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Antimicrobial, Proximate Composition, Mineral Content and Phytoconstituent Analysis of the Seed Extracts of *Buchholzia coriacea* (Wonderful Kola)

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ABSTRACT *Buchholzia coriacea* (wonderful kolanut plant) is a medicinal plant with its different parts used traditionally for treatment of various ailments including diabetes mellitus, hypertension, headache, rheumatism and the likes. This study was aimed at evaluating the antimicrobial activities of the seed extracts of *B. coriacea* against selected microorganisms (*Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis, Candida albicans* and *Microsporum audouinii*) using the agar diffusion method. Proximate composition, mineral content and the presence of bioactive phytochemicals were also investigated using standard procedures and GC-MS assay. The results of the inhibition zone diameter (IZD) ranged from 7.2 to 25 mm, 8 to 21.2 mm and 8 to 22 mm for the methanol, petroleum ether and chloroform extracts respectively. The minimum inhibitory concentration (MIC) showed that the methanol extract had better antimicrobial activity followed by the chloroform and petroleum ether extracts with the MIC values ranging from 0.2 to 4 mg/ml, 4.5 to 14 mg/ml and 4 to 13 mg/ml for the methanol, petroleum ether and chloroform extracts respectively. The IZD and MIC results further revealed the Gram-positive organisms to be more susceptible than the Gram-negative organisms whereas the fungus *C. albicans* showed intermediate susceptibility while the susceptibility of *M. audouinii* was near that of *P. aeruginosa* which was the least susceptible. Results of proximate composition include carbohydrate (61.49%), crude fibre (2.70%), crude fat (1.50%), crude protein (12.96%), moisture (1.36%), Ash (6.01%) and caloric value of 311.3 Kcal in the pulverized seed. The mineral analysis showed the presence of calcium (0.35%), magnesium (1.20%), phosphorus (1.01%), potassium (1.36%), sodium (0.84%), iron (0.19%) and zinc (1.03%). Qualitative and quantitative phytochemical screening results revealed the presence of glycosides, reducing sugars, saponins, anthracene derivatives, steroids, tannins, flavonoids and alkaloids in varying amount while gas chromatography and mass spectrometry (GC-MS) screening revealed the abundant presence of Cis-9-Hexadecenal, n-Hexadecanoic acid, 5-Hydroxymethyl furfural, 1-methyl-pyrrolidine-2-carboxylic acid, 6-octadecenoic acid, methyl ester, 4-Hydroxy-2-methyl pyrrolidine-2-carboxylic acid, 1,1-bicyclopentyl-2-one and hexadecanoic acid- methyl ester amidst the total of thirty-one compounds identified.

Keywords: Antimicrobial, phytoconstituent, proximate, mineral, *Buchholzia coriacea*, seed extracts.

Introduction

The history of therapeutic application of plants is as old as human existence on planet earth (Alani *et al*., 2005). Phyto-medicine or therapeutic uses of plants continue to maintain the advantage of being accessible, cheaper and widely acceptable option over their synthetic or orthodox counterpart of which adverse drug reaction and increasing microbial resistance are not uncommonly associated (Ekunsanmi, 2005; Habamu *et al*., 2010; Ibrahim and Fagbohun, 2014). Recently, the interest of drug developers and related scientists are now re-directed from the previous synthetic trend to the search for novel and more active plant derived compounds or products (Doughari *et al*., 2008; Josephs and Dowe 2016). Plants are known to produce many secondary metabolites which constitute an important source of microbicides, pesticides, many pharmaceutical drugs and herbal therapeutic preparations (Sindhu, 2009).

Buchholzia coriacea Engler. is a shrub or medium-sized tree also known as "musk tree" belonging to the family Capparidaceae. It is an ever green under storey tree of low land rain forest, growing up to 20 metres high occuring in West Africa from Guinea to West and East Cameroon and in Gabon (Habbu *et al*., 2010; Ibrahim and Fagbohun, 2014). The tree is also found in the southern part of Nigeria, Ghana and Liberia (Okoli *et al.,* 2010). The plant can be recognized by its compound pinnate leaves and the long narrow angular fruits containing large, usually aligned edible seeds commonly known as "wonderful kolanut" owing to its diverse ethnomedicinal applications (Ajaiyeoba *et al*., 2003; Amaechi, 2009). The bark of *B. coriacea* or "wonderful kolanut" plant can be made into a pulp for inhalation or into snuff to relieve headache, sinusitis, bronchitis, opthalmias, pleurisy, kidney pains and nasal congestion in Ivory Coast; for the treatment of smallpox or skin itching in Gabon and ear ache in Ghana (Ibrahim and Fagbohun, 2014). The pulped bark is applied to the chest to treat chest pains and also boils in Liberia (Sindhu, 2009). Also in Liberia, the seeds are used on skin eruption and internally for worms (Sindhu, 2009; Nweze, 2011). In Ivory coast, the crushed-up seeds are pasted over the stomach to shorten labor or speedup childbirth (Ajaiyeoba *et al.,* 2003). In Nigeria, the seed decoction is usually made in lime or local gin, hot water decoction is usually made by boiling in water all for the treatment of diabetes mellitus, hypertension, rheumatism, headache, cold, cough, catarrh and also regarded as brain food to promote memory by some trado-medical practitioners (Mbata *et al.*, 2009). Among other uses of the seeds include its use as stimulant, aphrodisiac, vessel dilator, fat burner, detoxifier, antiplasmodial, antihelmintics, analgesic, etc (Mbata *et al*., 2009; Okoli *et al.,* 2010). Plants are known to possess bioactive phytochemicals and comparative studies have shown some plant extracts to be nearly more active than conventional antibiotics (Ekunsanmi, 2005; Ndukwe *et al*., 2005; Kareem *et al*., 2012; Savoia, 2012). Bioactive compounds of plants are of immeasurable nutritional, therapeutic and ethnomedicinal importance (Savoia, 2012). The nutritional significance of edible plants is evaluated by their proximate composition of proteins, carbohydrates, lipids

(fats and oil), minerals, vitamins and water which are essential for the growth of the plant itself and by extension, other animals that feed on them (Mbata *et al*., 2009). Similarly, the ethnomedicinal and therapeutic significance is determined by a variety of phytoconstituent analysis involving series of scientific procedure all aimed at discovering novel plant derived bioactive compounds (Savoia, 2012). The original function of phytochemicals and secondary metabolites are to protect the plants against microbial attack and to aid their survival or adaptation and these functions by extension is transferred to man and other animals that utilizes or explores them for therapeutic purposes (Okwu, 2004; Savoia, 2012). Amidst numerous bioactivity assays, antimicrobial assay becomes imperative especially for *B. coriacea* and other plants with claims of being effective in treating ailments caused by infectious agents (microorganisms). Hence, plant extracts that can inhibit the growth of microorganisms or kill them and have negligible or no toxicity upon such studies becomes very attractive in quest for novel bioactive compounds (Masoko, et al., 2005; Nweze, 2011). Despite the huge exploration of many plants for novel bioactive compounds, such search or exploration is still considered to be in the embryonic stage especially owing to the large array of plant biodiversity (Ajaiyeoba *et al*., 2003; Vinothkumar *et al*., 2012; Kelembe *et al*., 2014). Therefore, the aim of this study is to evaluate the methanol, chloroform and petroleum ether seed extracts of *B. coriacea* for antimicrobial activity, proximate composition and the presence of bioactive plant metabolites.

Materials and Methods

Materials

Materials used include Sohxlet apparatus, table-sized autoclave, mettler weighing balance and glass wares of pyrex, England. All solvents were of the Analar grade, obtained from JHD, Guandgua Chemical Ltd., China. Microbiological media were obtained from Biotech., India and include Mueller Hinton agar, Nutrient broth, Sabouraud Dextrose agar and Sabouraud Dextrose broth.

Collection and Identification of Plant

The plant seed samples were collected from Ekpoma, Esan West Local Government area of Edo State and Identified by the Taxonomist Mr. Olufemi Shashanya of the Forestry Research Institute of Nigeria (FRIN), in Ibadan, Oyo state where a voucher specimen was made with assigned herbarium number: FHI110393.

Preparation of extracts

The seeds of *B. coriacea* were washed, chopped into pieces and oven dried at 55^{o}C. After drying, the pieces of seeds were pulverized into fine powder using a mechanical milling machine. After which approximately 250 g of the powdered plant was seperately extracted with 2000 ml of methanol, chloroform and petroleum ether using the Soxhlet apparatus. The extract was then concentrated to dryness at 55^{o}C using a thermostatically controlled water bath. The yields were weighed, percentage

yield calculated to be 13.41%, 10.49% and 7.23% corresponding to the methanonol, chloroform and petroleum ether extract respectively. The extracts were introduced into sterilized sample bottles and stored in the refrigerator at 4^{o}C for subsequent analyses (Habamu *et al.,* 2010).

Antimicrobial assay of the extracts

Overnight broth cultures of the selected pure clinical isolates of *Staphylococcus aureus*, *Klebsiella pneumoniae, Pseudomonas eruginosa*, *Bacillus subtilis*, *Candida albicans* and *Microsporum audouinii* were adjusted to 0.5 McFarland turbidity standard and further diluted (1:100 using normal saline solution) to yield microbial suspension of approximately 10^{6}cfu/ml; Antimicrobial sensitivity of the extracts were performed using the modified agar well diffusion method (Firas *et al*., 2008; Habamu *et al.,* 2010). Wells of 7 mm in diameter were made into uniformly streaked Mueller Hinton agar/Sabouraud dextrose agar plates. Each well was filled with 0.1 ml of the extract at varying concentrations. The same quantity of Tween-80 (10%) served as negative control while 0.1 mg/ml of Ciprofloxacin and 0.1mg/ml of Ketoconazole were used as positive controls for bacteria and fungi respectively. All plates were incubated in an upright position. However, bacteria plates were incubated overnight at 37^{o}C and fungal plates were incubated at room temperature (25^{o}C) for 72 hr. The absence or presence of growth was observed on the plates and the diameter of clear zone was measured in mm and recorded. The experiments were done in duplicates and the mean zones of inhibitions calculated.

Determination of Minimum Inhibitory Concentration's (MIC's) of the extracts

The MIC's of the extracts that showed activity against the organisms were determined by the agar dilution method (NCCLS, 2003; Lalitha *et al*., 2004). From the extract stock concentration of 100 mg/ml, lower concentrations were prepared by incorporation into the molten Mueller Hinton agar at different volumes to obtain a range of concentrations of between 0.1-18 mg/ml. Then a loop-full volume of one in hundred dilution (1:100) of 0.5MacFarland turbidity standard of microbial suspensions obtained from overnight broth were spotted on the surface of the agar plates at marked segment of the various plate concentrations of the test extracts and plates were incubated at 37^{o}C for 24 hours. The same procedure was repeated using Sabouraud agar but incubation was at room temperature (25±2^{o}C) for 48-72 hours after which the lowest concentration at which there was no observable bacterial/ fungal growth was recorded as the MICs.

Proximate analysis

The proximate analysis of the pulverized *B. coriacea* seeds was determined using standard method of analysis described by AOAC, (2007). The Caloric value (CV) was estimated by multiplying the values of carbohydrate, lipid and protein by factors of 4, 9 and 4 respectively and the total sum expressed in Kcal i.e kilocalories (AOAC, 2007; Imran *et al.*, 2007). Other proximate parameters evaluated include; Moisture content, crude fat, ash content, crude protein, crude fibre and carbohydrate.

All proximate values were carried out in triplicates and mean calculated and expressed in percentage.

Mineral content determination

The mineral composition (calcium, magnesium, phosphorus, potassium, sodium, iron and zinc) were determined using, analytical methods of atomic absorption spectrophotometer after ashing and dissolving the samples in 10% hydrochloric acid described by the Association of Official Analytical Chemists (AOAC, 2007).

Phytochemical screening

Qualitative and quantitative screening of the phytochemical components of the pulverized *B. coriacea* seed was carried out using the method outlined by Harborne (1998) to detect the presence of glycosides, alkaloids, saponins, tannins, flavonoids, anthracene derivatives, reducing sugars and steroids. The quantity of the stated parameters per weight of the dry mass of the pulverized seeds of *B. coriacea* was also determined and expressed in percentage.

GC-MS (Gas Chromatography Mass Spectrometry) Analysis

The GC-MS analysis was carried out according to the method described by Isahq *et al*. (2015). In the process, the carrier gas used was Helium at a flow rate of 1.2 ml/ min. The inlet temperature was maintained at 230^{o}C. The oven temperature was programmed initially at 50^{o}C for 5 minutes. Then programmed to increase to 300^{o}C at a rate of 10^{o}C ending with 25 minutes with a total run time of 45 minutes. The source temperature was maintained at 230^{o}C and the MS Quad at 150^{o}C. The ionization mode used was electron ionization mode at 70eV. Total Ion Count (TIC) was used to evaluate for compound identification and quantification. The Spectrum of the separated compound was compared with the database of the spectrum of known compound saved in the NIST02 Reference Spectra Library. Data analysis and peak area measurement was carried out using Agilent Chemstation Software.

Statistical Analysis

Statistical analysis was done using SPSS software version 16 (SPSS Inc. Chicago) and results presented in tables as mean ± standard error of mean. Paired t-Test and one-way analysis of variance (ANOVA) was used to compare data for level of significance.

Results

The Inhibition zone diameters (IZDs) recorded for the various concentrations of *B. coriacea* methanol, petroleum ether and chloroform seed extract as well as Ciprofloxacin and Ketoconazole (positive controls) are shown in Table 1, 2 and 3. The methanol extract, as shown in Table 1, recorded the highest range of IZDs (7.2±1.3 to 25.0±0.0 mm) while the petroleum ether extract (Table 2) showed IZDs range of

8.0±0.2 to 21.2±0.8 mm and the chloroform extract showed IZDs range of 8.0±0.0 to 22.0±1.0 mm in table 3. The petroleum ether extract and the chloroform extract showed a complete loss of activity at 1 mg/ml against all the test organisms but at a higher concentration of 10 mg/ml, their activities were established against all the test organisms except for the fungus, *M. audouinii* and the most resistant bacterium *P. aeruginosa*. However, at a higher concentration of 20 mg/ml, all the test organisms became susceptible to the inhibitory effect of the petroleum ether and the chloroform extract.

Results of the minimum inhibitory concentration (MIC) are presented in Table 4. The methanol extracts had the least set of MICs on the average, followed by the chloroform extract and the petroleum ether extract which showed the highest MIC against *P. aeruginosa*. The least set of MICs of the extracts against *K. pnemoniae, P. aeruginosa, B. subtilis, S. aureus, C. albicans* and *M. audouinii* were 0.6, 4, 0.2, 0.4, 0.6 and 0.8 mg/ml respectively for the methanol extract followed by 6, 13, 4, 5, 6 and 11 mg/ml respectively for the chloroform extract and the highest set were 6.5, 14, 4.5, 6, 6 and 12 mg/ml respectively. However, there was no large disparity between the activity of the petroleum ether and the chloroform extract.

Table 1: Antimicrobial activities of the methanol seed extract of *B. coriacea*

Organisms	Zones of Inhibition (mean ± S.E.M mm)						
	Concentrations (mg/ml)				CIP	KET	Tween-80
	1	10	20	40	0.1mg/ml	0.1mg/ml	(10%)
K. pneumoniae	8.6 ± 0.4	13.6±0.3	15.2±0.8	23.7±0.3	32.4±0.8	ND	0.00
P. aeruginosa	0.00	9.6 ± 0.8	13.5±1.0	18.2± 0.8	30.3±0.6	ND	0.00
B. subtilis	10.5±0.5	15.3±1.2	16.7±0.5	25.0±0.0	33.0±4.0	ND	0.00
S. aureus	9.7.0±1.0	14.0±0.0	15.8±0.6	24.0±0.2	33.5±0.5	ND	0.00
C. albicans	9.0 ± 0.5	13.2±0.3	14.7±0.8	23.3±0.6	ND	29.6±1.4	0.00
M. audouinii	7.2 ± 1.3	10.8±0.6	14.0±1.0	20.2±0.8	ND	27.5±0.5	0.00

Table 1: Antimicrobial activities of the methanol seed extract of *B. coriacea*
Key: S.E.M (Standard Error of Mean), ND **(**Not Determined), CIP (ciprofloxacin), KET (Ketoconazole)

Table 2: Antimicrobial activities of the petroleum ether extract

Organisms	Zones of Inhibition (mean ± S.E.M mm)						
	Concentrations (mg/ml)				CIP	KET	Tween-80
	1	10	20	40	0.1mg/ml	0.1mg/ml	(10%)
K. pneumoniae	0.00	8.0±0.2	12.5±0.5	19.0±0.0	32.7±0.3	ND	0.00
P. aeruginosa	0.00	0.00	9.0±0.0	17.2±0.3	31.5±1.5	ND	0.00
B. subtilis	0.00	9.2±0.8	14.6±0.3	21.2±0.8	34.4±0.6	ND	0.00
S. aureus	0.00	9.8±0.6	13.0±1.0	17.8±0.2	34.0±2.0	ND	0.00
C. albicans	0.00	9.8±0.2	12.3±1.6	17.2±0.3	ND	30.0±0.0	0.00
M. audouinii	0.00	0.00	10.3±0.2	15.0±1.0	ND	28.6±0.4	0.00

Key: S.E.M (Standard Error of Mean), ND **(**Not Determined), CIP (ciprofloxacin), KET (Ketoconazole)

Table 3: Antimicrobial activities of the chloroform extract

Organisms	Zones of Inhibition (mean ± S.E.M mm)						
	Concentrations (mg/ml)				CIP	KET	Tween-80
	1	10	20	40	0.1mg/ml	0.1mg/ml	(10%)
K. pneumoniae	0.00	8.0 ± 0.0	13.0±1.0	19.3±1.6	33.0±1.0	ND	0.00
P. aeruginosa	0.00	0.00	8.8 ± 0.6	19.0±0.0	31.5±0.5	ND	0.00
B. subtilis	0.00	10.0±0.5	15.3±0.6	22.0±1.0	34.0±2.0	ND	0.00
S. aureus	0.00	9.0 ± 0.0	15.0±1.0	18.0±1.5	34.5±0.5	ND	0.00
C. albicans	0.00	9.3 ± 0.2	14.0±1.0	18.0±1.0	ND	30.3±0.0	0.00
M. audouinii	0.00	0.00	10.8±0.6	16.6±2.3	ND	29.8±0.4	0.00

Key: S.E.M (Standard Error of Mean), ND **(**Not Determined), CIP (ciprofloxacin), KET (Ketoconazole)

Table 4: MICs of the extracts against the test organisms

Organisms	Methanol Extract	Petroleum ether extract	Chloroform Extracts
	MICs (mg/ml)		
K. pneumoniae	0.6	6.5	6
P. aeruginosa	4	14	13
B. subtilis	0.2	4.5	4
S. aureus	0.4	6	5
C. albicans	0.6	6	6
M. audouinii	0.8	12	11

The proximate composition of the pulverized (powdered) *B. coriacea* seed is presented in table 5. The parameters analyzed and values obtained were carbohydrate content 61.49±0.60 %, crude fibre 2.70 ± 0.30%, crude fat 1.50 ± 0.05%, crude protein 12.96±0.01%, moisture content 1.36 ± 0.36%, ash content 6.01 ± 0.03% and caloric value estimated to be 311.3±0.22 Kcal.

Table 5: Proximate composition of the pulverized *B. coriacea* seeds

Parameters	Values (Mean ± S.E)
Carbohydrate (%)	61.49±0.60
Crude fibre (%)	2.70 ± 0.30
Crude fat (%)	1.50 ± 0.05
Crude protein (%)	12.96±0.01
Moisture content (%)	1.36 ± 0.36
Ash content (%)	6.01 ± 0.03
Caloric value (kcal)	311.3±0.22

Key: S.E (standard error of mean)

Table 6 shows the results of mineral content of the pulverized seeds of *B. coriacea*. The seeds were found to contain calcium (0.35±0.81%), magnesium (1.20±0.42%), phosphorus (1.01±0.02), potassium (1.36±0.21%), sodium (0.84±0.37%), iron (0.19±0.41) and zinc (1.03±0.08%).

Table 6: Mineral content of the pulverized *B. coriacea* seeds

Parameters	Values (Mean ± S.E %)
Calcium (Ca)	0.35±0.81
Magnesium (Mg)	1.20±0.42
Phosphorus (P)	1.01±0.02
Potassium (K)	1.36±0.21
Sodium (Na)	0.84±0.37
Iron (Fe)	0.19±0.41
Zinc (Zn)	1.03±0.08

Key: S.E (standard error of mean)

The qualitative phytochemical analysis of the pulverized plant powder detected the presence of glycosides, reducing sugars, saponins, anthracene derivatives, steroids, tannins, flavonoids and alkaloids as presented in Table 7. Quantitatively, glycoside was found to be present in relatively high amount (2.3±0.02%), reducing sugar (moderate; 1.6±0.15%), saponins (high; 3.8±0.07%), anthracene derivatives (low; 0.3±0.41%), steroids (low; 0.4±0.81%), tannins (moderate; 1.4± 0.13%), flavonoids (high; 4.7±0.40%) and alkaloids (low; 0.3±0.76%).

Table 7: Phytochemical constituent of the pulverized seeds

Constituent	Status	Values (Mean ± S.E %)
Glycosides	+++	2.3± 0.02
Reducing sugars	++	1.6± 0.15
Saponins	+++	3.8± 0.07
Anthracene derivatives	+	0.3± 0.41
Steroids	+	0.4± 0.81
Tannins	++	1.4± 0.13
Flavonoids	+++	4.7± 0.40
Alkaloids	+	0.3± 0.76

Key: +++ (highly present), ++(moderately present), +(scantily present), - (absent), S.E (standard error of mean)

Results of the GC-MS (gas chromatography mass spectrometry) analysis are shown in Figure 1 and Table 8. The chromatogram reveals thirty-one chemical groups of compounds registered by each peak. the first peak detected the compound

3-pentanol, followed by peak 2(1-methyl-pyrrolidine-2-carboxylic acid), peak 3 (2-cyclopenten-l-one, 3-ethyl-2-hydroxyide), peak 4 (4h-pyran-4-one,2,3-dihydro-3,5-dihydroxy), peak 5 (1-pentanol, 2,2-dimethyl-), peak 6 is heptanoic acid, 3-hydroxy, methyl ester, peak 7 is 4h-pyran-4-one,3,5-dihydroxy-2 methyl-propan-2-ol, peak 8 (1,2-isopropyl-5-methyl cyclohexane), peak 9 (4-hydroxy-2-methyl pyrrolidine-2-carbolic), peak 10 (5-hydroxymethyl furfural) which was second to highest, peak 11 (pyrazine), peak 12 (1-methyl-pyrrolidine-2-carboxylic acid), peak 13 (1,1-bicyclopentyl-2-one), peak 14 (di-stachydrine), peak 15 (pentadecane), peak 16 (rolziracetam), peak 17 (Hexadecane), peak 18 (methyl ester), peak 19 (octanal), peak 20 (heptadecane), peak 21(6-hydroxyhexahydrocyclopenta[b]furan-2-c), peak 22 and 23 represent the same compound (cyclohexane,1,1-oxybis), peak 24 (4-hydroxy-2-methylpyrrolidine-2-carboxyl-hexadecanoic acid, peak 25 (methyl ester), peak 26 (cis-1,2-cyclododecanediol), peak 27 (cis-1,2-cyclododecanediol), peak 28 (6-octadecenoic acid), 29 (cis-9-hexadecenal) which had the highest peak, peak 30 (2-nonadecanone) and peak 31 (undecanal-2-methyl). However, the major compounds with the highest percentages and peaks include, Cis-9-Hexadecenal (12.58%; peak 29), n-Hexadecanoic acid (9.05%; peak 26), 5-Hydroxymethyl furfural (7.00%; peak 10), 1-methyl-pyrrolidine-2-carboxylic acid (6.55%; peak 12), 6-octadecenoic acid, methyl ester (5.63%; peak 28), 4-Hydroxy-2-methyl pyrrolidine-2-carboxylic acid (5.39%; peak 9), 1,1-bicyclopentyl-2-one (4.79; peak 13) and hexadecanoic acid- methyl ester (4.64%; 25).

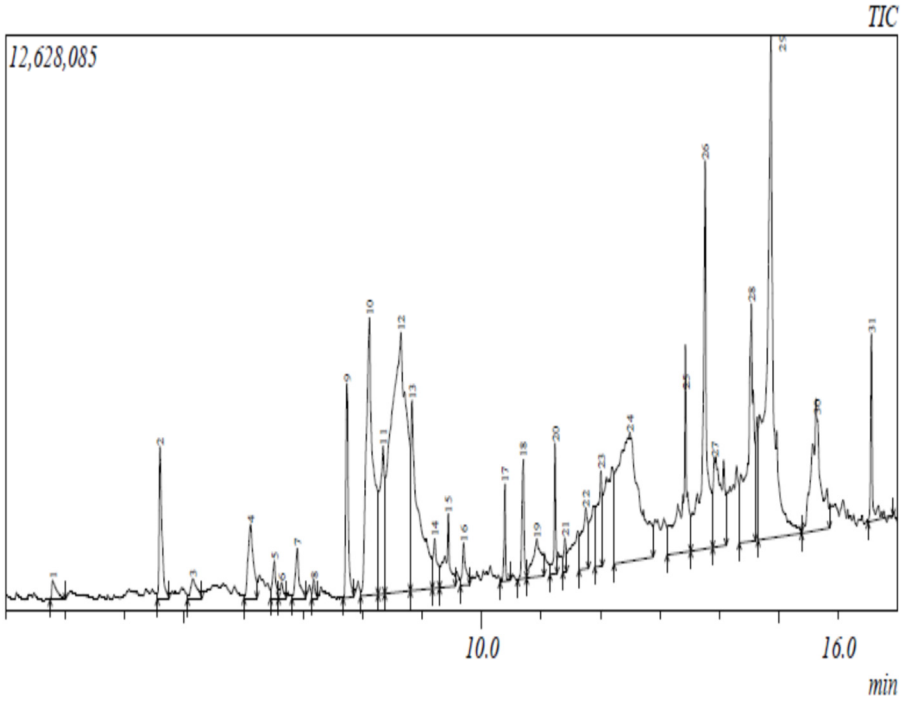

Figure 1: GC-MS sample chromatogram of the methanol seed extract of *B. coriacea*

Table 8: GC-MS Peaks and compounds represented in the Chromatogram

Compounds	Heights%	Peaks
3-pentanol	O.48	1
1-methyl-pyrrolidine-2-carboxylic acid	3.83	2
2-cyclopenten-l-one, 3-ethyl-2-hydroxyide	0.49	3
4h-pyran-4-one,2,3-dihydro-3,5-dihydroxy	1.87	4
1-pentanol, 2,2-dimethyl-	0.93	5
heptanoic acid, 3-hydroxy, methyl ester	0.40	6
4h-pyran-4-one,3,5-dihydroxy-2 methyl-propan-2-ol	1.26	7
1-2-isopropyl-5-methyl cyclohexane	0.46	8
4-hydroxy-2-methyl pyrrolidine-2-carbolic	5.39	9
5-hydroxymethyl furfural	7.00	10
octahydropyrrolo [1,2-a] pyrazine	3.72	11
1-methyl-pyrrolidine-2-carboxylic acid	6.55	12
1,1-bicyclopentyl-2-0ne	4.79	13
di-stachydrine	1.25	14
Pentadecane	1.83	15
Rolziracetam	1.07	16
Hexadecane	2.44	17
dl-proline, 5-oxo-,methyl ester	3.00	18
Octanal	0.95	19
Heptadecane	3.28	20
6-hydroxyhexahydrocyclopenta[b]furan-2-c	0.86	21
cyclohexane,1,1-oxybis	1.53	22
cyclohexane,1,1-oxybis	2.39	23
4-hydroxy-2-methylpyrrolidine-2-carboxyl-	3.24	24
hexadecanoic acid methyl ester	4.64	25
n-hexadecanoic acid	9.05	26
cis-1,2-cyclododecanediol	2.24	27
6-octadecenoic acid, methyl ester	5.63	28
cis-9-hexadecenal	12.58	29
2-nonadecanone	3.09	30
Undecanal-2-methyl	3.77	31

Discussion

One of the *in vitro* analytical method of determining antimicrobial activity of plant extracts and other agents is the establishment of inhibitory zone diameter (IZD) on seeded agar (Cheesbrough, 2006). The IZDs recorded for the *B. coriacea* methanol, petroleum ether and chloroform seed extract as well as Ciprofloxacin and Ketoconazole (positive controls) shows good activity against the test organism when compared to Tween-80 (negative control) which showed no activity (Table 1, 2 and 3). The activity shown were also concentration dependent. Extracts were considered active at zone of inhibition of >7 mm due to the diameter of the cork borer used to

make the agar wells. In a related study in which cork borer diameter of 9 mm was used by Ndukwe *et al*. (2005) and Usman *et al*. (2005), they considered activity of their test extract at IZD of > 9 mm. The IZDs varied with respect to the organisms and the various extracts with the methanol extract (Table 1) showing the highest range of IZDs (7.2±1.3 to 25.0±0.0 mm) followed by the petroleum ether extract (Table 2) with IZDs range of 8.0±0.2 to 21.2±0.8 mm and the chloroform extract IZDs (8.0±0.0 to 22.0±1.0 mm) in table 3. The petroleum ether and chloroform extracts are very similar and are both of lesser IZDs range than those recorded for the methanol extract and they showed a complete loss of activity at 1 mg/ml against all the test organisms but at a higher concentration of 10 mg/ml, their activities were established against all the test organisms except for the fungus, *M. audouinii* and the most resistant bacterium *P. aeruginosa*. However, at a higher concentration of 20 mg/ml, all the test organisms became susceptible to the inhibitory effect of the petroleum ether and the chloroform extract. In all concentration considered, the positive controls were observed to show higher inhibition zone diameters which may be attributed to the crude nature of the extracts as opposed to ciprofloxacin and ketoconazole (positive controls) which already are in their purified and compounded form. This result is in agreement with those reported by Ishaq *et al*. (2016) in which the methanol extract of *Cannabis indica* showed higher IZDs than the chloroform and petroleum ether extract. Similarly, Habbu *et al*. (2010) reported higher IZDs for the chloroform extract of *Spondias mombi* relative to the n-Butanol and petroleum ether extracts. In contrast however, Alani *et al*. (2005) reported higher IZDs for the petroleum ether extract of two selected Indian medicinal plants relative to their methanol and chloroform extracts.

The lower the MIC value the more potent the antimicrobial agent, conversely, the higher the MIC value, the less potent the antimicrobial agent (Cheesbrough, 2006; Dowe *et al*., 2016). *P. aeruginosa* followed by the fungus *M. audouinii* were least susceptible to the inhibitory effect of the plant extracts whereas *B. subtilis* was most susceptible (Table 4). The MIC results shows the Gram-positive organisms to be more susceptible to the inhibitory effect of the plant extracts compared to the Gram-negative organisms whereas *C. albicans, K. pneumoniae* and *S. aureus* showed intermediate susceptibility relative to the two extremes. In a related study, Oshomo and Idu (2012), reported lower MICs for *B. subtilis S. mutans*, *S. aureus* and *C. albicans* compared to other gram negative dental caries isolates. Some organisms are generally more susceptible than others depending largely on their structural framework; some such as gram negative bacteria are often more resistance than their gram positive counterpart due to an outer phospholipid membrane with structural lipopolysaccharides (LPS) components that reduce the cell wall penetration ability of antimicrobial compounds (Chessebrough, 2006; Rahman *et al.,* 2011).

The seed of *B. coriacea* is appreciably rich in carbohydrate, proteins and fat resulting in high caloric value all of which points to the energy rich nature of the seeds (Table 5). This result corroborates those obtained by Ibrahim and Fagbohun (2014) as they also reported good percentage of carbohydrate, protein, crude fat, etc. The moisture content (% dry matter; 1.36 ± 0.36) of the pulverized seeds of *B. coriacea* also known as wonderful Kola is close to the values (moisture content; 1.34± 0.02%) obtained by Amaechi (2009) for the same reference plant seed. The low moisture content is an indication that the seeds will last longer when stored than

most fruits and seeds with higher moisture content since deteriorating agents requires higher water activity for their proliferation. The ash content of 6.01 ± 0.03% obtained from this study is higher than 4.33% and 3.01% obtained by Ameachi (2009) and Eleyinmi *et al*. (2006) for *B. coriacea* (wonderful kola) and bitter kola respectively. The crude fibre (2.70 ± 0.30%) was higher than 1.7% obtained by Ameachi (2009) for same *B. coriacea* probably due to source variation. The crude fibre was however, lower than what was obtained in bitter kola (11.4%) by Eleynmi *et al*. (2006) and kolanut seeds (7.3%) by Jayeola, (2001). Adequate intake of dietary fibre had been reported by Ishida *et al.* (2000) to lower the serum cholesterol level, risk of coronary heart diseases, hypertension, constipation, and diabetes.

The low calcium (0.35±0.81%) content of the seeds (Table 6) reveals that the seeds would not be too useful for bone formation and other related functions of calcium. Calcium in consumption with phosphorous, magnesium, manganese are responsible for bone formation (Akinhanmi *et al*., 2008). Akinhanmi *et al*. (2008) further posited that for good calcium and phosphorous intestinal absorption, Calcium to Phosphorus ratio should be close to or up to 1. The sodium content (0.84±0.37%) is also low which is in accordance with the general observation of Aremu *et al*. (2005) that tropical crops carry subnormal concentration of sodium which is a reflection of low sodium content of the soils. The potassium content of the seeds (1.36±0.21%) was also low relative to most common fruits that are major sources of minerals and vitamins; nevertheless, it was highest among the minerals analysed which is in agreement with the report of Aremu *et al.* (2005) that potassium is the predominant mineral generally reported in Nigerian agricultural products. Sodium and potassium plays significant role in electrical potential and contributes to salt balance which is vital in reducing high blood pressure as recommended by FND (2002). Magnesium is a component of chlorophyll and has been reported to be involved in maintaining the electrical potential and activation of some enzyme systems in plants (Jayeola, 2001; Ibrahim and Fagbohun, 2014). Iron contributes to blood functions and zinc is involved in normal function of immune system and is a component of over 50 enzymes in the body (Okaka and Okaka 2006).

The result of the qualitative and quantitative phytochemistry shows the presence in appreciable quantity of bioactive secondary plant metabolites (Table 7). Similar results were obtained in a study by Ajayeoba *et al* (2003) and Mbata *et al* (2009) in which the presence of alkaloids, saponins, tannins, reducing sugar, phenols, carbohydrates and flavonoids were detected in the methanol seed extract of *B. coriacea* in related quantities. Glycosides are molecules in which a sugar is bound to another functional group via a glycosidic bond, the sugar molecule may be hydrolysed by enzymatic action enabling the chemical to be available for use. This is highly important in enabling *in vivo* bioactivity of some plants that seem inactive upon *in vitro* bioassay (Mbata *et al*., 2009; Nobmann *et al*., 2009; Josephs and Dowe 2016). The antimicrobial properties of tannins, alkaloids, flavonoids and saponins has been established and reported (Okwu, 2004; Adekunle and Ikumapayi, 2006; Kalembe *et al*., 2014). Saponins has bitter taste, foaming property and serve as mild detergent that solubilizes cell permeability barriers and consequent lysing of bacterial and fungal cells (Okwu, 2004; Dowe *et al*., 2016). Alkaloids are one of the most valuable plant secondary metabolite in therapeutic sense. Pure isolates of alkaloids and their synthetic derivatives are used as basic medicinal agents for their analgesic, and anti-

bacteriocidal properties (Okwu, 2004). Phenolics are a member of a group of aromatic compounds with weakly acidic properties. The presence of phenols is considered to be potentially toxic to the growth and development of pathogens (Okwu and Okwu 2004). They contribute to the antioxidant properties of the seeds and may play a role in inactivating carcinogens and inhibiting the expression of mutagens in experimental animals (Okwu, 2004; Rahman *et al*., 2011). Anthracene derivatives are a group of aromatic ring compounds with two or more ketone substitutions. The presence of tannins which is a mixture of esters of gallic acid with glucose was confirmed in the powdered plant. They have an astringent property with many physiologic activities and a wide range of anti-infective action (Okwu, 2004; Manikandan *et al*., 2006). Flavonoids was confirmed to be present in the plant extracts and has been found to play some important pharmacologic roles against diseases, such as cardiovascular diseases, cancer, inflammation and allergy and other oxidative stress related to diseases (Habbu, *et al.,* 2010). The broad-spectrum activities of the plant extracts are traceable to the presence of flavonoids. They are known to be synthesized by plants in response to microbial infection and have been found *in vitro* to be effective against a wide array of microorganisms (Harborne, 1998; Manikandan *et al*., 2006; Habbu, *et al.,* 2010).

The Chromatogram (Figure 1 and Table 8) showed that the first peak was the compound 3-pentanol known to protect plant from pathogenic invasion and now used for therapeutic treatment of plants for pest control puposes (kalembe *et al.,* 2014), followed by peak 2(1-methyl-pyrrolidine-2-carboxylic acid), peak 3 (2-cyclopenten-l-one, 3-ethyl-2-hydroxyide), peak 4 (4h-pyran-4-one,2,3-dihydro-3,5-dihydroxy), peak 5 (1-pentanol, 2,2-dimethyl-), peak 6 is heptanoic acid, 3-hydroxy, methyl ester which has been reported by Habbu *et al*. (2010) to possess antimicrobial and antifungal activities. From the total of 31 compounds detected and identified by the GC-MS assay, the major compounds with the highest percentages and peaks include, Cis-9-Hexadecenal (12.58%; peak 29), n-Hexadecanoic acid (9.05%; peak 26), 5-Hydroxymethyl furfural (7.00%; peak 10), 1-methyl-pyrolidine-2-carboxylic acid (6.55%; peak 12), 6-octadecenoic acid, methyl ester (5.63%; peak 28), 4-Hydroxy-2-methyl pyrolidine-2-carboxylic acid (5.39%; peak 9), 1,1-bicyclopentyl-2-one (4.79; peak 13) and hexadecanoic acid- methyl ester (4.64%; 25). The compound cis-9-hexadecenal with the highest peak is related to oleic acid which aids the functioning of the nervous system while rolziracetam has been reported to reduce epileptic seizures (Okwu, 2004). According to Durling *et al*. (2009), 5-Hydroxymethylfurfural (HMF), a heat-induced food toxicant present in a vast number of plants, has been suggested to be genotoxic after being bioactivated by the sulfotransferase SULT1A1. It has been shown to possess DNA damaging effect either activated or otherwise in microbial cells. Also, 1,1-bicyclopentyl-2-one and distachydrine are known to possess antiinflamatory, antimicrobial and antioxidant properties (Ajayeoba *et al*., 2003; Mbata *et al*., 2009).

Conclusion

This study has shown that the methanol, petroleum ether and chloroform seed extract of *B. coriacea* had a concentration dependent activity against the test organisms as opposed to the negative control (10% Tween-80). The methanol extract was more

active, followed by the chloroform extract and the petroleum ether extract which was least active. However, the difference in activity between the petroleum ether extract and chloroform extract was not statistically significant but the difference in activity between either of the petroleum ether or the chloroform extract and the methanol extract was statistically significant (*$P < 0.05$). There is the presence of several bio-active plant compounds or metabolites which correlates with the antimicrobial properties of the plant. The positive findings from this study supports the continuous ethnomedicinal uses of the plant as well as suggestive of the plant as a promising raw material that requires full exploitation in the scientific world and health related industries.

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