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Research Article
Preliminary Studies on the Seed Oil of *Caryota mitis*: Proximate Composition, Phytochemical Screening and Evaluation of Antimicrobial Activity

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Abstract

Background and Objective: There are many varieties of seeds and nuts that can produce oils for food, nutraceuticals, skin care products, aromatherapies, fuels and industrial lubricants. In this present study, the oil from the seed of *Caryota mitis* was extracted by soxhlet extraction and investigated to promote its quality aspects for biodiversity. **Methodology:** The structural characterization of the oil was carried out using physico-chemical analysis and spectroscopic means such as FR-IR, ^{1}H and ^{13}C-NMR as well as mass spectral data. **Results:** The phytochemical screening of the oil from *Caryota mitis* seed species revealed the presence of phytosterols, triterpenes, alkaloids, flavonoids and saponins. The proximate analysis showed the moisture content to be 42%, while crude fibre, carbohydrate, crude fat, crude protein and ash content was found to be 34.30, 11.92, 5.30, 4.64 and 1.84%, respectively on dry matter basis. The mineral content determination revealed the presence of iron (3.10±0.02 mg kg^{-1}), manganese (0.11±0.01 mg kg^{-1}), sodium (154.15±1.98 mg kg^{-1}) and potassium (127.04±1.21 mg kg^{-1}) while cadmium, nickel and lead were not detected. **Conclusion:** The *in vitro* antimicrobial screening on the oil, showed that it has promising antimicrobial potential on the organism tested but it was not as active as the clinical standards used. This oil is therefore, a candidate for further study in terms of the cytotoxicity profile and its ability to be used as functional food for proper exploration of its nutraceutical endowment.

Key words: *Caryota mitis* seed oil, *in vitro* screening, secondary metabolites, nutritive value, soxhlet extraction

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

INTRODUCTION

Over the years, there is a considerable attention in the exploitation of plants for medicinal research in Africa and beyond[1]. The use of medicinal plants for the curing of illnesses and treatment of infectious diseases could be dated back to antiquity[2]. Concomitantly, there is an increase in data and huge patronage to herbal products round the world. In fact, in recent times, scientists are increasingly becoming involved in the screening of such plants with the aim of establishing their potential antimicrobial effects and identifying the compounds responsible for the antimicrobial properties[2]. The medicinal properties of *Caryota* sp., are due to the presence of flavonoids such as astragalin, kaempferol, quercetin, myricetin[3]. From ancient days, the various part of this plant serves as therapeutic agents. The flower is used to treat gastric ulcer, migraine and headache. The root is used as teeth ailment while the palm heart leaves is used locally for the control of diabetes and in ayurvedic medicine[4]. In ayurveda recommends the use of *Caryota* sp., for seminal weakness and urinary disorders, the juice is applied on the forehead for hemicranias. In traditional medicine porridge prepared from *Caryota* sp., flower is used to treat gastric ulcer, migraine headaches, snake bite poisoning as well as rheumatic swellings[4]. Out of 56 wild fruit samples subjected to Ferric Reducing Antioxidant Power (FRAP) assay, *Caryota mitis* was reported to be the 5th and 7th best antioxidant and highest total phenolic content, respectively[5]. Immunotherapeutic effects of recombinant *Caryota mitis* profilin (rCmP)-loaded PLGA nanoparticles revealed them to be very efficient in the treatment of allergic asthma[6].

Furthermore, the clustered fishtail palm, *Caryota mitis* Lour., is native from Burma to the Malay Peninsula Southeast Asia, which grows in clusters of 25-40 ft high, with green-gray trunks topped by dense tufts of fan-shaped, dull green leaves, jagged at the apex and nodding at the tips. Fishtail palms can thrive in light conditions from full sun to deep shade, requiring only that its soil be well-drained and reasonably fertile. It has a moderate to rapid growth rate and should be located outdoors in a sheltered location protected from cold[7]. This clump-growing group of palms has medium green leaf blades which are divided into many segments, each of which resembles the tail of a fancy goldfish[7]. This palm is commonly used for horticultural purpose and environmental beautification[8]. The x-ray crystallographical analysis of raphides isolated from the palm of *Caryota mitis* were reported to contain calcium oxalate monohydrate (whewellite)[8] which appear to play a central role in a variety of important functions, including tissue calcium regulation, protection from herbivory and metal detoxification[9].

The search for new good quality but economical sources of protein, oil and energy has emerging to be a major concern of governments and other institutions charged with the task for food and nutrition in many countries of the developing world. Hence, the seed oil of *Caryota mitis* for nutritional evaluation via proximate determination and possible antimicrobial potential was explored in order to ascertain its possible application as alternative source of vegetable oil for human consumption as well as its diversity in livestock feed formulation.

MATERIALS AND METHODS

Materials

Plant material collection: The dry fruits from *Caryota mitis* were collected from the environment of the Covenant University, Ota, Ogun State, Nigeria on the 23rd of July, 2015 between the hours of 6.30 am and 8.30 am at a prevailing temperature of 26±2°C. The plants were identified and authenticated at the Department of Biological Sciences, Covenant University where a voucher specimen with herbarium number CU-0017 was available for reference purposes.

Chemicals and equipment: All chemical compounds and reagents used were obtained from Sigma-Aldrich Chemical Companies except n-hexane and methanol which were procured from British Drug House (BDH) Chemicals. However, these chemicals were made available by Department of Chemistry, Covenant University. Solvents used were of analytical grade and were used directly without further purification unless otherwise stated. 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 solid state in KBr disc over the range of 400-4000 cm^{-1} using the Bruker FT-IR spectrophotometer while the mass spectra were obtained with Electron Spray Ionization (ESI) technique 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 with the coupling constants (J) of distinct multiplicity patterns measured in hertz (Hz). 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.

Methods

Extraction and isolation: The bean seed was crushed into powder using milling machine. About 2 g of the crushed seed was neatly wrapped inside Whatman filter paper and mounted on soxhlet apparatus. Oil from the seeds of *Caryota mitis* was extracted through repeated washing or percolation with n-hexane under reflux over a period of 6 h with the aid of soxhlet apparatus. The procedure described by Association of Official Analytical Chemists[10], was adopted to achieve this. Then, n-hexane solvent was removed by the use of rotary evaporator at reduced pressure to afford golden yellow oil.

Phytochemical screening method: Phytochemical analysis for the confirmation of secondary metabolites was carried out on the seed oil sample aforementioned using standard procedures as described by Trease and Evans[11] and Harborne[12]. 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 AOAC[10], while nitrogen was determined by the micro-kjeldahl method[13] 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[14]. Fat content was determined using the procedure of AOAC[10] and n-hexane as solvent. Carbohydrate was determined by difference.

Qualitative determination of protein and carbohydrates

Biuret test: To the test sample was added 6% NaOH solution and violet colour appears when small drops of 1% $CuSO_4$ solution was added, which indicated the presence of protein[15].

Molisch's test: To the extract solution, was add few drops of alcoholic α-naphthol. Afterward, 1 mL of concentrated sulphuric acid was added slowly through the sides of the tube, purple color which turns to violet color ring was appears at the junction of the solution confirmed the presence of carbohydrate[16].

Determination of minerals content: Five grams seed was pulverized and turned 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 iron (Fe), manganese (Mn), cadmium (Cd), nickel (Ni) and lead (Pb) by atomic absorption spectrometry

Principle: In this technique the atoms of an element are vaporized and atomized in the flame. The atoms then absorb the light at a characteristic wavelength. The source of the light is a hollow cathode lamp, which is made up of the same element, which has to be determined. The lamp produces radiation of an appropriate wavelength, which while passing through the flame is absorbed by the free atoms of the sample. The absorbed energy is measured by a photo-detector read-out system. The amount of energy absorbed is proportional to the concentration of the element in the sample.

Procedure: The digested sample was analyzed for mineral contents by atomic absorption spectrophotometer (Hitachi model 170-10). Different electrode lamps were used for each mineral. The equipment was run for standard solutions of each mineral before and during determination to check that it is working properly. The dilution factor for all minerals except P and Mg was 100. For determination of Mg, further dilution of the original solution was done by using 0.5 mL original solution and enough distilled water was added to it to make the volume up to 100 mL. The concentrations of minerals recorded in terms of parts per million (ppm) were converted to milligrams (mg) of the minerals by multiplying the parts per million (ppm) with dilution factor and dividing by 1000.

Determination of sodium (Na) and potassium (K) by flame photometer

Principle: The flame photometer measures the emission of radiant energy when atoms of an element return to their ground state after their excitation by the high temperature of the flame. The degree of emission is related to the concentration of element in the solution.

Procedure: The Na and K analysis of the sample were done by the method of flame photometry. The same wet digested food sample solutions as used in AAS were used for the determination of Na and K. Standard solutions of 20, 40, 60,

80 and 100 milli equivalent L^{-1} were used both for Na and K. The calculations for the total mineral intake involve the same procedure as given in AAS.

Bioassay of antimicrobial screening

Determination of antibacterial activity: The agar-well diffusion assay as described by Vollekova *et al.*[17] 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 of the sample 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 sample solution. The plates were incubated at 28°C for 72 h. The resultant zone of inhibition of the sample were observed and measured using a transparent meter rule.

Minimum Inhibitory Concentration (MIC): This test was according to Vollekova *et al.*[17] method was employed. 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 AND DISCUSSION

Phytochemistry: Table 1 showed the phytochemical constitutents of *caryota mitis* seed oil sample. Medicinal plants are part and parcel of human society to combat diseases from the dawn of civilization. The seed of *Caryota mitis* was harvested from the fishtail tree from premises of Covenant University Campus, Nigeria, carefully treated and the oil was extracted using n-hexane and the pictorial view for this stepwise processing for the extraction of the process was as shown in Fig. 1. Thus, in the present investigation, the oil from the seed of *Caryota mitis* was subjected to phytochemical screening and the result showed the presence of variety of primary and secondary metabolites (Table 1). The phytochemical test was carried out using the standard procedures adopted from earlier works of Trease and Evans[11] and Harborne[12] in order to identify the phyto-constituents of the oil. The number of positive sign indicated the intensity of reactions that reflects the quantity present (Table 1). The results of the phytochemical screening showed that two secondary metabolites namely phytosterols

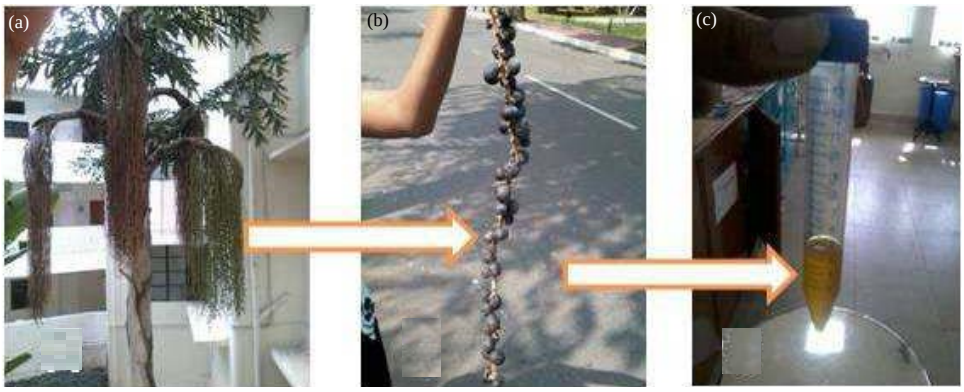

Fig. 1(a-c): Pictorial view showing the processing stages for oil (a) Fishtail palm tree (b) dry seed of *Caryota mitis* obtained from the tree (c) oil extracted from the seed of *C. mitis*

Table 1: Result of phytochemical screening of the seed oil of *Caryota mitis*

Phytochemical constituents	Test result
Phytosterols	+++
Triterpenes	+++
Alkaloids	++
Flavonoids	++
Saponins	+
Cardiac glycosides	-
Anthraquinone	-
Tannins	-
Phlabotannins	-
Cyanogenetic glycosides	-

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

Table 2: Result of the proximate analysis of the extracted seed oil of *Caryota mitis*

Component	Value (g %)
Crude protein	4.64±0.01
Crude fibre	34.30±0.12
Carbohydrate	11.92±0.08
Moisture content	42.00±0.19
Ash content	1.84±0.02
Crude fat	5.30±0.05

Values are Mean±SD of triplicate determination

and tripterpenes were present in high concentrations in the seed oil sample while alkaloids and flavonoids were available in moderate concentration. Although, saponin was present in trace amount, the oil sample of *Caryota mitis* was void of tannins, phlabotanins, anthraquinone, cardiac glycosides and cyanogenetic glycosides. The absence of the five phytochemicals might be as a result of highly polar functionalities presence in them which made them to lack solubility compatibility with non-polar nature of the lipid and solvent used for the extraction (n-hexane). The availability of high amount of phytosterol suggested that the oil might be useful for easy lowering of cholesterol level in human body. This is because the use of plant sterol/stanol capsules or tablets offers a practical option compared with traditional food applications because it provides a vehicle that can be easily incorporated into a cholesterol-lowering regimen without impacting dietary macronutrient distribution[18]. High content of triterpenes might influence the regulation of metabolism and energy homeostasis which is in agreement with the earlier findings of Haridas *et al.*[19]. It is reported that avicins, a family of apoptotic triterpene electrophiles are known to regulate cellular metabolism and energy homeostasis, by targeting the mitochondria[19]. The presence of moderate concentration of alkaloids might influence the usage of the oil sample in corrosion inhibition study due to availability of the nitrogen bases in the core structures of most alkaloids. The moderate content of favonoids might lead to the enhancement of free oxygen scavenging ability of the oil sample for better antioxidant efficiency.

Proximate composition: Table 2 showed the result of proximate analysis of the oil sample. 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. Hence, the proximate analysis was carried out on the extracted oil in order to know its suitability in dietary (Table 2). The high moisture content (42.00%) provides for greater activity of water soluble enzymes and co-enzymes needed for metabolic activities[20] of this oil. The dry matter component was 58.00% which was the summed total of crude fibre (34.30%), carbohydrate (11.92%), crude fat (5.30%), crude protein (4.64%) and ash content (1.84%). Apart from the moisture content, crude fiber has the highest composition (34.3%) herein. This is because crude fibre which is the insoluble fraction of carbohydrate in a define concentration of alkalis or acid, is a total sum of cellulose, hemicellulose and lignin. The carbohydrate (11.92%) is categorized as the nitrogen free extract (extract) which comprises of the easily digestible carbohydrates such as sugar, starch and organic acids. This high amount of carbohydrate 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.*[21]. The crude fat (5.30%) reported herein is closely similar to the value reported to be found in Stevia leave (4.34%) by Tadhani and Subhash[22]. The contraction of muscular walls of the digestive tract is stimulated by crude fiber, thus counteracting constipation[23]. Crude fiber also plays a very crucial role in the protection against cardiovascular disease, colorectal cancer and obesity. The moderate level of crude protein (4.64%) was noticed in the sample. Hence, its intake can contribute to the formation of hormones which controls a variety of body functions such as growth, repair and maintenance. The Recommended Dietary Allowance (RDA) for protein is 56 g for individual weighing 70 kg and 46 g for adult weighing 50 kg, children[24] may consume 2 kg day^{-1}. Hence, the result of the proximate analysis showed that oil from seed of *Caryota mitis* is good source of carbohydrate and protein. Although, the protein content is low, yet it will be of great economic importance and a potential replacement for the commonly consumed vegetable oil by human. This is because the plant is a moderate source of protein and according to

Table 3: Result of mineral content determination of seed oil

Mineral	Value (mg kg^{-1})
Iron (Fe)	3.10±0.02
Manganese (Mn)	0.11±0.01
Cadmium (Cd)	0.00±0.00
Nickel (Ni)	0.00±0.00
Lead (Pb)	0.00±0.00
Sodium (Na)	154.15±1.98
Potassium (K)	127.04±1.21

Values are Mean±SD of triplicate determinations

Table 4: Result of physicochemical parameters of seed oil of *Caryota mitis*

Parameters	Obtained value
Yield (%)	5.30
Colour	Brownish yellow
Cloud point (°C)	10
Melting point (°C)	70-74
Boiling point (°C)	260
Refractive index	1.477
Density (g cm^{-3})	0.93
Free fatty acid	32.96
Acid value	16.48
Saponification value (mg g^{-1})	18.75
Iodine value	105.20
Peroxide value	-
pH	4.72

Pamela *et al.*[25], proteins from plant sources have lower quality but their combination with many other sources of protein such as animal protein may result in adequate nutritional value. The chemical test used to confirm the presence of carbohydrate and protein are Molisch and Biuret test, respectively. The procedure for the Molisch's test was carried out according to the procedure of Gangwal *et al.*[16] while, the procedure described by Suneetha *et al.*[15] was adopted for the Biuret's test. The chemistry of Molisch's test involves dehydration of carbohydrate by action of concentrated mineral acid to produce aldehyde. The purple color observed was as a result of condensation of the aldehyde with phenolic functionality of the two molecules of α-naphthol.

Mineral content determination: Table 3 showed the result of mineral content determination of seed oil. The test was carried out on five micronutrients which were iron, manganese, cadmium, nickel, lead and two macronutrients which were sodium and potassium (Table 3). The iron content of the oil samples investigated was 3.10±0.02 mg kg^{-1}. Since iron plays crucial roles in haemopoiesic, control of infection and cell mediated immunity[26] and its deficiency has been described as the most prevalent nutritional deficiency, then the intake of this oil might help fight iron deficiency anemia which is estimated to affect more than one billion people worldwide[27]. The manganese value was determined to be 0.11±0.01 mg kg^{-1}. This value was not above the expected standard for human consumption since most people need an intake of between 2 and 3 mg day^{-1}. The manganese was present in trace amount in the oil sample which was appropriate as the case should be for typical micronutrient. The mineral manganese, in trace amount is essential for normal brain and nerve function and it plays an important role in helping the body form bones, tissue and sex hormones[28]. High levels of manganese in the body, particularly in the brain, are associated with neurological disorders. Symptoms of manganese toxicity include headaches, tremors, loss of appetite, muscle rigidity, leg cramps and hallucinations. Some people with manganese toxicity may get extremely irritable and be prone to acts of violence[28]. Cadmium, nickel and lead were not detected. This was suitable for the sample because they were toxic metals. It should be noted that the zero value of these mineral mean that they might be present in infinitesimal amount which was below the machine detectable limit. This also buttresses the fact that this oil is safe for consumption since these three metals which pose serious health hazardous to mankind were below the detectable limit in the oil sample. For instance, the excess of cadmium can result in hypertension and nephritis while lead toxicity is commonly accompanied by hyperuricaemia and/or gout[29]. Two macronutrients determined were sodium and potassium and their compositions in the oil sample were 154.15±1.98 and 127.04±1.21 mg kg^{-1}, respectively. The measurement of sodium and potassium was crucial because they are important intracellular and extracellular cations, respectively. The high Na level might be an added advantage since it is involved in the regulation of plasma volume, acid-base balance, nerve and muscle contraction[30].

Physicochemical parameter determination: Table 4 showed the result of the physicochemical parameters of the oil sample wherein parameters such as colour, refractive index, density, acid value, iodine value and saponification values were evaluated. 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[31]. The refractive index of the oil was 1.477 which fell within the range of the expected standard according to ASTM value[32] which ranged from 1.476-1.479. Others could be attributed to the presence of other components of the crude oil mixture. Considering the physico-chemical parameters, the low values of PV are indicative of low levels of oxidative rancidity of the oils and also suggest strong presence or high levels of antioxidant[33]. This could be corroborated by the presence of flavonoid in moderate intensity (++) as free radical and singlet-oxygen scavenger which also assists body system

Table 5: Data of spectroscopic characterization for seed oil of *Caryota mitis*

Spectroscopic technique	Data obtained
FT-IR	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}
^{1}H NMR	(400 MHz, $CDCl_3$) δ: 5.41-5.31 (m, 5H), 4.30-4.26 (dd, J_1 = 4.30 Hz, J_2 = 8.00 Hz, 1H, CH-CH=CH), 4.16-4.11 (dd, J_1 = 5.90 Hz, J_2 = 8.00 Hz, 1H, CH=CH-CH), 2.78-2.74 (t, J = 12.72 Hz, 2H, CH_2-CH_2), 2.34-2.28 (m, 4H, 2×CH_2), 2.06-1.97 (m, 6H, 3×CH_2), 1.58 (s, 3H, CH_3-OCO), 1.29-1.24 (d, J = 12.72 Hz, 32H, CO-$(CH_2)_{16}$-CH), 0.90-0.85 (m, 6H, 2×CH_3)
^{13}C NMR	^{13}C-NMR (100 MHz, $CDCl_3$) δ: 173.8, 171.8, 130.5, 130.0, 127.6, 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
Mass spectrum	MS: in m/z (rel. %): 508.97 (M^+, 34.5%), 466.95 (100%), 354.37 (10.0%), 275.00 (22.5%), 270.03 (35.0%)

against oxidative stress. 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.*[34]. The oil is yellow in colour and has density of 0.93 g cm^{-3} which was the same with that of *Carytoa urens*. This oil is less dense than water. Density and other gravities are important parameters for diesel fuel injection systems the values must be maintained within tolerable limits to allow optimal air to fuel ratios for complete combustion. High-density biodiesel or its blend can lead to incomplete combustion and particulate matter emissions[35]. The cloud point and the melting point of oil were determined to be 10 and 70-74°C, respectively. This could give prediction if there is tendency congealing nature of the oil at low temperature. The acid value was found to be 16.48 mg KOH g^{-1} which is double the value earlier reported for *Caryota urens* by Girisha *et al.*[36]. 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. The free fatty acid was 32.96. This value was further corroborated by the pH of the oil sample which was acidic being 4.72. Iodine value is 105.20 g/100 g which was in agreement with the value reported for *Caryota urens* by Girisha *et al.*[36]. High iodine value indicates high 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[36]. 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. The iodine value obtained places the oil in the non-drying groups. This oil may find application as a raw material in industries for the manufacture of vegetable oil-based ice cream[31]. 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[37]. The low saponification is an indication of the fact that this oil may not be suitable for detergent manufacturing.

Structural characterization via spectroscopic means: Table 5 showed the spectroscopic characterization of the extracted seed oil of *Caryota mitis* with the aid of 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.*[38] 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 which was downfield with respect to TMS position at exactly chemical shift of 5.41-5.31 ppm followed by a doublet of doublet at 4.30-4.26 ppm which was one proton of olefinic bond with coupling constants of 4.30 Hz 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 5.90 and 8.00 Hz. The two doublet of doublet signals of CH protons have the same value of 8.00 Hz for their second coupling constant, meaning that they are neighbours to each other. The triplet at δ 2.78-2.74 ppm having 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 2.34-2.28 ppm was due to the presence of four proton atoms which was equivalent to two methylene (2×CH_2) while the six proton multiplet at δ 2.06-1.97 ppm was for three methylene (3×CH_2). A singlet at δ 1.58 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

Table 6: *In vitro* antimicrobial activity of the seed oil of *Caryota mitis*

	Diameter of zone of Inhibition (mm) at different concentrations in (mg mL^{-1})								
Organisms	100	50	25	12.5	6.25	3.625	Gentamicin (10 mg mL^{-1})	Clotrimazole (10 mg mL^{-1})	Minimum inhibitory concentration (mg mL^{-1})
Klebsiella sp.	12.0	7.0	R	R	R	R	26.0	-	50.0
Escherichia coli	11.0	8.0	5.0	R	R	R	22.0	-	25.0
Micrococcus sp.	10.0	8.0	4.0	R	R	R	28.0	-	25.0
Staphylococcus aureus	9.0	6.0	4.0	R	R	R	18.0	-	25.0
Candida albican	17.0	12.0	5.0	R	R	R	-	24.0	25.0
Aspergillus niger	R	R	R	R	R	R	-	10.0	R

R: Resistance

at δ 1.29-1.24 ppm as thirty two protons doublet (16×CH_2) with coupling constant of 12.73 Hz. The most shielded signal was a multiplet at δ 0.90-0.85 ppm which was attributed to the six protons (2×CH_3). The ^{13}C-NMR spectrum of the sample was run at 100 MHz frequency in the presence of $CDCl_3$ solvent and the result is likewise shown in Table 5. It showed the presence of 32 carbon atoms. The most de-shielded signals at δ 173.8 and 171.8 ppm were due to the presence of carbonyl of ester and was in agreement with the earlier reported value by Al-Kawkabani *et al.*[39] who investigated the synthesis of novel 2H,8H-pyrano[2,3-f]chromene-2,8-dione based scaffolds under tandem knoevenagel condition. On the other hand, the most shielded signal was found at d 13.5 ppm which was due to 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 with the high degree of accuracy because it did not exceed ±0.20 unit difference. The base peak observed at m/z 466.95 (100%) was as a result of loss of 42 mass unit which accounted for the loss of propene molecule (M-42) or (M-$CH_3CH=CH_2$).

Antimicrobial activity: Table 6 showed the result of antimicrobial endowmwnt of the seed oil sample. Antimicrobial sensitivity testing was carried out using agar-well diffusion method against the following test organisms: *Klebsiella* sp., *Escherichia coli* (Enteropathogenic), *Micrococcus* sp., *Staphylococcus aureus*, *Candida albicans* and *Aspergillus niger*. Minimum Inhibitory Concentration (MIC) of the seedoil of *Caryota mitis* was determined using broth dilution method as described by Vollekova *et al.*[17]. The antimicrobial activity of the sample was evaluated using four bacterial and two fungal isolates while gentamicin and clotrimazole were used as the clinical standard antibacterial and antifungal agents, respectively. The general sensitivity testing was carried out using agar diffusion method with the zones of inhibition measured in millilitres (mm). The choice of the 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[40]. It has similar mechanism of action as streptomycin and other aminoglycoside antibiotic. Justification for the choice of organisms include the fact that *Klebsiella* sp., constitutes a major public health threat, being one of the most common causes of hospital and community acquired infections. *Escherichia coli* and *Staphylococcus aureus* are intestinal bacteria often implicated in several gastrointestinal disorders. Gastrointestinal diseases caused by *E. coli* are the most frequent causes of death in developing countries[41]. The sample from the seedoil of *Caryota mitis* studied herein inhibited the growth of *Klebsiella* sp., at 100 and 50 mg mL^{-1} concentration with zones of inhibition (ZOI) of 12 mm and 7 mm, respectively whereas *Klebsiella* sp., was discovered to develop resistance against the sample at concentration of 25-3.125 mg mL^{-1} (i.e., concentration ≤25 mg mL^{-1}). The Gram negative organism, *E. coli* was sensitive to the efficacy of the oil sample at concentrations of 100 mg mL^{-1} (ZOI = 11 mm), 50 mg mL^{-1} (ZOI = 8 mm) and 25 mg mL^{-1} (ZOI = 5 mm) while this pathogenic organism was resistant to the oil sample at concentrations ≤12.5 mg mL^{-1}. The last two bacteria which were *Micrococcus* sp. and *Staphylococcus aureus* had similarity in their response to the efficacy of the oil sample in that they were sensitive to the efficacy of the oil sample at three concentrations (100, 50 and 25 mg mL^{-1}) whereas the two aforementioned bacteria showed resistance at three lower concentrations which were 12.5, 6.25 and 3.125 mg mL^{-1}. For zone of inhibition detail, *Micrococcus* sp. and *Staphylococcus aureus* were sensitive to the oil sample at concentrations of 100 mg mL^{-1} (ZOI = 10 mm), 50 mg mL^{-1} (ZOI = 8 mm), 25 mg mL^{-1} (ZOI = 4 mm), 100 mg mL^{-1} (ZOI = 9 mm), 50 mg mL^{-1} (ZOI = 6 mm) and 25 mg mL^{-1} (ZOI = 4 mm), respectively. Although, the oil sample was active on the four bacteria with

zone of inhibition ranging from 4-12 mm but it was less active than gentamicin standard having zone of inhibition ranging from 18-26 mm against the four bacteria.

In addition, the commonest agents of fungal infections include *Candida*, *Aspergillus* and *Cryptococcus* since they are ubiquitous and commensal fungal colonizers[42]. *Candida albican* is the cause of majority of cases of oropharyngeal candidiasis in patients with Sjögren's syndrome, diabetes mellitus, HIV infections and AIDS as well as head and neck cancers[43]. In view of these challenges among others, the antifungal potential of the oil sample was investigated against *Candida albican* and *Aspergillus niger* by carrying out the *in vitro* antifungal screening alongside the clotrimazole standard antifungal agent. Considering the sensitivity testing on two fungi, the oil sample was active on *Candida albican* at concentrations of 100 mg mL^{-1} (ZOI = 17 mm), 50 mg mL^{-1} (ZOI = 12 mm) and 25 mg mL^{-1} (ZOI = 5 mm) while this fungus was resistant at concentration of 12.5, 6.25 and 3.125 mg mL^{-1}. Contrariwise, *Aspergillus niger* was completely resistant to the biological potential of the sample throughout (i.e., from concentration of 100-3.125 mg mL^{-1}), since it was resistant even at 100 mg mL^{-1} which was the highest concentration in this present study. Due to broad spectrum of activity observed during the general sensitivity testing, the Minimum Inhibitory Concentration (MIC) test was carried out in order to actualize the lowest concentration of the sample at which the growth of the sensitive organism was inhibited. The MIC values of the oil against the growth of all the bacteria was 25 mg mL^{-1} except for *Klebsiella* sp., wherein the MIC was 50 mg mL^{-1} which was two folds less active as compare to others. The MIC investigative screening on the two fungi showed the lowest growth inhibitory concentration to be 25 mg mL^{-1} against *Candida albican* while *Aspergillus niger* showed resistance to bioactive endowment of the *Caryota mitis* seed oil sample.

CONCLUSION

The seed oil of *Caryota mitis* was successfully analyzed and expatiated for its phyto-constituent, proximate compositions and mineral composition. The sample was screened against four bacteria and two fungi in order to investigate the antimicrobial properties. Different chemical, physical and biological methods have been developed to obtain the information needed. It is worthwhile to note that consumption of numerous types of edible plants as sources of food could be beneficial to nutritionally marginalized population especially in developing countries where poverty and climate is causing havoc to the rural populace. The data obtained herein provides information, which can be utilized for the comparative assessment of nutritional worth and medicinal property of this oil with other common oils. Based on the array of information obtained from the proximate analytical data of the oil studied, it could be a potential replacement for some common edible oil. However, since secondary metabolite content may vary as a function of multiple factors, such as environmental conditions and harvest period, reproduction of this analysis over a long period of time is needed before the effectiveness of our method is totally demonstrated.

REFERENCES

1. Nwinyi, O.C., N.S. Chinedu, O.O. Ajani, C.O. Ikpo and K.O. Ogunniran, 2009. Antibacterial effects of extracts of *Ocimum gratissimum* and *Piper guineense* on *Escherichia coli* and *Staphylococcus aureus*. Afr. J. Food Sci., 3: 22-25.
2. Aibinu, I., T. Adenipekun, T. Adelowotan, T. Ogunsanya and T. Odugbemi, 2007. Evaluation of the antimicrobial properties of different parts of *Citrus aurantifolia* (lime fruit) as used locally. Afr. J. Tradit. Complement. Altern. Med., 4: 185-190.
3. Ambika, V., R. Arivudainambi and D. Sukumar, 2012. Investigations on *Caryota urens*. Int. J. Scient. Res., 1: 5-7.
4. Vaishnavi, R. and V. Suneetha, 2013. Phytochemical analysis on *Caryota urens* (fishtail palm) fruit from VIT university campus for pharmaceutical use. Der Pharmacia Lettre, 5: 71-75.
5. Fu, L., B.T. Xu, X.R. Xu, X.S. Qin, R.Y. Gan and H.B. Li, 2010. Antioxidant capacities and total phenolic contents of 56 wild fruits from South China. Molecules, 15: 8602-8617.
6. Xiao, X., X. Zeng, X. Zhang, L. Ma and X. Liu *et al*., 2013. Effects of *Caryota mitis* profilin-loaded PLGA nanoparticles in a murine model of allergic asthma. Int. J. Nanomed., 8: 4553-4562.
7. Gilman, E.F. and D.G. Watson, 1993. Caryota spp. Fishtail palm. Fact Sheet ST-124, November 1993, pp: 1-3. http://hort.ufl.edu/database/documents/pdf/tree_fact_sheets/carsppa.pdf
8. Snyder, D.S., G.M. Hatfield and K.F. Lampe, 1979. Examination of the itch response from the raphides of the fishtail palm *Caryota mitis*. Toxicol. Applied Pharmacol., 48: 287-292.
9. Nakata, P.A., 2003. Advances in our understanding of calcium oxalate crystal formation and function in plants. Plant Sci., 164: 901-909.
10. AOAC., 1999. Official Methods of Analysis. 21st Edn., Association of Official Analytical Chemists (AOAC), Washington, DC., USA.

11. Trease, G.E. and W.C. Evans, 1987. Pharmacognosy. 13th Edn., Brailliar Tiridel Can. Macmillan Publishers, USA.
12. Harborne, J.B., 1973. Phytochemical Methods. Chapman and Hall Ltd., London, UK., pp: 49-188.
13. Pearson, D., 1976. The Chemical Analysis of Food. 7th Edn., Churchill, London, pp: 7-11.
14. Kirk, R.S. and R. Sawyer, 1991. Pearson's Composition and Analysis of Foods. 9th Edn., Longman, London, UK., ISBN-13: 9780582409101, Pages: 708.
15. Suneetha, V., B. Mishra, R. Gopinath, S.R. Shrestha and G K.B. Kartik *et al*., 2012. Screening and Identification of degradable products by pectin lyase producing *Actinomycetes* from katpadi and chittoor fruit industrial waste enriched soil samples. Asian J. Microbiol. Biotechnol. Environ. Sci., 14: 405-412.
16. Gangwal, A., S.K. Parmar and N.R. Sheth, 2010. Triterpenoid, flavonoids and sterols from *Lagenaria siceraria* fruit. Der Pharmacia Lettre, 1: 307-317.
17. Vollekova, A., D. Kostalova and R. Sochorova, 2001. Isoquinoline alkaloids from *Mahonia aquifolium* stem bark are active against *Malassezia* spp. Folia Microbiologica, 46: 107-111.
18. Maki, K.C., A.L. Lawless, M.S. Reeves, M.R. Dicklin, B.H. Jenks, E. Shneyvas and J.R. Brooks, 2012. Lipid-altering effects of a dietary supplement tablet containing free plant sterols and stanols in men and women with primary hypercholesterolaemia: A randomized, placebo-controlled crossover trial. Int. J. Food Sci. Nutr., 63: 476-482.
19. Haridas, V., Z.X. Xu, D. Kitchen, A. Jiang, P. Michels and J.U. Gutterman, 2011. The anticancer plant triterpenoid, avicin D, regulates glucocorticoid receptor signaling: implications for cellular metabolism. PLoS One., Vol. 6. 10.1371/journal. pone.0028037
20. Iheanacho, K.M.E. and A.C. Udebuani, 2009. Nutritional composition of some leafy vegetables consumed in Imo State, Nigeria. J. Applied Sci. Environ. Manage., 13: 35-38.
21. Bhattacharjee, S., A. Sultana, M.H. Sazzad, M.A. Islam, M.M. Ahtashom and Asaduzzaman, 2013. Analysis of the proximate composition and energy values of two varieties of onion (*Allium cepa* L.) bulbs of different origin: A comparative study. Int. J. Nutr. Food Sci., 2: 246-253.
22. Tadhani, M. and R. Subhash, 2006. Preliminary studies on *Stevia rebaudiana* leaves: Proximal composition, mineral analysis and phytochemical screening. J. Med. Sci., 6: 321-326.
23. Samydurai, P. and V. Thangapandian, 2012. Nutritional assessment, polyphenols evaluation and antioxidant activity of food resource plant *Decalepis hamiltonii* Wight and Arn. J. Applied Pharmaceut. Sci., 2: 106-110.
24. Jones, M.M., D.O J ohnson, J.T. Netlerville, J.I. Wood and M.D. Joesten, 1985. Chemistry and Society. 5th Edn., Saunders College Publishers, USA., pp: 521-577.
25. Pamela, C.C., A.H. Richard and R.F. Denise, 2005. Lippincotts illustrated Reviews Biochemistry. 3rd Edn., Lippincott Williams and Wilkins, Philadelphia, pp: 335-388.
26. Bhaskaram, P., 2001. Immunobiology of mild micronutrient deficiencies. Br. J. Nutr., 85: S75-S80.
27. Trowbridge, F. and R. Martorell, 2002. Forging effective strategies to combat iron deficiency. Summary and recommendations. J. Nutr., 132: 875S-880S.
28. Wolfenden, E., 2016. Taking too much manganese. March 5, 2016. http://www.livestrong.com/article/520170-taking-too-much-manganese/
29. Gonick, H.C., 2008. Nephrotoxicity of cadmium and lead. Indian J. Med. Res., 128: 335-352.
30. Akpanyung, E.O., 2005. Proximate and mineral composition of bouillon cubes produced in Nigeria. Pak. J. Nutr., 4: 327-329.
31. Oderinde, R.A., I.A. Ajayi and A. Adewuyi, 2009. Characterization of seed and seed oil of *Hura crepitans* and the kinetics of degradation of the oil during heating. Electron. J. Environ. Agric. Food Chem., 8: 201-208.
32. ASTM International, 2002. Standard test method for oxidation onset temperature of hydrocarbons by differential scanning calorimetry. ASTM E-2009-02, Annual Book of Standards, Section 12.10, ASTM International, West Conshohocken, PA., USA., pp: 734-738.
33. Eze, S.O.O., 2016. Physico-chemical properties of oil from some selected underutilized oil seeds available for biodiesel preparation. Afr. J. Biotechnol., 11: 10003-10007.
34. Akpambang, V.O.E., I.A. Amoo and A. Izuagie, 2008. Comparative compositional analysis on two varieties of melon (*Colocynthis citrullus* and *Cucumeropsis edulis*) and a variety of almond (*Prunus amygdalus*). Res. J. Agric. Biol. Sci., 4: 639-642.
35. Galadima, A., Z.N. Garba and B.M. Ibrahim, 2008. Homogeneous and heterogeneous transesterification of groundnut oil for synthesizing methyl biodiesel. Int. J. Pure Applied Sci., 2: 138-144.
36. Girisha, S.T., K. Ravikumar, B.R. Mrunalini and V. Girish, 2014. Comparative study of extraction methods and properties of non edible oils for biodiesel production. Asian J. Plant Sci. Res., 4: 28-35.
37. Singh, R.K. and S.K. Padhi, 2009. Characteristic of Jatropha oil for the preparation of biodiesel. Nat. Prod. Radiance, 8: 127-132.
38. Lewis, R.N., R.N. McElhaney, W. Pohle and H.H. Mantsch, 1994. Components of the carbonyl stretching band in the infrared spectra of hydrated 1,2-diacylglycerolipid bilayers: A reevaluation. Biophys. J., 67: 2367-2375.
39. Al-Kawkabani, A., B. Boutemeur-Kheddis, M. Makhloufi-Chebli, M. Hamdi, O. Talhi and A.M. Silva, 2013. Synthesis of novel 2H,8H-pyrano [2,3-f] chromene-2,8-diones from 8-formyl-7-hydroxy-4-methylcoumarin. Tetrahedron Lett., 54: 5111-5114.

40. Voet, D. and J.D. Voet, 2004. Biochemistry. 3rd Edn., John Wiley and Sons, Hoboken, NJ., USA., ISBN-13: 9780471193500, pp: 1341-1342.
41. Ajani, O.O., T.F. Owoeye, G.I. Olasehinde, D.K. Akinlabu, F.E. Owolabi and O.Y. Audu, 2016. Characterization, proximate composition and evaluation of antimicrobial activity of seed oil of *Bauhinia tomentosa*. J. Biol. Sci., 16: 102-111.
42. Sanglard, D. and T.C. White, 2006. Molecular Principled of Antifungal Drug Resistance. In: Molecular Principles of Fungal Pathogenesis, Heitman, J., S.G. Filler, J.E. Edward and A.P. Mitchell (Eds.). ASM Press, Washington, DC., pp: 197-212.
43. Filler, S.G., D.C. Sheppard and J.E. Edwards Jr, 2006. Molecular Basis of Fungal Adherence to Endothelial Epithelial Cells. In: Molecular Principles of Fungal Pathogenesis, Heitman, J., S.G. Filler, J.E. Edward and A.P. Mitchell (Eds.). ASM Press, Washington, DC., ISBN: 9781555813680, pp: 187-196.