International Journal of Research in Engineering and Applied Sciences(IJREAS)
Available online at http://euroasiapub.org/journals.php
Vol. 7 Issue 3, March-2017, pp. 17~26
ISSN (O): 2249-3905, ISSN(P): 2349-6525 | Impact Factor: 7.196 | Thomson Reuters Researcher ID: L-5236-2015

Preliminary Studies on the Secondary Metabolites of *Buchholzia Coriacea* (Wonderful Kola) Seed Ethanol Extract by GC-MS Analysis

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Abstract

Plants are the major sources of drugs. Natives all over the world use plants in different ways to treat diseases. Man, wild and domestic animals selectively eat these plants when they are sick. Our research interest is to find the phytochemicals in the seed of *Bucholzia coriacea* (Wonder cola) seed that produces its medicinal activity. Gas Chromatography-Mass Spectrometry (GC-MS) analysis was used for screening the secondary metabolites in its seed as a preliminary study. The extract was prepared using Soxhlet extraction method with ethanol as solvent. It was concentrated in a rotary evaporator. The molecular mass of the phytochemicals were established based on the fragmentation pattern in the mass spectra. The chromatogram showed twelve peaks representing the presence of twelve phytochemicals in the extract. The compounds were proposed based on comparison with National Institute of Standards and Technology (NIST) database. The suggested compounds are Beta-vinyl acrylic acid (10.62%), 2-methyl-pyrrolidine-2-carboxylic acid (33.60%), 1,2-benzenedicarboxylic acid, diethyl ester (0.89%), 14-ketopentadecanoic acid (0.68%), methyl 14-methylpenta decanoate (1.10%), norlinolenic acid (4.26%), hexadecanoic acid (13.26%), 9,12-octadecadienoic acid (5.64%), 9-octadecynoic acid (9.84%), linoelaidic acid (12.87%), micropine (1.02%) and anandamide (6.22%).The bioactivity studies showed that *Bucholzia coriacea* seed could be a urinary acidifier, inhibitor of uric acid production, catechol-O-Methyl transferase inhibitor which could control chronic infection like Parkinson's disease. We therefore recommend the isolation and total characterization of *B. coriacea* in order to confirm the secondary metabolites present.

Keywords: Bioactivity, extraction, gas- chromatography, *Bucholzia coriacea,* mass- spectrometry

INTRODUCTION

B. coriacea belongs to the family Capparaceae. It is commonly known as 'Wonderful kola' due to the medicinal properties of its seed and is widely seen in the rain forests of Nigeria, Cameroon, Liberia, Central African Republic, Congo, Ivory Coast and Gabon (Ezekiel and Onyeoziri, 2009; Mbata *et al*., 2009). Traditionally it is known in Nigeria as 'uke' or 'Okpokolo' in Igbo, 'owi' in Edo and 'uworo' or 'Aponmu' in Yoruba (Ezekiel and Onyeoziri, 2009) while in other countries it is known as 'Ndo' in Mende (Sierra Leone),'Doe-fiah' in Kru-basa (Liberia), 'Eson-bese' in Akan-asante (Ghana), 'Banda' in Munga (West Cameroons), 'Essonbossi' in Central Africa and 'Kola Pimente' in French (Anowi *et al*., 2012; Koudogbo *et al*., 1972).

The plant is a medium sized evergreen tree growing up to 20m high with a smooth dark brown bark, wide leaves that are glossy and leathery arranged spirally and clustered with conspicuous cream white flowers in racemates at the end of the branches (Akpanyung *et al*., 1995; Culpeper, 1995). *B. coriacea* has a variety of medicinal uses which includes treatment of migraine when applied topically on the head (Erhirhie *et al*., 2015), anti-helminthic activity of the leaves and seed (Kameswararao *et al.,* 2003) as well as antimicrobial properties (Nweze *et al*., 2006; Ejikeugwu *et al*., 2014). Obembe *et al.,* 2012 results suggested that the extract of *Buchholzia coriacea* may have antifertility effects in male rats, the site of action most probably the epididymis . In Gabon *B. coriacea* is sometimes cultivated as both a medicinal and fetish plant (Lemmens, 2013). *B. coriacea* seed was shown to possess significant antidiabetic potential and also reduced lipid peroxidation in diabetic rats (Ezeigbo, 2011; Chinaka *et al*., 2012), while its leaf extract demonstrated anti-inflammatory activity (Ezike *et al*., 2015). Our research therefore is aimed at studying the medicinal potentials and secondary metabolites in the ethanol extract of *B. coriacea* seed by GC-MS analysis. GCMS analyses has been used by several researchers to demonstrate the presence of primary and secondary metabolites in plant extracts (Igwe *et al.*, 2016; Yan-qun *et al*., 2013; Igwe *et al.*, 2016b; Divya and Subba, 2013; Igwe *et al.*, 2016c). The pictorial view of ***B. coriacea*** is shown in Figure 1.

Fig -1: Pictorial view of *B. coriacea*

MATERIAL AND METHODS

a- Plant Materials

Fresh seeds of *B. coriacea* was harvested at Asaba in Delta State, Nigeria. The seeds were identified at the Taxonomy section of College of Natural,Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike, Nigeria.

b- Preparation of Plant Extract

B. coriacea was dried in a shady place for 15 days and pulverized to powder using electrical grinder. Extraction was performed using soxhlet method (Jensen, 2007). Thirty five grams (36 g) of powdered sample was introduced into the extraction chamber of the soxhlet extractor using ethanol as solvent at a temperature of 70° C for 48 hrs. At the end of the extraction, the extract was concentrated in a rotary evaporator. The extract was sent for GC-MS analysis.

c- GC-MS analysis of B. coriacea

GC-MS QP2010 Plus (Shimadzu, Japan) was used in the characterization. The identification of the photochemical in the sample was carried out using a QP2010 gas chromatography with Thermal Desorption System, TD 20 coupled with mass spectrometry (Shimadzu). The ionization voltage was 70eV. Gas chromatography was conducted in the temperature programming mode with a Restek column (0.25 mm, 60m, XTI-5).The initial column temperature was 80°C for 1min, and then increased linearly at 70°C min-1 to 220°C, held for 3 min followed by linear increased temperature 10°C min-1 to 290°C for 10 min.The temperature of the injection port was 290°C and the GC-MS interface was maintained at 290°C .The sample was introduced via an all-glass injector working in the split mode, with helium carrier gas low rate of 1.2 ml min^{-1}.

d- Identification of secondary metabolites in B. coriacea

The GC-MS chromatogram of ethanol extract of *B. coriacea* was compared with the database of National Institute of Standards and Technology (NIST), NIST08.LIB (Stein, 1990), WILEY8.LIB (McLafferty, 1986) and with published literature. The name, molecular weight, formula, structure and bioactivities of the compounds were ascertained.

RESULTS AND DISCUSSION

B. coriacea gas chromatogram is presented in Figure 2. The mass spectra of *B. coriacea* seed is show in Figure 3.

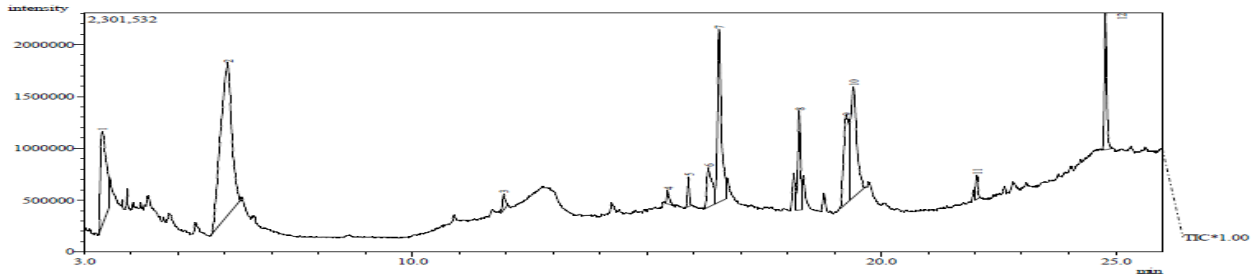

Figure 2: Gas chromatogram of *B. coriacea* (wonderful kola) seed ethanol extract

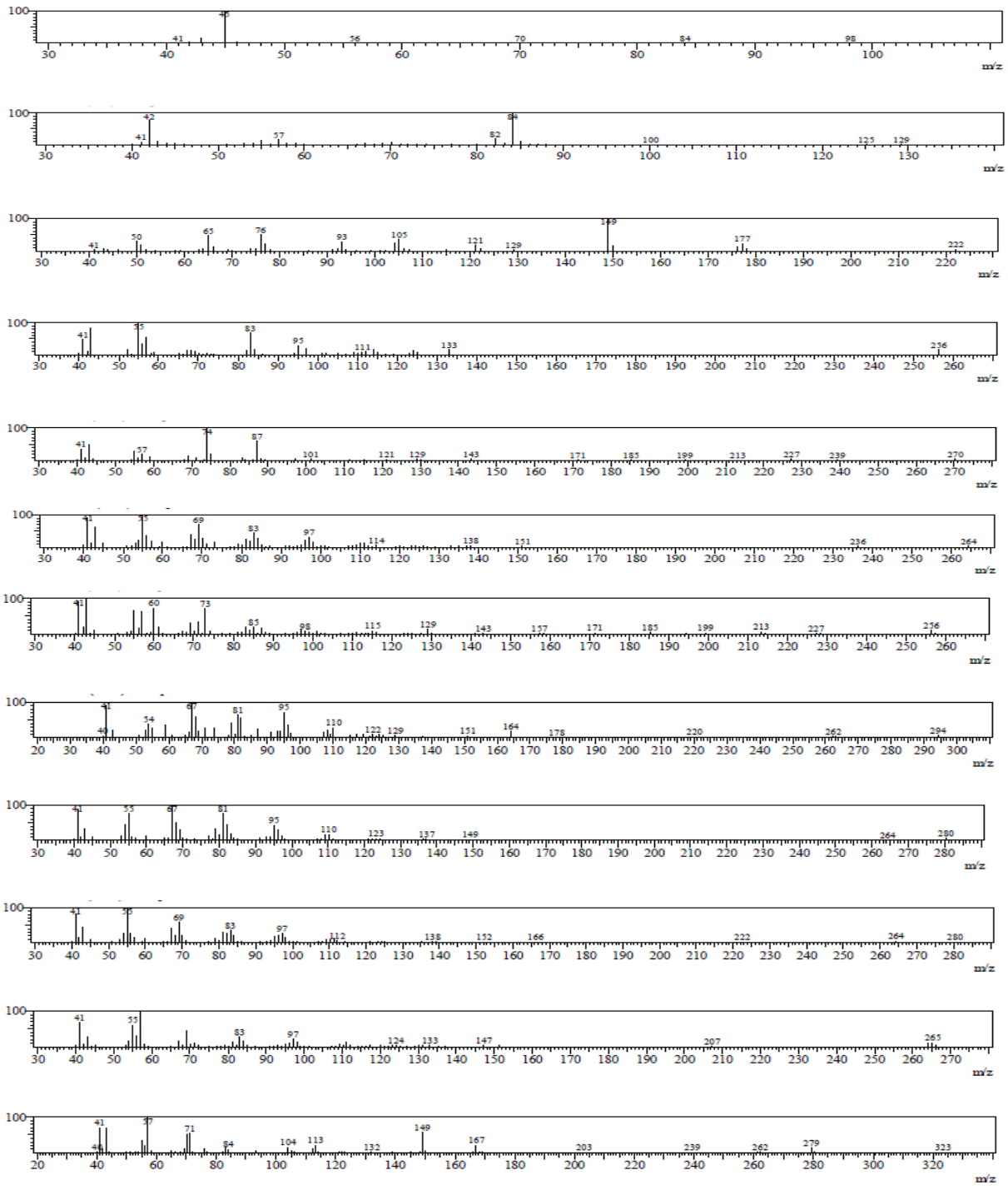

Figure- 3: shows mass spectra of *B. coriacea* seed

Table -1 Compounds and suggested bioactivity of *B. coriacea* (wonderful kola) seed ethanol extract

S/No	Name of Compound	Retention time	Peak Area (%)	Molecular weight	Molecular formula	Bioactivity
1	Beta-vinyl acrylic acid	3.389	10.62	98.09	$C_5H_6O_2$	Antiamyloid-Beta, Beta-2-Receptor-Agonist, Beta-Glucuronidase-Inhibitor
2	2-Methyl-pyrrolidine-2-carboxylic acid	6.059	33.60	129.15	$C_6H_{11}NO_2$	Methyl-Guanidine-Inhibitor
3	1,2-Benzenedicarboxylic acid, diethyl ester	11.952	0.89	222.23	$C_{12}H_{14}O_4$	Acidifier
4	14-ketopentadecanoic acid	15.449	0.68	256.38	$C_{15}H_{28}O_3$	Acidulant
5	Methyl 14-methylpentadecanoate	15.890	1.10	270.45	$C_{17}H_{34}O_2$	Catechol-O-Methyltransferase-Inhibitor
6	Norlinolenic acid	16.317	4.26	264.40	$C_{17}H_{28}O_2$	Increase Aromatic Amino Acid Decarboxylase Activity
7	Hexadecanoic acid	16.544	13.26	256.42	$C_{16}H_{32}O_2$	Arachidonic acid-Inhibitor
8	9,12-Octadecadienoic acid	18.251	5.64	294.47	$C_{19}H_{34}O_2$	Inhibit Production of Uric Acid
9	9-Octadecynoic acid	19.258	9.84	280.44	$C_{18}H_{32}O_2$	Urine-Acidifier
10	Linoelaidic acid	19.406	12.87	280.44	$C_{18}H_{32}O_2$	Acidifier
11	Micropine	22.040	1.02	265.39	$C_{16}H_{27}NO_2$	Not found
12	*Anandamide*	24.783	6.22	323.28	$C_{20}H_{37}NO_2$	Neurotransmitter

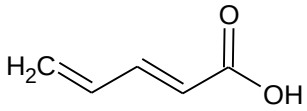

Figure- 4: Beta-Vinyl acrylic acid

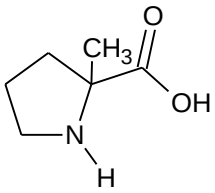

Figure- 5: 2-Methyl-pyrrolidine-2-carboxylic acid

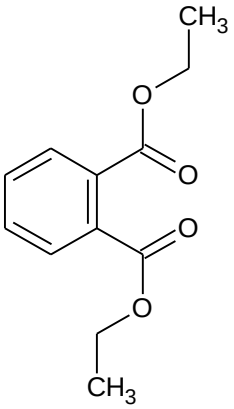

Figure- 6: 1,2-Benzenedicarboxylic acid, diethyl ester

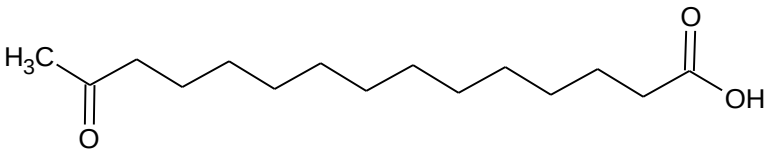

Figure- 7: 14-ketopentadecanoic acid

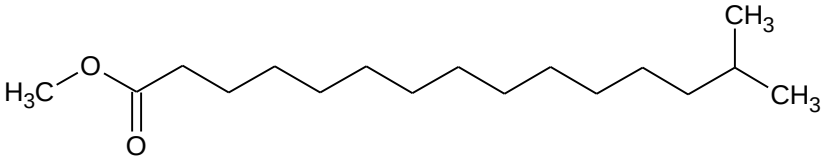

Figure- 8: Methyl 14-methylpentadecanoate

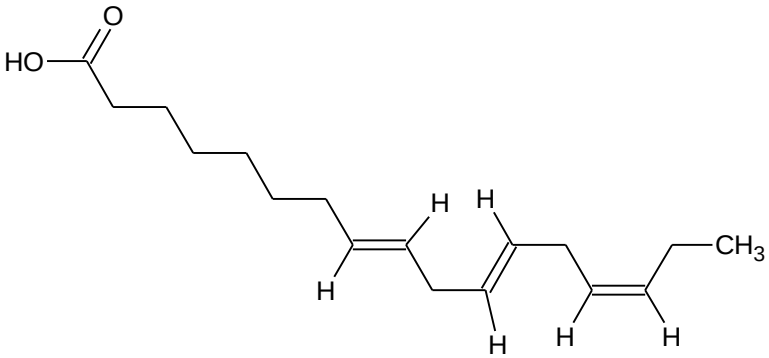

Figure- 9: Norlinolenic acid

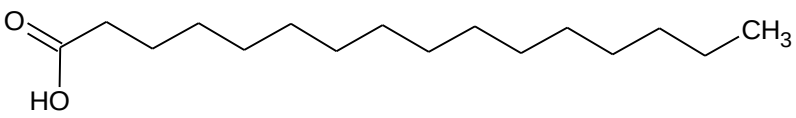

Figure- 10: Hexadecanoic acid

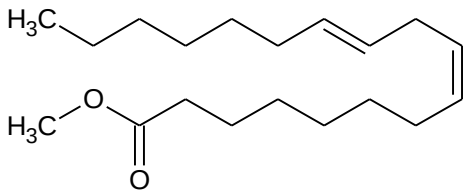

Figure- 11: 9,12-Octadecadienoic acid

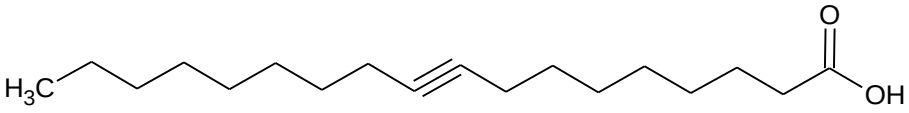

Figure- 12: 9-Octadecynoic acid

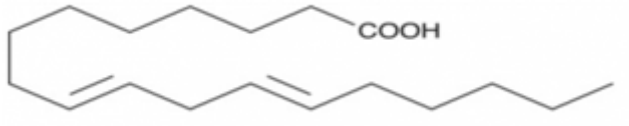

Figure- 13: Linoelaidic acid

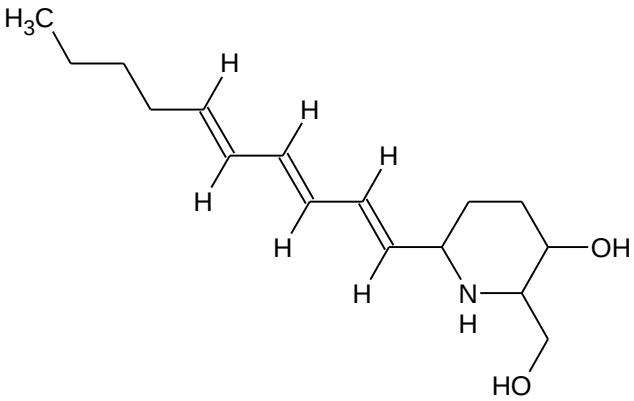

Figure- 14: Micropine

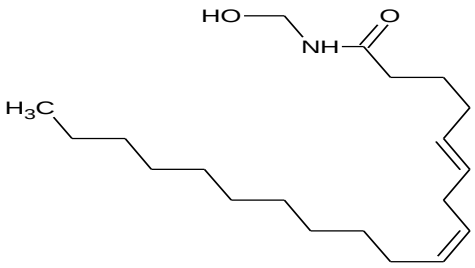

Figure 12: *Anandamide*

The GC-MS gas chromamatogram showed 12 peaks representing 12 phytochemicals in *B. coriacea* (wonderful kola) seed ethanol extract (Figure 2). The molecular weight is equivalent to the molecular ions in the mass spectra . The name, retention time, peak area percentage, molecular weight, molecular formular and bioactivity of *B. coriacea* (wonderful kola) seed ethanol extract are shown in Table 1 while their molecular structures are seen in Figures 4- 15. 2-Methyl-pyrrolidine-2-carboxylic acid with the highest peak area % of 33.60 and a retention time of 6.059 is a Methyl-Guanidine-Inhibitor (Duke, 1996). Methyl-Guanidine inhibitor, inhibits the action of the hydrolase enzyme methyguanidinase which is an enzyme that catalyzes the hydrolysis of methylguanidine to urea (Nakajima, 1980). Hexadecanoic acid at (RT: 16.544), (PA:13.26%) was the second abundant compound in the extract. It is an arachidonic acid inhibition. Beta-vinyl acrylic acid is an antiamyloid-beta-2-receptor agonist and beta-glucuronidase inhibitor. activity as analyzed by GC-MS. 9-Octadecynoic acid and Linoelaidic acid (RT: 19.258; 19.406) and (PA 9.84%; 12.87%) respectively are urine acidifier (Duke, 1996). Acidifiers are chemicals that reduce the pH of the body. Acidifiers are needed for food digestion especially in patients suffering from achlorhydria. These patients are not able to secret HCl for food digestion. These phytocompounds will be beneficial since it increases gastric acid when ingested. The compound 9,12-Octadecadienoic acid with peak area % 5.64 may inhibit uric acid production. Uric acid inhibitor is a compound that inhibits the acidification of urine minimizing the risk of formation of uric acid stones and deposition of uric acid crystals in the joints such as the toe and knee joints that form gout thereby reducing episodes of sharp pain in the affected joints (Muhammad, 2013). Methyl 14-methylpentadecanoate is a known catechol-O-methyl transferase inhibitor. A chronic infection like Parkinson's disease is treatable with a catechol-O-methyl-transferase inhibitors (Burkhard *et al*., 2001). Since methyl 14-methylpentadecanoate and 9,12-octadecadienoic acid, methyl ester are inhibitors of catechol-O-methyl-transferase (Duke, 1996), they may be effective in the treatment of Parkinson's disease. Catechol-O-methyltransferase is involved in the degradation of neurotransmitters but the inhibitors oppose the degradation of neurotransmitters. COMT is involved in the degradation of catecholamine (dopamine, noradrenaline and adrenaline) which are neurotransmitters. Anandamide, another compound with lower concentration in the extract (PA: 6.22%) and at (RT: 24.783) is responsible for neurotransmission action.

ACKNOWLEDGEMENT

We are grateful **ACKNOWLEDGEMENT**
to National Research Institute for Chemical Technology [NARICT], Zaria, Nigeria, for running the GC-MS analysis and Mrs Rose Sangodare for her effort to safe guard the sample for accurate result.

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