Original Research Article

Proximate analysis and phytochemical profile of Brachystegia eurycoma leaves

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 (NIST[05]) library.

Result: The proximate analysis result showed *B. eurycoma* leaves to be abundant in parameters evaluated in the order of $31.47\pm0.43\%$ Carbohydrate $> 15.15\pm0.04\%$ Ash $> 14.45\pm0.15$ crude fibre $> 13.83\pm0.32$ protein $> 13.14\pm0.22$ moisture $> 1.97\pm0.01$ fat. Qualitative phytochemical analysis detected alkaloid, saponin, tanin, 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%), Hexadecanoic acid, (2.97%), Benzofuran, 2,3-dihydro-(1.94%), Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) (1.92%).

Conclusion: This result reveals the potentials of *B. eurycoma* leaves in food, pharmaceutical, cosmetic and nutraceutical industry.

Comment [DJ1]: Proximate analysis is common term for food analysis. Can you justify how the term is used for a plant here? From my search on its terminology it is defined as investigating of moisture, volatile matter, fixed carbon (by difference), and ash. The term **proximate analysis** does not include determinations of chemical elements or determinations other than those **named**. How about considering the use of "physicochemical" instead of "proximate analysis"?

Comment [DJ2]: kindly look into inconsistency on capitals used here. Also need clarification in text on the 05 in NIST05 if not remove altogether here.

Comment [DJ3]: Can specify it is for w/w or w/v here
Comment [DJ4]: Should have been quantitative for better output
Comment [DJ5]: Wrong spelling usedtannin

Comment [DJ6]: GCMS is best for volatile compound detection hence not giving full picture of all compounds present. Should include the word "volatile"

Comment [DJ7]: Kindly replace as "The presence of bioactive compounds based on this preliminary investigation potentially makes this plant as a candidate for food, pharmaceutical, cosmetic and nutraceutical industries.

Keywords: *Brachystegia eurycoma*, Gas chromatography mass spectrometry (GC-MS), phytochemical, nutraceutical, Desulphosinigrin, drug discovery.

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 drug based on retention time and fragmentation pattern. The fragmentation pattern for a drug is unique and is therefore an identifying characteristic of the drug [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 **Comment [DJ8]:** If the keyword already been used within the title not necessary to re-use here. Choose others for better hit.

Comment [DJ9]: Kindly include review papers identifying usefulness of micro-compounds in plants instead

Comment [DJ10]: This is my concern on the use of the term proximate analysis as yours is plant and more meant for health related biological activities and not for food related.

Comment [DJ11]: It is to identify volatile compounds mainly in samples. Kindly change if seen fitting to your purpose. Drugs is not done here by GCMS. 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 antiinflamatory, 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 its possible nutritional and nutraceutical potentials.

MATERIALS AND METHODS

Plant material

B. eurycoma leaves were collected from Abakiliki, Ebonyi State, Nigeria, authenticated at the University of Port Harcourt Herbarium, Port Harcourt, Nigeria. The leaves were rinsed in distilled water, air-dried and ground into powder. **Comment [DJ12]:** Hence it is part of food preparation and qualifies as an additive. You need to therefore make decision whether it is fine to state you are performing proximate analysis. I leave the decision entirely to you.

Comment [DJ13]: Any voucher specimen deposited in any herbarium. If none who is the botanist to authenticate. Include the name here.

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.

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].

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], Evans [24] and Harborne [25].

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 0.25 μ m 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. <u>A total</u>, 1 μ l of the fractions was diluted in 200 μ l dichloromethane and then injected into the GC-MS [26, 27].

Comment [DJ14]: Since proximate analysis is meant for food as such you need to include a justification why conducted the analysis to a plant instead within introduction or discussion.

Comment [DJ15]: This is the normal conduct of herbs or medicinal plants and not proximate analysis. So this is okay. Hope you understand why I query about proximate analysis above.

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 (NIST) database to ascertain the name, molecular weight and structure of the components of the test materials.

RESULT

The result of proximate analysis shows that the leaves of *B. eurycoma* to contain $31.47\pm0.43\%$ carbohydrates, $15.15\pm0.04\%$ ash, $14.45\pm0.15\%$ crude fiber, $13.83\pm0.32\%$ protein, $13.14\pm0.22\%$ moisture, $1.97\pm0.01\%$ fat and 198.87 ± 0.56 Kcal/100g (Table 1).

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

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

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

Phytochemical	Type of test	Ethanol leaf extract of B.
components		eurycoma

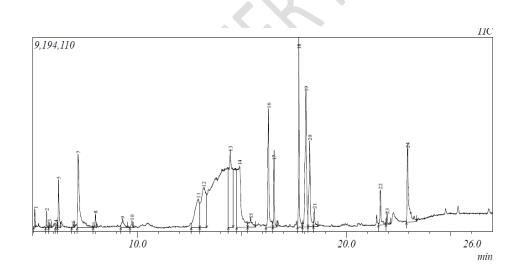
Comment [DJ16]: Why now not using the NIST05 here? Be consistent

Comment [DJ17]: Provide within footnote how the proximate analysis conducted for each so that readers know more details

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A 111-: J-	M	
Alkaloids		+
		+
	Dragendroff's test	+
Phenol	Ferric Chloride	+
saponin	Frothing	+
Tannin	Ferric Chloride	+
Coumarin	alcoholic sodium hydroxide	-
Quinine	potassium hydroxide	+
Anthraquinone	Borntrager's	-
steroids	Libermann- Burchard	-
Protein	Million's	et 🔰
Xanthoprotein	General test	+
Cardiac glycoside	Keller kiliani	+
Flavonoid	sodium hydroxide	+
Diterpene	Copper acetate	+
	saponin Tannin Coumarin Quinine Anthraquinone steroids Protein Xanthoprotein Cardiac glycoside Flavonoid	Wanger's test Dragendroff's testPhenolFerric ChloridesaponinFrothingTanninFerric ChlorideCoumarinalcoholic sodium hydroxideQuininepotassium hydroxideAnthraquinoneBorntrager'ssteroidsLibermann- BurchardProteinMillion'sXanthoproteinGeneral testCardiac glycosideKeller kilianiFlavonoidsodium hydroxide

The GC-MS chromatogram obtained from ethanol extract of *B. eurycoma* shows the presence of



24 distinct peaks (figure 1).

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

The 24 phytochemicals identified in the order of their peaks are presented in figure 2.

Comment [DJ19]: include as asterisk to indicate to which reference this methid been obtained from.

Peak Report TIC											
Peak#	R.Time	I Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name	
1	5.102	5.050	5.142	1572759	0.50	868088	1.60	1.81		Furan-3-carboxaldehyde, 2-methoxy-2,3-dil	
2	5.660	5.625	5.767	1578299	0.50	785361	1.45	1.99		4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy	
3	5.806	5.767	5.883	821421	0.26	271589	0.50	3.02	V	2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-	
4	6.100	6.075	6.183	986554	0.31	233514	0.43	4.22	V	Hydroquinone	
5	6.237	6.183	6.333	6085352	1.94	2283993	4.22	2.66	V	Benzofuran, 2,3-dihydro-	
6	6.935	6.867	6.983	618211	0.20	190340	0.35	3.25	V	Pipridine, 1-chloroacetyl-	
7	7.170	7.125	7.892	22647479	7.23	3485442	6.44	6.26	V	Hydroquinone	
8	8.002	7.892	8.042	1741729	0.56	675050	1.25	2.58	V	Benzene, 2-methoxy-1,3,5-trimethyl-	
9	9.299	9.208	9.508	3093625	0.99	401110	0.74	7.71	V	Tetrahydrofuran-2-one, 3-[1-fluoroethyl]-5-	
10	9.747	9.650	9.817	1409666	0.45	338811	0.63	4.16		9-[2-Deoxybetad-ribohexopyranosyl]pur	
11	12.916	12.575	12.983	20727848	6.61	1374145	2.54	15.08	V	Ethyl .alphad-glucopyranoside	
12	13.177	12.983	13.325	32406722	10.34	1895570	3.50	17.10	V	Desulphosinigrin	- {
13	14.454	14.350	14.583	41452388	13.23	3597376	6.65	11.52	V	3-O-Methyl-d-glucose	÷
14	14.900	14.750	15.283	38929701	12.42	2879052	5.32	13.52	V	3-O-Methyl-d-glucose	1
15	15.433	15.283	15.650	4468492	1.43	429751	0.79	10.40	V	3-O-Methyl-d-glucose	ï
16	16.281	16.150	16.483	20727534	6.61	5542816	10.24	3.74	V	n-Hexadecanoic acid	Ŀ
17	16.547	16.483	16.683	9309686	2.97	3395336	6.27	2.74	V	Hexadecanoic acid, ethyl ester	ŀ
18	17.732	17.667	17.900	23743294	7.58	8683680	16.05	2.73	V	Phytol	
19	18.072	17.900	18.167	32593772	10.40	6241435	11.53	5.22	V	cis-9-Hexadecenal	
20	18.250	18.167	18.417	18472090	5.89	4009206	7.41	4.61	V	9,12-Octadecadienoic acid (Z,Z)-	
21	18.473	18.417	18.650	3301278	1.05	767072	1.42	4.30	V	Octadecanoic acid, ethyl ester	
22	21.634	21.533	21.900	6010628	1.92	1633519	3.02	3.68	V	Hexadecanoic acid, 2-hydroxy-1-(hydroxyn	
23	21.950	21.900	22.117	1542228	0.49	538818	1.00	2.86	V	Bis(2-ethylhexyl) phthalate	
24	22.933	22.867	23.367	19114576	6.10	3592028	6.64	5.23	V	Oleoyl chloride	
				313355332	100.00	54113102	100.00			1	

Comment [DJ20]: No need for the exact results given by the GCMS equipment. By right you can process further from raw data to be more professional. Check some journals with GCMS results to understand further.

Figure 2. Phytochemicals present in ethanol leaf extract of B. eurycoma

The first compound identified with less retention time (5.102 min) was Furan-3-carboxaldehyde 2-methoxy-2,3-dihydro- whereas Oleoyl chloride was the last compound which took longest retention time (22.933 min) to identify. The phytochemicals identified in order of most abundant to least abundant based on percentage peak are presented in table 3, showing their molecular formular and structure.

Comment [DJ21]: Spelling error

	Name of compound	Molecular formula	Structure	Molecular weight	Peak Area (%)
1	3-O-Methyl-d-glucose	C ₇ H ₁₄ O ₆	_о_Сон	194	13.23
2	3-O-Methyl-d-glucose	$C_{7}H_{14}O_{6}$	но он он	194	12.42
3	cis-9-Hexadecenal ; 9-Hexadecenal, (Z)-	C ₁₆ H ₃₀ O	но он он	238	10.40
4	Desulphosinigrin	C ₁₀ H ₁₇ NO ₆ S	ОН	279	10.34
		05			
5	Phytol	$C_{20}H_{40}O$		296	7.58
6	Hydroquinone	$C_6H_6O_2$	ОН	110	7.23
•			OH	•••	
7	n-Hexadecanoic acid	$C_{16}H_{32}O_2$		256	6.61
8	Ethyl .alphad- glucopyranoside	$C_8H_{16}O_6$	HO OH HO OH	208	6.61

9	Oleoyl chloride	C ₁₈ H ₃₃ C _{lO}	f f f f f f f f f f f f f f f f f f f	300	6.10
10	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$		280	5.89
11		$C_{18}H_{36}O_2$		284	2.97
12	ethyl ester ; 2,3-dihydro- Benzofuran Coumaran	C ₈ H ₈ O		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	$C_{7}H_{14}O_{6}$	-O OH	194	1.43
15	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	но он	312	1.05
16	Tetrahydrofuran-2-one, 3-[1-fluoroethyl]-5-[[2- hydroxypropyl]benzene ethyl-	C ₁₇ H ₂₃ FO ₃	, , , ,	294	0.99
			OT OH		

17 Benzene, 2-methoxy- C ₁₀ H ₁₄ O 1,3,5-trimethyl-	150	0.56
18 Furan-3- C ₆ H ₈ O ₃ carboxaldehyde 2-methoxy-2,3- dihydro-	128	0.50
19 4H-Pyran-4-one $C_6H_8O_4$ 0 2,3-dihydro-3,5- dihydroxy-6-methyl-	ОН	0.50
20 Bis(2-ethylhexyl) $C_{24}H_{38}O_4$ phthalate	390	0.49
, Li	\sim	
21 9-[2-Deoxybetad- ribohexopyranosyl]puri n-6(1H)-one	282	0.45
	он	
22 Hydroquinone C ₆ H ₆ O ₂ OH	110	0.31
23 2-Cyclopenten-1-one $C_7H_{10}O_2$	126	0.26
3-ethyl-2-hydroxy-	_OH	0.20
	Un	
24 Piperidine, 1- C ₇ H ₁₂ C ₁ NO chloroacetyl-	o 161	0.20
N	C1	
	b	

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 fibre > 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%) [29]. 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 RDA of fiber is 19-25% for children, 21-38% for adult, 28% for pregnant mothers and 29% for breast-feeding mothers [30]. 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 [31]. *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% [32] but low moisture content is indicative of a longer shelf life. It is a general observation that leafy vegetables have low lipid content [31]. The low lipid content of *B. eurycoma* leaves is in agreement with this observation.

Comment [DJ22]: Check whether correct as

"fiber" or "fibre

Comment [DJ23]: Need full meaning before can use abbreviation

Comment [DJ24]: Is B. eurycoma leaves been used as vegetable or any other food ? If yes than more meaningful to mention so.

Comment [DJ25]: Italics please

Preliminary phytochemical screening detected the presence of alkaloid, saponin, tanin, diterpenes, phenol, quinine, flavonoid, protein, xanthoprotein and cardiac glycoside. Alkaloids are diuretic in nature, they affect the nervous system and reduce appetite [33]. 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 [34]. Tannins are antiinflammatory, antidiarrheal, haemostatic, antiviral, antibacterial, antidiarrheal and antihemorrhoidal compounds which has been reported to relief sore throat, fatigue and skin ulcer [35]. Diterpenes are antimicriobial and anti-inflammatory [36]. Quinine has many medicinal applications due to its fever-reducing, painkilling and antiinflammatory properties [37]. Many flavonoids have been shown to have antioxidative activity, free radical scavenging capacity, antihypertensive, -hepatoprotective, anti-inflammatory, antiviral and anticancer activities [38]. Cardiac glycosides have cardiotonic activity, are antiviral, anticancer and antiproliferative effects [39].

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-

Comment [DJ26]: spelling

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dihydroxy-6-methyl-). <u>Such findings on sugar content constituents are in agreement with the</u> proximate analysis of detecting carbohydrates but more detail description the types of constituents by GCMS.

Comment [DJ28]: Kindly include this statement

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 [28]. 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].

The extent of current studies has indicated presence of many useful constituents with known biological activities however more compounds could be still be found in this plant as the detection only related to volatile compounds by GCMS. It is not yet conclusive to identify this plant for its usefulness for such biological activities until been studied quantitatively. Hence the work is preliminary to suggest this plant determined to contain potential biological activities based on the content found. This is expected to provide useful information on a plant to be selected for example for antioxidant activity and consequently attempt to isolate the bioactive compound responsible toward such capability via bioassay guided isolation.

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Conclusion

This study showed that *B. eurycoma* leaves are nutritious and contains important phytochemicals with several biological activities revealing its potentials use in food, pharmaceutical, cosmetic and nutraceutical industry.

References

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