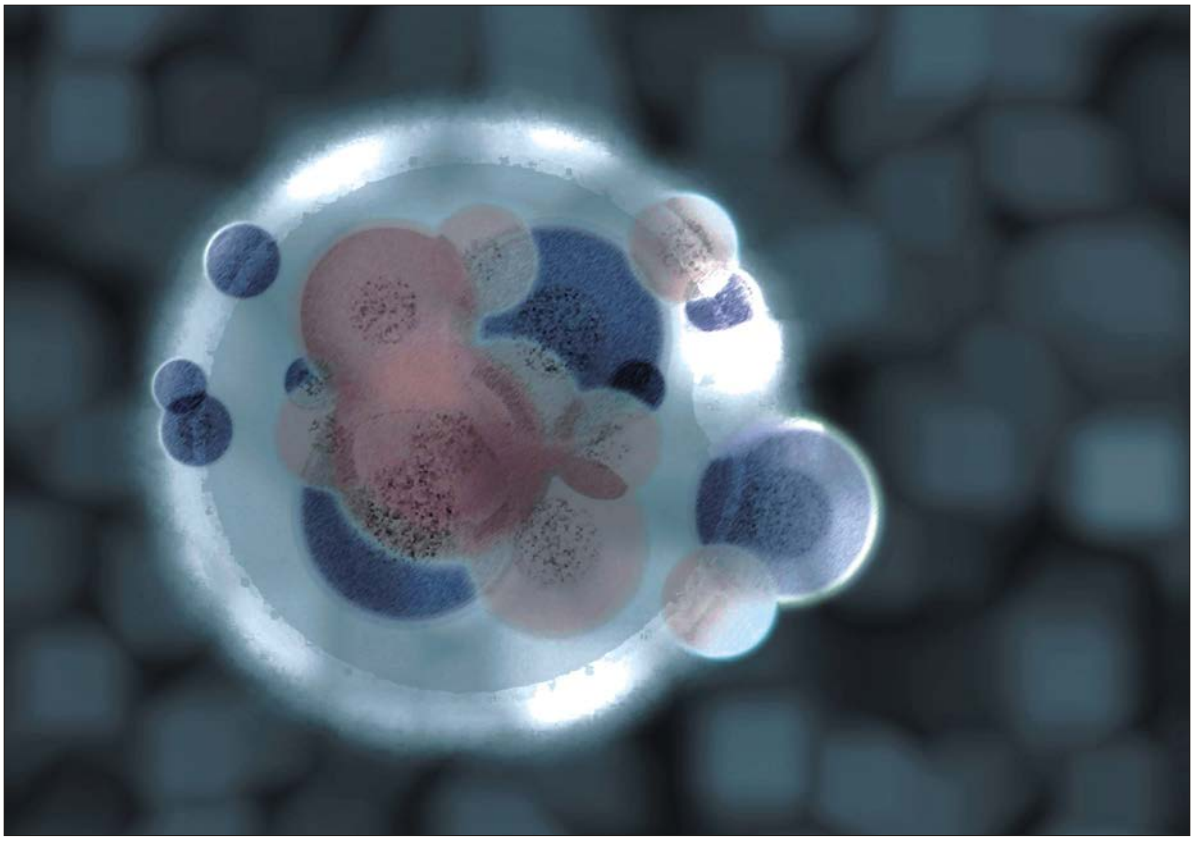

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Research Article
Characterization, Proximate Composition and Evaluation of Antimicrobial Activity of Seed Oil of *Bauhinia tomentosa*

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Abstract

Background: This study was carried out to investigate proximate composition, phytochemical profile and antimicrobial activity of the spectroscopically characterized seed oil of *Bauhinia tomentosa*. **Materials and method:** The characterization was carried out using FT-IR, mass spectra, ^{1}H- and ^{13}C-NMR. **Results:** The results from the proximate analysis showed the presence of crude protein 30.36±0.98%, crude fibre 26.00±0.69%, carbohydrate 25.32±0.57%, moisture content 12.04±0.39%, ash content 4.00±0.15% and fat content 2.28±0.09%. The phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, terpenes, cardiac glycosides, sterols, anthraquinones and tannins in varying degrees. The mineral determination showed that the seed oil contained iron (3.10±0.01 mg kg^{-1}), manganese (0.38±0.01 mg kg^{-1}), while cadmium (0.0 mg kg^{-1}), lead (0.0 mg kg^{-1}) and nickel (0.0 mg kg^{-1}) were not detected. The extracted seed oil was investigated for antimicrobial efficiency against four bacterial isolates and two fungal, wherein gentamicin and clotrimazole were the clinical standard antibiotic and antifungal agents, respectively. **Conclusion:** The antimicrobial activity result revealed the sample to be bioactive and of great pharmaceutical potential with MIC value of 6.25 and <3.625 mg mL^{-1} against *Escherichia coli* and *Candida albican*, respectively. Due to high nutritional values and broad antimicrobial properties, the seed oil of *Bauhinia tomentosa* has nutraceutical potentials, which might pave way for its use as an alternative nutrient source for mankind or for industrial purposes.

Key words: Nutrition, antimicrobial activity, *Bauhinia tomentosa*, nutraceutical, infrared spectroscopy

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The medicinal applications of plant and other agricultural products could be dated back to antiquity because nature has provided a complete storehouse of remedies to cure ailment of mankind[1]. Bauhinia genus consists of nearly 200 species of plants[2] among which *Bauhinia tomentosa* L., belonging to the family Leguminosae (Fabaceae) was well known as a small evergreen medicinal and avenue tree[3]. The relatively large Bauhinia genus consisting of trees, climbers and shrubs are distributed in a wide range of geographic locations[4] and thrive well in slightly alkaline well-drained soil in moderate watering condition. The adult plants can tolerate a moderate amount of frost but the seedlings and younger plants should be shielded from the frost[5]. *Bauhinia tomentosa*, commonly called yellow bell orchid tree is usually a scrambling, many-stemmed shrub or small tree reaching 4 m (max. 8) in height, the branches often drooping with many slender twigs[6]. The bark is grey and smooth or slightly hairy on young branches, becoming brown and smooth on the older stems. *Bauhinia tomentosa* is easily cultivated from the seed and its germination is usually within 7-15 days[7].

Several parts of this tree have been used in ayurveda to prepare indigenous medicines and to treat common disease conditions, such as cough and jaundice[3]. The flowers are used as a laxative, while the roots are carminative. The bark is used in addition to other drugs to treat snake bites and wounds from other venomous animals[8]. Wound healing properties of the chloroform extract of the leaves of *Bauhinia* sp. was recently reported by Ananth *et al*.[9]. This genus is known as a prolific source of structurally diverse secondary metabolites. The fruit is said to be diuretic, the seed is eaten in India as tonic and aphrodisiac, while its bud and flower are used for dysentery treatment[10]. The dried leaf and flower bud of *B. tomentosa* and a decoction of the root and bark are used medicinally by the African doctors of South Africa[11]. Folklore history emphasizes the use of *B. tomentosa* for cancer therapy. Recent study demonstrated *Bauhinia* sp. to be very efficacious against peptic ulcer, which is a gastrointestinal disorder induced by various factor, such as *Helicobacter pylori*, aspirin, indomethacin and alcohol among others[12]. Bauhinia statins was identified as antineoplastic agent with new and unusual cancer cell growth inhibitory potential[13]. Scientifically, *B. tomentosa* has been proved to possess wide range of pharmacological potential, which include but not limited to antidiabetic[14], thyroid stimulating and antihypothyroidism[15], anti-inflammatory[16], antimalarial[17], larvicidal[18], antioxidant[19] and immunomodulatory[20] activities.

Furthermore, oil seeds are oil bearing crops, such as soybean, cottonseed, sunflower, palm and rape seed oil. Many oils and fats for human consumption or for industrial purposes are derived from plants. Indeed, seeds constitute essential oil reserves of nutritional, industrial and pharmaceutical importance[21]. Dr. Stephen De Felice (1992) defined nutraceutical as "Any substance that may be considered a food or part of a food and which provides medical or health benefits including the prevention and treatment of disease"[22]. Oil seeds are widely used in food, feed and industrial applications. A food supplement "Kachnar" is made out of this plant as a gargle for sore throat treatment, as a paste for skin diseases or internally as a remedy for diarrhea[23]. The energy and nutrient contributions of foods by food group to dietary intake are known to differ among race/ethnic groups of children[24] and suggest further intake differences may exist by processing level due to the changes that foods undergo during processing. The aim of this present study is to investigate the nutritional quality and antimicrobial activity of the characterized seed oil of *Bauhinia tomentosa* in order to identify its nutraceutical potential for human consumption.

MATERIALS AND METHODS

Sample seed collection: The mature *Bauhinia tomentosa* L., seeds were collected from the Campus of Covenant University Ota, Ogun State, Nigeria in the early morning of the 23rd of July, 2015 between the hours of 6 and 8 am at a prevailing temperature of 27±2°C. The plants were identified and authenticated at the Department of Biological Sciences, Covenant University, where a voucher specimen was kept with number CU-0014 at the University Herbarium for reference purposes.

Extraction and isolation: The *Bauhinia tomentosa* seed was crushed into powder using milling machine. About 97.27 g of the crushed seed was neatly wrapped inside whatman filter paper and mounted in the thimble compartment of the soxhlet apparatus for extraction process according to the procedure described by Association of Official Analytical Chemists[25]. It was extracted with n-hexane over a period of 6 h and the hexane was removed at reduced pressure using rotary evaporator and carefully controlled low temperature in order to avoid decomposition and loss of heat labile metabolites that might be bioactive.

Instrumental analysis: All chemical compounds and reagents used were obtained from Sigma-Aldrich Chemical Companies but were made available by Department of Chemistry, Covenant University. Solvents used were of analytical grade and used directly without further purification. Melting points of the compounds were determined in open capillary tubes on stuart melting point apparatus and were uncorrected. The IR spectra were run in KBr disc using the Bruker FT-IR spectrophotometer, while the mass spectra were obtained using Q-trap 2000 spectrometer at 70 eV. The ^{1}H-NMR and ^{13}C-NMR of the compounds were run on JEOL delta NMR ECX 400 spectrometer machine at 400 and 100 MHz, respectively. The chemical shifts were measured with reference to tetramethylsilane (TMS) as internal standard and the solvent used was $CDCl_3$. The pH was monitored using portable pH Meter Model PHB4. All drying were conducted at reduced pressure with DHG-9023A vacuum oven. Organic solvents were concentrated and removed with a RE-2000B Buchi Rotary Evaporator at reduced pressure.

Spectral data for the seed oil sample: The FT-IR (KBr): 2974 (CH aliphatic), 2822 (CH aliphatic), 2485 (CO_2), 1751 (C=O ester), 1599 (C=C), 1038 (C-O alkoxy) cm^{-1}. The ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.36-5.30 (m, 5H), 4.30-4.26 (dd, J_1 = 4.28 Hz, J_2 = 8.00 Hz, 1H, CH-CH=CH), 4.16-4.11 (dd, J_1 = 5.96 Hz, J_2 = 8.00 Hz, 1H, CH=CH-CH), 2.78-2.74 (t, J = 12.72 Hz, 2H, CH_2-CH_2), 2.34-2.31 (m, 4H, 2×CH_2), 2.03-1.99 (m, 6H, 3×CH_2), 1.61 (s, 3H, CH_3-OCO), 1.29-1.24 (d, J = 12.72 Hz, 32H, CO-$(CH_2)_{16}$-CH), 0.88 (m, 6H, 2×CH_3). The ^{1}H-NMR (100 MHz, $CDCl_3$) δ: 173.3, 172.8, 130.3, 130.1, 127.8, 68.8, 62.2, 34.1, 32.0, 31.6 (3×CH_2), 29.8 (2×CH_2), 29.4 (2×CH_2), 29.2 (2×CH_2), 27.3 (3×CH_2), 25.7 (3×CH_2), 24.9, 22.7 (CH_3), 14.2 (CH_3), 13.5 (CH_3) ppm. The MS: in m/z (rel. %): 508.97 (M^+, 34.5%), 466.95 (100%), 354.37 (10.0%), 275.00 (22.5%) and 270.03 (35.0%).

Phytochemical screening method: Phytochemical analysis for the confirmation of secondary metabolites were carried out on the seed oil sample aforementioned using standard procedures as described by Trease and Evans[26] and Harbone[27]. The chemical tests showed the presence of various phytoconstituents like alkaloids, flavonoids, saponins, triterpenes, cardiac glycosides, sterols, anthraquinones, tannins and absence of cyanogenetic glycosides.

Proximate analysis procedure: Moisture, crude fat, crude fat and crude fibre were determined in accordance with the official methods of the Association of Official Analytical Chemists[25], while nitrogen was determined by the micro-kjeldahl method[28] and the percentage of nitrogen was converted to crude protein by multiplying by 6.25. The total ash was determined as described by Kirk and Sawyer[29]. Fat content was determined using the procedure of AOAC[25] and n-hexane as solvent. Carbohydrate was determined by difference.

Determination of minerals content: About 5 g seed was powdered and tuned to ash. The resulting ash was dissolved in 25 mL of dilute HCl (1:1) and incubated for 20-25 min on hot water bath. The solution was filtered. The filtrate was collected and made the volume to 100 mL with water in a volumetric flask and 10 mL of this solution was diluted to 100 mL with distilled water. The diluted solution was used for the estimation of minerals such as iron, manganese, cadmium, nickel and lead by atomic absorption spectrophotometer from Thermofisher Scientific.

Determination of antibacterial activity: The agar-well diffusion assay as described by Vollekova *et al.*[30] was used to determine the growth inhibition of bacteria by the seed oil. The tests were carried out by using a stock concentration of 100 mg mL^{-1} prepared by dissolving 1 g of the ethanol extract into 10 mL of distilled water. Nutrient agar was prepared and 25 mL each was poured into sterile petri dish. This was allowed to solidify and dry. The solidified nutrient agar was inoculated with the suspension of the test bacterial isolates. Using a sterile cock-borer of 4 mm diameter three equidistant holes per plate were made in the set agar. Thereafter, the wells (holes) were filled with 0.2 mL of the extract solution. The plates were incubated at 37°C for 24 h. The resultant zone of inhibition (3 replicates) of the different plant extracts were observed and measured using a transparent meter rule.

Antifungal activity: The fungal suspension in potato dextrose broth was used to surface inoculate freshly prepared potato dextrose agar in petri dishes. Using a sterile cock-borer of 4 mm diameter, three equidistant holes per plate were made in the inoculated agar plate. Then, the wells were filled with 0.2 mL of the extract solution. The plates were incubated at 28°C for 72 h. The resultant zone of inhibition of the different plant extracts were observed and measured using a transparent meter rule.

Minimum Inhibitory Concentration (MIC): The MIC test was carried out according to the procedure of Vollekova *et al.*[30]. The seed oil sample was prepared to the highest concentration of 100 mg mL^{-1} (stock concentration) in sterile distilled water and serially diluted (two-fold) to a working concentration ranging from 50.000-3.125 mg mL^{-1} each of which was mixed with equal amount of nutrient broth. The dilutions were inoculated with 0.2 mL suspension of the test organisms. After 24 h of incubation at 37°C, the test tubes were observed for turbidity. The least concentration where no turbidity was observed was determined and noted as the Minimum Inhibitory Concentration (MIC) value.

RESULTS

There are various agricultural policies formulated to curtail the food security challenges. Unfortunately, these policies have not yielded the desired result of increase food production[31]. The dietary supplements and nutraceutical market were projected to achieve a global market size of about Rs. 90 billion in 2013 at a CAGR of 20.24%[32]. However, this target was not met due to challenge of food shortage. In order to bridge the unmet gap between food supply and population growth especially in the developing countries, there is a continuous need for study for novel high quality but inexpensive sources of food. This has always been a major challenge of the agencies involved in the provision of adequate food. Thus, it is conceivable to evaluation the nutritive value and pharmaceutical properties of seed oil of *Bauhinia tomentosa* in order to establish its nutraceutical potential. First and foremost, the seed of *Bauhinia tomentosa* was crushed and extracted with n-hexane using soxhlet extraction according to the procedure of Association of Official Analytical Chemists[25]. The isolate obtained from the seed oil thereof was then characterized for structural elucidation using spectroscopic means, which include FT-IR, ^{1}H-NMR and ^{13}C-NMR as well as mass spectra. Hence, the result of the spectral data for the seed oil sample was shown. The study of techniques for phytochemical screening played a significant role in giving the solution to systematic problems on the one hand and in the search for additional resources of raw materials for pharmaceutical industry on the other hand. Thus, the quantitative phytochemical constituents of the sample was investigated using standard procedures[26,27] in order to identify the secondary metabolites present and the result was as shown in Table 1. Protein-energy malnutrition is among the most serious problems tropical developing countries are facing today. It has become imperative for local governments along with food researchers and nutritionists to study for cheap, reliable and safe plant-based resources to accomplish the demand for protein-rich foods. The proximate analysis was carried out on the extracted oil in order to know its suitability in dietary and the result is as shown in Table 2. The result of the physicochemical parameters of the oil sample was as shown in Table 3, wherein parameters such as colour, refractive index, density, acid value, iodine value and saponification values were evaluated. The result of mineral element composition of the seed oil, in mg kg^{-1} dry matter was as presented in Table 4. The result of *in vitro* antimicrobial

Table 1: Result of phytochemical screening of the seed oil of *Bauhinia tomentosa*

Phytochemical constituent	Test result
Alkaloids	+++
Flavonoids	+++
Saponins	++
Terpenes	++
Cardiac glycosides	++
Sterols	++
Anthraquinone	+
Tannins	+
Cyanogenetic glycosides	-

Number of positive sign indicates the intensity of concentration, +++: High concentration, ++: Moderate concentration, +: Low concentration and -: Negative result

Table 2: Result of the proximate analysis of the extracted seed oil of *Bauhinia tomentosa*

Components	Value (g %)
Crude protein	30.36±0.98
Crude fibre	26.00±0.69
Carbohydrate	25.32±0.57
Moisture content	12.04±0.39
Ash content	4.00±0.15
Crude fat	2.28±0.09

Values are Mean±SD of triplicate determination

Table 3: Result of physicochemical parameters of seed oil of *Bauhinia tomentosa*

Parameters	Value obtained
Yield (%)	18.23 (%)
Colour	Brownish yellow
Cloud point	10°C
Melting point	70-74°C
Boiling point	260°C
Refractive index	1.476
Density	1.50
Free fatty acid	17.60
Acid value	8.80
Saponification value (mg g^{-1})	18.75
Iodine value	82.20
Peroxide value	-
pH	4.72

Table 4: Result of mineral content determination of seed oil seed oil of *Bauhinia tomentosa*

Mineral	Value (mg kg^{-1})
Iron (Fe)	3.10±0.01
Manganese (Mn)	0.38±0.01
Cadmium (Cd)	0.00±0.00
Nickel (Ni)	0.00±0.00
Lead (Pb)	0.00±0.00

screening of the seed oil sample against four bacteria and two fungi was as presented in Table 5, wherein gentamicin and clotrimazole were used as the clinical reference for the antibacterial and antifungal activity evaluation, respectively.

DISCUSSION

The percentage consumption of common vegetable oil shown in Fig. 1 established the fact that the seed oil of *Bauhinia tomentosa* has not been explored for possible human consumption as an alternative to common vegetable oil. The spectroscopic characterization of the extracted seed oil sample for the proposed structure of the isolate (Fig. 2) was carried out using FT-IR, ^{1}H-NMR and ^{13}C-NMR as well as mass spectral data. The IR spectrum of the sample exhibited the absorption bands at 2974 and 2822 cm^{-1} due to the presence of C-H stretching vibration of CH_2 and CH_3 aliphatic, while the band at 2485 cm^{-1} depicted the presence of CO_2 functionality. The stretching frequency of C=O of ester was found at 1751 cm^{-1}, which was further confirmed by the presence of C-O of alkoxy group at 1038 cm^{-1}. The carbonyl frequency herein reported (1751 cm^{-1}) was further confirmed by comparing it with the findings of Lewis *et al.*[33] who documented the various C=O stretching absorption vibration frequencies in infrared spectra to range between 1685-1758 cm^{-1}. The absorption band noticed at 1599 cm^{-1} depicted the presence of C=C of olefinic, which was an evidence that there was unsaturation. The presence of C=C was chemically confirmed by addition of bromine water, wherein the change in colour was observed as a clear indication of presence of unsaturation.

The ^{1}H-NMR spectrum of the sample showed the presence of five protons downfield at 5.36-5.30 ppm followed by a doublet of doublet at 4.30-4.26 ppm, which was one

Table 5: *In vitro* antimicrobial activity of the seed oil of *Bauhinia tomentosa*

	Diameter of zone of inhibition (mm) at different concentrations (mg mL^{-1})								
Organism	100	50	25	12.5	6.25	3.625	Gent. (10 mg mL^{-1})	Clot. (10 mg mL^{-1})	MIC (mg mL^{-1})
Klebsiella sp.	22.0	18.0	10.0	R	R	R	28.0	-	25.0
Escherichia coli	15.0	13.5	11.0	10.5	10.0	R	23.0	-	6.25
Streptococcus sp.	14.0	13.0	12.0	10.0	R	R	20.0	-	12.5
Staphylococcus aureus	12.0	11.0	10.0	R	R	R	18.0	-	25.0
Candida albican	21.0	19.0	17.0	11.0	10.0	10.0	-	32.0	<3.125
Aspergillus niger	R	R	R	R	R	R	-	12.0	

RR: Resistance, Gent.: Gentamicin, Clot.: Clotrimazole and MIC: Minimum inhibitory concentration

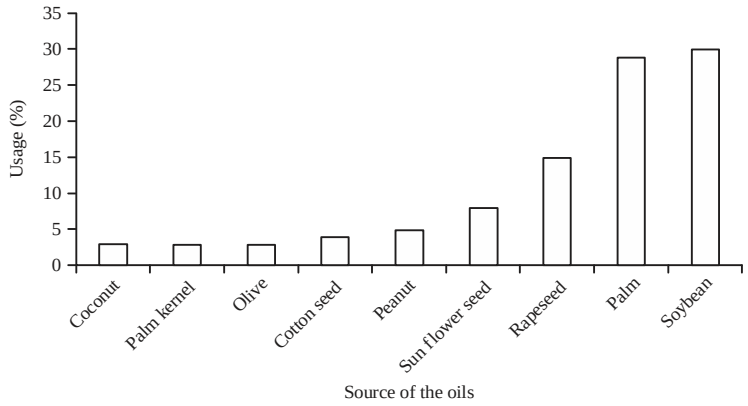

Fig. 1: Pictorial representation of world common vegetable oil consumption

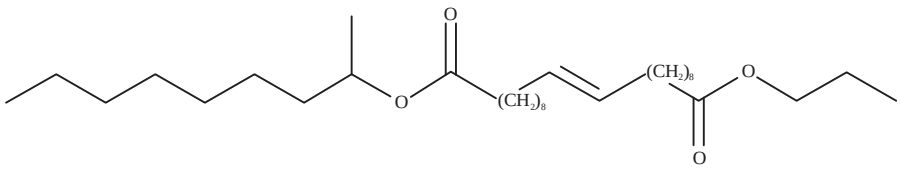

Fig. 2: Structure of the characterized isolate from the *Bauhinia tomentosa* oil

proton of olefinic bond with coupling constants of 4.28 and 8.00 Hz, while the second one proton of olefinic bond resonated at chemical shift value of 4.16-4.11 ppm with coupling constant of 5.96 and 8.00 Hz. The two doublet of doublet signal of CH proton have the same value of 8.00 Hz for their second coupling constant, meaning that they are neighbouring group to each other. The triplet at δ 2.78-2.74 ppm with coupling constant value of 12.72 Hz was due to the presence of a methylene in the neighbourhood of two protons environment. The multiplet at δ 2.34-2.31 ppm was due to the presence of four proton atoms, which was equivalent to two methylene ($2\times CH_2$), while the six proton multiplet at δ 2.03-1.99 ppm was for three methylene ($3\times CH_2$). A singlet at δ 1.66 ppm was an up-field signal for three protons, which depicted the presence of CH_3 linked to alkoxy carbonyl of ester. The penultimate signal to TMS region up-field resonated at δ 1.29-1.24 ppm as 32 protons doublet ($16\times CH_2$) with coupling constant of 12.75 Hz. The most shielded signal was a multiplet at δ 0.88 ppm, which was attributed to the six protons ($2\times CH_3$). The result of ^{13}C-NMR spectrum of the sample ran at 100 MHz frequency showed the presence of 32 carbon atoms. The most de-shielded signals at δ 173.3 and 172.8 ppm were due to the presence of carbonyl of ester and was in agreement with the earlier reported value by Al-Kawkabani *et al.*[34] investigated the synthesis of novel 2H,8H-pyrano [2,3-f]chromene-2,8-dione based scaffolds under tandem knoevenagel condition, while the most shielded signal at δ 13.5 ppm was as a result of the presence of CH_3 attached to aliphatic carbon chains. The result of the mass spectrum of the seed oil sample showed the molecular ion peak at m/z 508.97 (34.5%) which correlated well with the molecular mass (508.82) of the proposed compound. The base peak observed at m/z 466.95 (100%) was as a result of loss of propene molecule (M-42).

Phytochemical tests were carried out for the above mentioned plant seed oil using the standard procedures[26,27] in order to identify the phyto-constituents of the oil. The result of the phytochemical screening is as shown in Table 1. The number of positive sign indicated the intensity of reactions that reflects the quantity present (Table 1). The various preliminary chemical tests showed the most abundant phyto-constituents were alkaloids and flavonoids, while saponin, terpenes, cardiac glycosides and sterols (phytosterol) were present in moderate concentrations. Analysis of the oil sample showed the presence of anthraquinone but in low concentration, whereas cyanogenetic glycosides and tannin were absent (Table 1). The absence of these two phyto-constituents being anti-nutrient was an indirect indication that the oil would be rich in nutritive values and parameters. These phyto-consituents are secondary metabolites, which are biologically active and may be applied in nutrition and as pharmacologically-active agents[35]. The presence of cardiac glycoside may support against cardiovascular disorder since cardiac glycosides are clinically used in the treatment of congestive heart failure and as anti-arrhythmic agents due to their strong inhibitory activity toward the ubiquitous cell surface enzyme Na^+/K^+-ATPase[36]. In order to formulate, produce and market quality diets it is necessary to gain information about substance classes that contribute to the energy content and special compounds, which influence digestibility. Each medicinal plant species has its own nutrient composition besides having pharmacologically important phytochemicals.

Nutrients are essential for the physiological functions of human body. Such nutrients and biochemicals like carbohydrates, fats and proteins play an important role in satisfying human needs for energy and life processes[37]. Hence, the proximate analysis was investigated in order to ascertain the nutritive importance of the sample for possible human consumption as well as in livestock feed meal formulation (Table 2). The moisture content was 12.04±0.39%, which allow for improved activity of water soluble enzymes and co-enzymes needed for metabolic activities of this oil[38]. Moisture content is among the most vital and mostly used measurement in the processing, preservation and storage of food[39]. Thus, this low moisture content implies that the product possess high shelf life. The dry matter component was 87.96±2.48%, which was the summed total of crude protein (30.36±0.98%), crude fibre (26.00±0.69%), carbohydrate (25.32±0.57%), ash content (4.00±0.15%) and crude fat (2.28±0.09%).

The protein content of the seed was found to be 30.36±0.98%, which shows that the seed oil can serve as a source of protein considering the level of protein deficiency in the society today. Its intake can contribute to the formation of hormones, which controls a variety of body functions such as growth, repair and maintenance. The crude fibre was 26.00±0.69%. In the intestinal tract, fiber resists being broken down by enzymes; although, part of it may be metabolized by bacteria in the lower gut. Fiber is characterized by low or no nutritional value, but because of its effect on the digestive system, it is thought to help with such problems as diabetes and high levels of blood cholesterol[40]. This high crude fibre might aid in combating gastrointestinal disorder, since, individuals with high intakes of dietary fiber appear to be at significantly lower risk for developing coronary heart disease, stroke, hypertension, diabetes, obesity and certain

gastrointestinal diseases[41]. The carbohydrate is categorized as the nitrogen free extract (extract), which comprises of the easily digestible carbohydrates such as sugar, starch and organic acids. The amount of carbohydrate herein was 25.32±0.57%, which conferred on the oil, significant roles to human health. This is because, apart from the supply of energy, carbohydrates are also needed in numerous biochemical reactions not directly concerned with energy metabolism as earlier reported by Bhattacharjee *et al.*[42]. The ash content (4.00±0.15%) was higher than value reported by Gul and Safdar[43] for cinnamon, while the crude fat (2.28±0.09%) was almost close to the values reported for cinnamon by Khanum *et al.*[44]. Although, the oil yield was low to be considered as oilseed for commercial purposes but their use may not be discouraged, as they are important nutritionally.

The extracted oil was analyzed for chemical properties such as: Iodine, peroxide, acid and saponification values, while specific gravity, viscosity refractive index and colour were examined for the physical properties (Table 3). The refractive index of the oil (at room temperature) was determined with Abbe refractometer[45] and the specific gravity measurement (also carried out at room temperature), using specific gravity bottle[46]. The state and colour of the oil were noted, using visual inspection at room temperature[46]. Viscosity and yield were determined, following the method described by the Association of Official Analytical Chemists[25]. Results are expressed as the means of two separate determinations. The extracted oil was obtained in 5% yield from the sohxlet apparatus after continue extraction with hexane (boiling point = 50-60°C) for 6 h using standard procedure[25]. The extracted oil was then stored at 5°C under argon atmosphere prior to further analysis in order to create inert environmental, which helps in the prevention of oxidation on standing. The Peroxide Value (PV) of the seed oil was zero. This value was a clear indication of extremely low level of oxidative rancidity of the oil and also suggested strong presence or high levels of antioxidant. It was also a pointer to the fact that the oils may not be easily susceptible to deterioration as earlier reported by Akpambang *et al.*[47]. The refractive index which is the ratio of the velocity of light in vacuum to the velocity of light in a medium is an indication of the level of saturation of the oil[46]. The refractive index of the oil was 1.476, which fell within the range of the expected standard according to ASTM value which ranged from 1.476-1.479[48]. Others could be attributed to the presence of other components of the crude oil mixture.

In addition, the acid value was found to be 8.8 mg KOH g^{-1} which is an acceptable range for further use, acid value measures the presence of corrosive free fatty acids and oxidation products, this is actually an important variable in considering the quality of oil because the lower the free fatty acid, the better the quality of oil, the acceptable limit for edible oils is ≤10[49]. Refining may improve its quality for industrial purposes[46]. Iodine value is 82.2/100 g; moderate iodine value indicates moderate level of unsaturation of fats and oils. Oils with iodine value above 125 are classified as drying oils; those with iodine value 110-140 are classified as semidrying oils[50]. Iodine value is a measure of the degree of unsaturation in an oil and it is an identity characteristic of native oil. This value could be used to quantify the amount of double bonds present in the oil, which reflects the susceptibility of oil to oxidation. The saponification value is 18.75 mg KOH g^{-1}, this was lower than other oil such as Jathropa (196 mg KOH g^{-1}) and Mahua (190 mg KOH g^{-1}). However, this saponification value falls just below the range expected of some non edible oils reported by Singh and Padhi[51]. The low saponification is an indication of the oil, which may not be suitable for detergent manufacturing.

From the result of mineral content analysis, iron (3.10±0.01 mg kg^{-1}), manganese (0.38±0.01 mg kg^{-1}), while cadmium (0.00±0.00 mg kg^{-1}), lead (0.00±0.00 mg kg^{-1}) and nickel (0.00±0.00 mg kg^{-1}) were not detected. In detail, iron is an important trace element in the human body, it plays crucial roles in haemopoiesic, control of infection and cell mediated immunity[52]. Iron was determined to be 3.104 mg g^{-1}, which is moderately high value. Since, the consequences of iron deficiency include anemia, which affect more than one billion people worldwide[53], reduced work capacity, impairments in behaviour and intellectual performance and decrease resistance to infection[54], then regular intake of this oil might boost human chance against this deficiency. Manganese, like many metallic minerals is toxic in large dosages. The manganese (0.38±0.01 mg kg^{-1}) is present in trace amount in the oil sample and below the expected standard for human consumption, since most people need an intake of between 2 and 3 mg day^{-1}. Cadmium, nickel and lead were not detected in the sample. This might be because they were present in minute level, which was below the machine detectable limit. This also buttresses the fact that this oil is safe for consumption since these three metals, which poses serious health hazardous to mankind were absent in the oil sample. For instance, the excess of cadmium can result in hypertension and nephritis[55]. While cadmium toxicity presents as a generalized disorder of proximal tubular function, including increased uric acid clearance and hypouricaemia, lead toxicity (except in children) is commonly accompanied by hyperuricaemia and/or gout[55].

Antimicrobial sensitivity testing was carried out *in vitro* using agar-well diffusion method against the following test organisms: *Staphylococcus aureus*, *Streptococcus* sp.,

Escherichia coli (Entero pathogenic), *Klebsiella* sp., *Candida albicans* and *Aspergillus niger*. Minimum Inhibitory Concentration (MIC) of the seed oil of *Bauhinia tomentosa* was determined using broth dilution method as described by Vollekova *et al*.[30]. The media were inoculated with test organisms and DMSO was used as negative control. The positive control on bacteria and fungi were gentamicin and 1% clotrimazole, respectively. The zones of inhibition were measured after 24 h of incubation. The result of antimicrobial screening (sensitivity testing) using serial dilution method at varying concentrations (100, 50, 25, 12.5, 6.25 and 3.125 mg mL^{-1}) on the six organisms with Zones of Inhibition (ZOI) duly recorded in millimetre (mm) is as shown in Table 4. The choice of use of gentamicin as clinical standards is based on the fact that at low concentrations, gentamicin only inhibits growth of the bacteria through induction of prokaryotic ribosomes to misread mRNA[56]. Gentamicin also prevents initiation of protein synthesis and leads to death of microbial cells. Also in humans, they have structurally different ribosomes from bacteria, thereby allowing the selectivity of this antibiotic for bacteria. Gentamicin inhibits bacterial growth by inhibiting protein biosynthesis. Its mechanism of action is similar to that of streptomycin and other aminoglycoside antibiotics. The *in vitro* screening of the oil sample against *Klebsiella* sp. at 100 mg mL^{-1} resulted in growth inhibition with ZOI of 10 mm, while the strongest activity against *Klebsiella* sp. was observed at 25 mg mL^{-1} having the ZOI of 10 mm. As regarding activity of the sample against second Gram negative bacterial isolate *E. coli*, noticeable ZOI of 15, 13.5, 11, 10.5 and 10 mm were obtained for the screening concentrations of 100, 50, 25, 12.5 and 6.26 mg mL^{-1}, respectively. *Streptococcus* sp. was sensitive to inhibitory efficiency of the tested sample at four various concentrations of 100, 50, 25 and 12.5 mg mL^{-1} with the ZOI values of 14, 13, 12 and 10 mm. Antifungal screening was carried out on two fungi, which were *Candida albican* and *Aspergillus niger* alongside with clotrimazole clinical standard of the antifungal screening, the better activity of the oil sample was found against *Candida albican* while *A. niger* was not susceptible to the sample but had consistent resistance at all concentrations used. This was because the oil sample was able to inhibit the growth of *Candida albican* at all concentrations used; 100, 50, 25, 12.5, 6.25 and at <3.125 mg mL^{-1} with the ZOI of 21, 19, 17, 11, 10 and 10 mm, respectively. Finally, the last column of Table 5 showed the Minimum Inhibitory Concentration (MIC) results of the tested seed oil sample of *Bauhinia tomentosa* as compared to that of standard against the six organisms. It was noticed that the seed oil sample was more potent than gentamicin (MIC = 10 mg mL^{-1}) against *E. coli* (MIC = 6.25 mg mL^{-1}), while it was less potent than gentamicin (MIC = 10 mg mL^{-1}) against *Streptococcus* sp. (MIC = 12.5 mg mL^{-1}), *Klebsiella* sp. (MIC = 25 mg mL^{-1}) and *Staphylococcus aureus* (MIC = 25 mg mL^{-1}). Lastly, the highest potency of the sample was observed against *Candida albican* at MIC<3.125 mg mL^{-1}, which made it three folds more active than clotrimazole standard antifungal agent.

CONCLUSION

It was discovered that if oil from *Bauhinia tomentosa* is consumed in sufficient amount it would contribute greatly towards meeting human nutritional requirement for normal growth and adequate protection against diseases arising from malnutrition. The phytochemical screening of the seed oil revealed the presence of alkaloids, flavonoids, saponins, triterpenes, sterols, tannins, cardiac glycosides as secondary metabolites, which might have contributed to its biological activity. The sample had broad activity spectrum against both gram positive and gram negative bacteria screened as well as the fungi with least MIC<3.125 mg mL^{-1}.

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