with the rate of 6.5°C/min and held for ten minutes. The temperature of the injector and detector were set up to 280°C and 300°C, respectively. Helium gas was used as a carrier gas. 1 µl of the fractions was diluted in 200 µl dichloromethane and then injected into the GC-MS [26,27].

2.6 Identification of Spectral

Interpretation of mass-spectrum was done by comparing the mass spectrum of the unknown component with spectrum of known components in the National Institute Standard and Technology (NIST05) database to ascertain the name, molecular weight and structure of the components of the test materials.

3. RESULTS

The result of proximate analysis shows that the leaves of *B. eurycoma* to contain 31.47±0.43% carbohydrates, 15.15±0.04% ash, 14.45±0.15% crude fiber, 13.83±0.32% protein, 13.14±0.22% moisture, 1.97±0.01% fat and 198.87±0.56 Kcal/100g (Table 1).

Table 1. Proximate composition of *B. eurycoma* leaves

Parameter	Unit	*B. eurycoma*
Carbohydrate (NFE)	%	31.47±0.43
Ash	%	15.15±0.04
Crude fibre	%	14.45±0.15
Protein	%	13.83±0.32
Moisture	%	13.14±0.22
Fat	%	1.97±0.01
Energy level	Kcal/100g	198.87±0.56

Qualitative phytochemical analysis revealed the presence of alkaloid, saponin, tannin, diterpenes, phenol, quinine, flavonoid, protein, xanthoprotein and cardiac glycoside in the leaves of *B. eurycoma*. Coumarin, steroids and anthraquinone were not detected (Table 2).

The GC-MS chromatogram obtained from ethanol extract of *B. eurycoma* shows the presence of 24 distinct peaks (Fig. 1).

Table 2. Qualitative phytochemical evaluation of *B. eurycoma* leaves

	Phytochemical components	Type of test	Ethanol leaf extract of *B. eurycoma*
1	Alkaloids	Mayer's Test	+
		Wanger's test	+
		Dragendroff's test	+
2	Phenol	Ferric Chloride	+
3	saponin	Frothing	+
4	Tannin	Ferric Chloride	+
5	Coumarin	alcoholic sodium hydroxide	-
6	Quinine	potassium hydroxide	+
7	Anthraquinone	Borntrager's	-
8	steroids	Libermann- Burchard	-
9	Protein	Million's	+
10	Xanthoprotein	General test	+
11	Cardiac glycoside	Keller kiliani	+
12	Flavonoid	sodium hydroxide	+
13	Diterpene	Copper acetate[25]	+

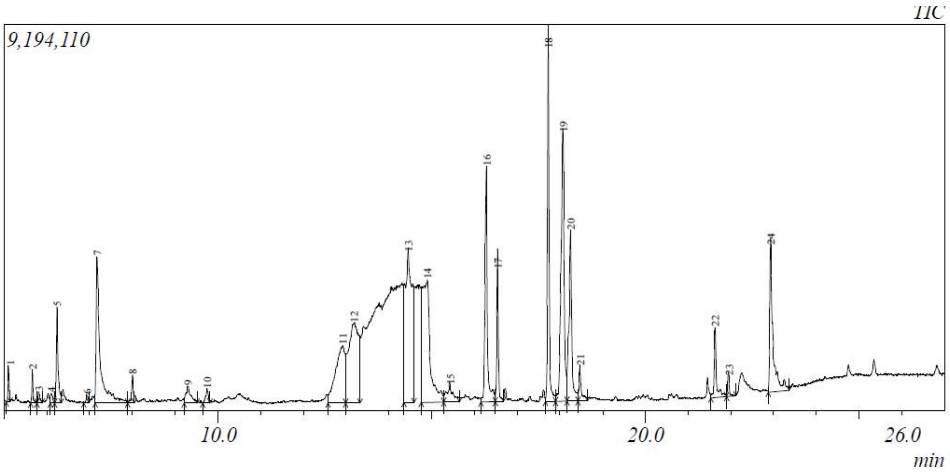
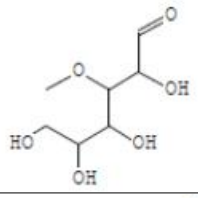
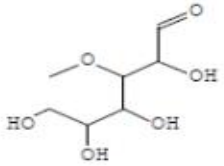
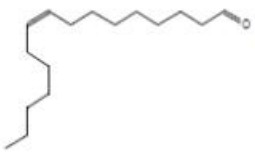
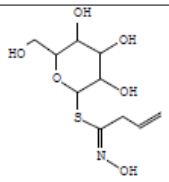
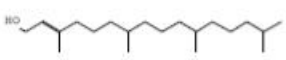
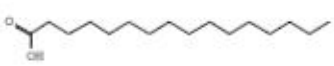
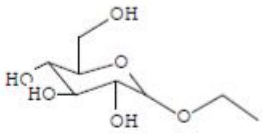
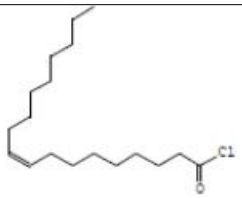
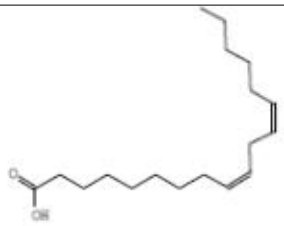
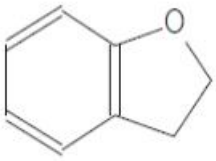
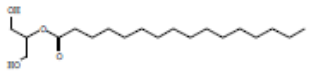
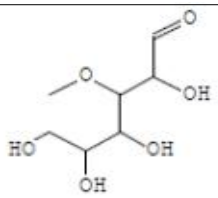
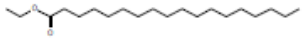
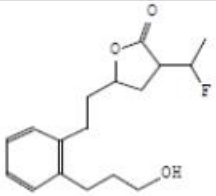
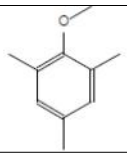
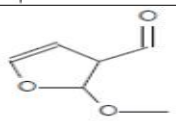
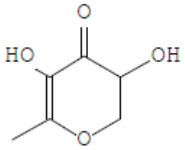
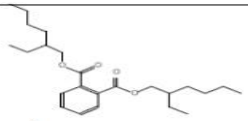
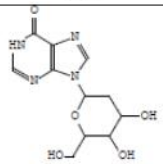
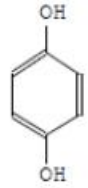
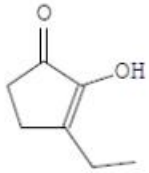
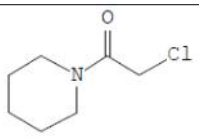


Fig. 1. GC-MS chromatogram of ethanol leaf extract of *B. eurycoma*

Table 3. Molecular formula and structure of phytochemicals identified in *B. eurycoma*

	Name of compound	Molecular formula	Structure	Molecular weight	Peak Area (%)
1	3-O-Methyl-d-glucose	$C_7H_{14}O_6$		194	13.23
2	3-O-Methyl-d-glucose	$C_7H_{14}O_6$		194	12.42
3	cis-9-Hexadecenal ; 9-Hexadecenal, (Z)-	$C_{16}H_{30}O$		238	10.40
4	Desulphosinigrin	$C_{10}H_{17}NO_6S$		279	10.34
5	Phytol	$C_{20}H_{40}O$		296	7.58
6	Hydroquinone	$C_6H_6O_2$		110	7.23

7	n-Hexadecanoic acid	$C_{16}H_{32}O_2$		256	6.61
8	Ethyl .alpha.-d-glucopyranoside	$C_8H_{16}O_6$		208	6.61
9	Oleoyl chloride	$C_{18}H_{33}C_{lO}$		300	6.10
10	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$		280	5.89
11	Hexadecanoic acid, ethyl ester ;	$C_{18}H_{36}O_2$		284	2.97
12	2,3-dihydro-Benzofuran Coumaran	C_8H_8O		120	1.94
13	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester Palmitin, 2-mono-	$C_{19}H_{38}O_4$		330	1.92
14	3-O-Methyl-d-glucose	$C_7H_{14}O_6$		194	1.43
15	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$		312	1.05
16	Tetrahydrofuran-2-one, 3-[1-fluoroethyl]-5-[[2-hydroxypropyl]benzeneethyl-	$C_{17}H_{23}FO_3$		294	0.99

17	Benzene, 2-methoxy-1,3,5-trimethyl-	$C_{10}H_{14}O$		150	0.56
18	Furan-3-carboxaldehyde 2-methoxy-2,3-dihydro-	$C_6H_8O_3$		128	0.50
19	4H-Pyran-4-one 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$		144	0.50
20	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$		390	0.49
21	9-[2-Deoxy-.beta.-d-ribohexopyranosyl]purin-6(1H)-one	$C_{11}H_{14}N_4O_5$		282	0.45
22	Hydroquinone	$C_6H_6O_2$		110	0.31
23	2-Cyclopenten-1-one 3-ethyl-2-hydroxy-	$C_7H_{10}O_2$		126	0.26
24	Piperidine, 1-chloroacetyl-	$C_7H_{12}C_lNO$		161	0.20

The phytochemicals identified in order of most abundant to least abundant based on percentage peak are presented in Table 3, showing their molecular formula and structure.

4. DISCUSSION

The findings from proximate analysis of this study shows *B. eurycoma* leaves to have the abundance of the parameters evaluated to be in Carbohydrate > Ash > crude fiber > protein > moisture > fat order. This shows *B. eurycoma leaves* are a better source of carbohydrates and fiber than proteins. Animals depend on carbohydrates among other macromolecules for generation of energy and some intermediates required for certain biological processes and the sustenance of life. The amount of carbohydrates (31.47%) is appreciable and similar to popular edible vegetables like *Celusia argenta (32.80%) and Corchorus olitorius* (31.30%) [28]. The ash content of the leaves (15.15%) is considerable showing that the leaves contain important mineral elements as the ash content of any sample is an index of mineral content.

The Recommended Dietary Allowance (RDA) of fiber is 19-25% for children, 21-38% for adult, 28% for pregnant mothers and 29% for breast-feeding mothers [29]. With crude fibre of 14.45%, *B. eurycoma* leaves will make a poor source of dietary fiber in human nutrition. Plant foods that provide more than 12% of its calorific value from protein is considered good source of protein [30]. *B. eurycoma* leaves meet this requirement, making it a good source of protein. Moisture content was lower when compared with that of common vegetable such as *Telfairia occidentalis 98%, Talinum triangulare* 91%, *Moringa oleifera* 87% and *Vernonia amygdalinan* 87% [31] but low moisture content is indicative of a longer shelf life. It is a general observation that leafy vegetables have low lipid content [30]. The low lipid content of *B. eurycoma* leaves is in agreement with this observation.

Preliminary phytochemical screening detected the presence of alkaloid, saponin, tannin, diterpenes, phenol, quinine, flavonoid, protein, xanthoprotein and cardiac glycoside. Alkaloids are diuretic in nature, they affect the nervous system and reduce appetite [32]. The ingestion of saponins as a part of the human diet have been linked with a variety of effects on health, including reducing blood cholesterol levels. They have also been reported to have pharmacological activities such as anti-inflammatory, antifungal, antibacterial, anti-parasitic, anti-cancer and antiviral activities [33]. Tannins are antiinflammatory, antidiarrheal, haemostatic, antiviral, antibacterial, antidiarrheal and antihemorrhoidal compounds which has been reported to relief sore throat, fatigue and skin ulcer [34]. Diterpenes are antimicriobial and anti-inflammatory [35]. Quinine has many medicinal applications due to its fever-reducing, painkilling and anti-inflammatory properties [36]. Many flavonoids have been shown to have antioxidative activity, free radical scavenging capacity, antihypertensive, hepatoprotective, anti-inflammatory, antiviral and anticancer activities [37]. Cardiac glycosides have cardiotonic activity, are antiviral, anticancer and antiproliferative effects [38].

GC-MS analysis of ethanol leaf extract of *B. eurycoma* revealed the presence of 24 phytochemical compounds; Amongst which were the sugar moiety (3-O-Methyl-d-glucose and Ethyl .alpha.-d-glucopyranoside), aldehyde (cis-9-Hexadecenal, Furan-3-carboxaldehyde-2-methoxy-2,3-dihydro-), Glucosinolates (Desulphosinigrin), Diterpene (phytol), phenol (Hydroquinone, 2,3-dihydro- Benzofuran), fatty acids esters (n-Hexadecanoic acid, 9,12-Octadecadienoic acid (Z,Z)-, Hexadecanoic acid, Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl), Octadecanoic acid) and Flavonoid (4H-Pyran-4-one-2,3-dihydro-3,5-dihydroxy-6-methyl-). The detection of sugar moieties by GC-MS analysis is in agreement with the high amount of carbohydrate observed in proximate analysis.

Phytochemical and ethnobotanical database has ascribed several pharmacological or biological activities such as anti-inflammatory, antioxidant, antilipidemic, antihistaminic, antimicrobial amongst others to majority of the compounds identified [39]. 3-O-Methyl-d-glucose, the most abundant component in *B. eurycoma*, is a non-metabolizable glucose analog, used as a proxy for d-glucose uptake in *in vivo* absorption studies and as a preservative [40,41].

Research findings have reported the anticancer and antimicrobial nature of desulphosinigrin [42]; antioxidant and antifungal activities of 4H-Pyran-4-one,2,3-,dihydro-3,5-dihydroxy-6-methyl-[43-45]; 5-Alpha reductase inhibitor, anti-androgenic, anticancer, antioxidant, hypocholesterolemic, acne reductive, anti-inflammatory and anti-eczemic effect of 9,12-Octadecadienoic acid (Z,Z)- [46-48], as well as the anti-inflammatory, anti-cancer, cytotoxic and antimicrobial properties of n-Hexadecanoic acid and Hexadecanoic acid [49-51].

Phytol, a building block of chlorophyll, is among the twenty four compounds identified in the present study. It is used in the manufacture of synthetic vitamins E and K_1. It has been reported to have antinociceptive, antioxidant, anti-inflammatory, antiallergic, immunostimulant, antimicrobial, antischistosoma, cancer preventive, sedative and anxiolytic effects [52-60]. Hydroquinone occurs naturally in various medicinal plants [61,62], it has been reported to be allelochemic, antimicrobial, antihepatomic, antilithic, antimelanomic, antimelasmic, uroantiseptic , antimitotic, and antipertussive [63-65]. Benzofuran, 2,3-dihydro is a coumaran and research has shown that it possesses anti-inflammatory, antidiarrheal, antileishmanial, immunomodulatory, antimicrobial and anti-helminthic activities [66-68]. Octadecanoic acid is a flavouring agent which is hypocholesterolemic [69]. 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one, a flavonoid with antifungal, antioxidant [70-71].

5. CONCLUSION

It could be seen, based on constituents detected in this preliminary study, that *B. eurycoma* leaves are nutritious and contains volatile phytochemicals with known biological activities revealing it as a potential candidate in food, pharmaceutical, cosmetic and nutraceutical industries. This research serves as bases for more research on *B. eurycoma* leaves.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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