World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

Check for updates

Isolation and characterization of the bioactive components in *Tetracarpidium conophorum* as dissolvent in methanol

Henrrietta Ogadimma Asuzu-Samuel [1,*] and John Godson Nnamdi [2]

[1] *Biomedical Technology, School of Science Laboratory Technology, University of Port Harcourt, Nigeria.*
[2] *Biochemistry/ Chemistry Technology, School of Science Laboratory Technology, University of Port Harcourt, Nigeria.*

World Journal of Advanced Research and Reviews, 2023, 20(03), 1878–1883

Publication history: Received on 21 November 2023; revised on 27 December 2023; accepted on 29 December 2023

Article DOI: https://doi.org/10.30574/wjarr.2023.20.3.2739

Abstract

This study evaluate bioactive compounds present in the methanol extracts of raw *Tetracarpidium conophorum* (African walnut) with their molecular formulae, molecular weights, Retention Times (RT) and Peak areas (%), are shown in Table 1. The GC-MS chromatograms detected 13 peaks of bioactive compounds for extract as shown in Figure 2.The height of the peak indicates the relative concentration of the components present in the extracts. These compounds mainly comprised esters, alcohols, hydrocarbons and ketones. The major chemical constituents identified in the raw extract was 9,12,15-octadecatrienoic acid, (Z, Z,Z)- (67.61%), other compounds identified were n-hexadecanoic acid (4.92%); 9,12-octadecadienoic acid, methyl ester (0.27%); 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)- (1.38%); heptadecanoic acid, 16-methyl-, methyl ester (0.10%); 9,12,15-octadecatrienoic acid, ethyl ester, (Z,Z,Z)- (17.15%); ethyl 9,12,15- octadecatrienoate (5.21%); 9,12,15-octadecatrien1-ol, (Z,Z,Z)- (2.85%); 9,12-octadecadienoic acid (Z,Z)- (9.55%); methyl 8,11,14 heptadecatrienoate (4.35%); octadecanoic acid (3.87%); bis(2-ethylhexyl) phthalate (0.11%) and squalene (0.23%).

Keywords: Bioactive; Methanol; *Tetracarpidium conophorum;* Isolation and Characterization

1. Introduction

African walnut (*Tetracarpidium conophorum*) is a member of the Euphorbiaceae family. It is found in the wet parts of Eastern and Western Nigeria as well as Western Africa in general. It is known in Eastern Nigeria as Ukpa (Igbo), Western Nigeria as "Awusa or Asala" (Yoruba), "Okhue or Okwe" (Edo) (Ayoola et al., 2011). *Tetracarpidium conophorum* is a climbing shrub of about 10-20ft long, it is normally planted under an indigenous tree so that can provide strong support for the heavy weight of the climber when fully established on the crown of the tree, and in some cases where they cannot be harvested manually; they are left till full maturation after which the pods fall off and are picked, removed from the rotten pods, washed and sold in the market (Dauda et al., 2020). The objective of isolation and characterization of the bioactive component of *Tetracarpidium conophorum* in disolvent methanol is to extract some chemicals from the *Tetracarpidium conophorum* seed, this extraction of the chemical act as: antioxidants, anticancer, antimicrobial property. The nut is whitish upon cracking from the shell and there is usually a thin layer in between two halves of the nut when it is divided into two equal parts (Ayoola et al., 2011). It also contains a very high composition of vitamin E, most especially, gamma-tocophenol (Kanu et al., 2015). It was reported that hot aqueous extract from *Tetracarpidium conophorum* nut could help to protect rats against castor oil-induced diarrhea; the inhibitory effect was attributed to the presence of some secondary metabolites and which also justified ethno medicinal use (Nwachoko and Jack 2015). The seeds of *Tetracarpidium conophorum* are used in Nigeria to increase sperm counts in men. Nwauzoma and Dappa (2013) reported that *Tetracarpidium conophorum* boiled seeds can also be eaten to improve sperm count in men. It was proved that the extract of *Tetracarpidium conophorum* seed increases the viability and sperm output of male albino rats

* Corresponding author: H.O. Asuzu-Samuel

and this suggests that the seed could be included in the formulation of male fertility drugs (Ikpeme et al., 2014). However, Dada and Aguda, (2015) reported that the *Tetracarpidium conophorum* seed powder has potential pro-fertility properties in male Clarias gariepinus (African sharp tooth catfish) that could be exploited in fish production as a feed additive for the improved reproductive performance of male African catfish. All parts of *Tetracarpidium conophorum* have been used ethnomedically, including the stem bark, leaves, seeds and roots. The bark is used by local people as a mild laxative (Janick *et al.,* 2008). The seed kernel, when eaten raw, has a bitter taste like the kola nut and is considered to be a tonic and aphrodisiac (Aiyeloja et al., 2006). Customarily, drinking water immediately after eating the edible nut has a bitter taste principle, which might be due to the presence of some alkaloid-containing compounds in the plant. The African walnut contains protein, vitamins, magnesium and is a good source of antioxidants (Kim *et al.,* 2002). The nut oil contains 48% – 50% dry weight of oil, is golden yellow in colour, with a taste and odour resembling linseed oil (Enujiugha 2003; Enujiugha et al., 2003; Negi et al., 2011). The oil is highly rich in linolenic acid (64%),palmitic and stearic acids (15%) and oleic acid (11%). It is also rich in polyunsaturated fatty acids such as α-linolenic acid and it contains mono-saturated fatty acids (Kanu *etal.,* 2015). Animashaun, Togun & Hughes (1994) used affinity chromatography techniques to isolate the agglutinin I and II of disulphide-bonded 70 kDa and 34 kDa of monomeric protein, respectively, which are referred to as isolectins from *Tetracarpidium conophorum* seed extracts. Asaolu (2009) quantified the amino acid composition of *Tetracarpidium conophorum* seed nuts and the total essential amino acid (274 mg/g) crude protein out of the total amino acids (573 mg/g). Glutamic acid (134 mg/g) had the highest amino acid and leucine (0.32) had the lowest essential amino acid score.

(Nkwonta 2015) indicated the presence of essential and non-essential fatty acids, namely palmitate, oleate, stearate, linoleate, arachidate and α-linoleate. The physicochemical characteristics, fatty acids and triglycerols (TG) of the nut oil (Type 1 and Type 2) content varied between 55.75% and 61.62%, while the ash values varied between 8.40% and9.68% (Tchiegang *et al.,* 2001) acid was linolenic acid (69.47% – 70.39%) as determinedby capillary gas chromatographic analysis and the triacylglycerol profile obtained by reversed phase liquid chromatography showed 10 TG, with three major ones identified as trilinolenin (33.39% – 47.67%), dilinolenic-linoleic (12.19% – 27.15%) and dilinolenic-olein (18.46% – 37.71%) (Tchiegang et al., 2001). (Nwaoguikp *et al*., 2012) established the phytochemical and biochemical composition of varieties of walnut (boiled and mashed wet nuts and dried powdered nuts). Saponins (8.37, 5.03 mg/kg) were the highest constituent of the mashed wet nuts and the dried powdered nuts, respectively. This suggested a role of the seed nuts in health and nutrition. (Ekwe *et al.,* 2013) reported the proximate composition of the African walnut (*Tetracarpidium conophorum*) on wet basis, which revealed protein (14.92%), oil (45.84%), crude fibre (1.14%), ash (3.52%) and carbohydrate (15.38%), while the anti-nutritionalfactors revealed tannins (0.89 mg/100 g), oxalate (1.28 mg/100 g), phytic acid (3.105 mg/100 g), trypsin inhibitors (1.84 mg/100 g), saponin (985.0 mg/100 g) and alkaloid (40.91 mg/100 g). Arinola and Adesina (2014) stated that the seed nut is a rich source of protein and fat but high heat could reduce the protein, ash and crude fibre content of the nut.

2. Materials and methods

2.1. Sample Collection

Fresh nuts of *Tetracarpidium conophorum* was purchased from the Ondo State of Nigeria and were identified by Dr. Chimezie Ekeke of the Department of Plant Science and Biotechnology, University of Port Harcourt and with plate number given as UPH/V/307. The Walnut hard tusk was peeled off while the seeds of the walnut were harvested, completely drained, separately coarse, cut into small particles and allowed to air- dried at room temperature. They were monitored daily and shuffled to prevent decay and enable complete drying. At the end of the four weeks one kilogram of all fully dried nuts were grinded into powdered form using electrical blender which yielded (735.52g). This research was carried out in the Department of Pharmacognosy and Phylotherapy Research Laboratory of the University of Port Harcourt.

2.1.1. Maceration of T. Conophorum sample

The powdered T.conophorum was macerated using a macerating jar and methanol as the maceration solvent. Maceration occurred three times every 24hrs. 1500ml of methanol was used for each of the maceration and filtered every day.

2.1.2. Extraction of oil from Tetracarpidium conophorum sample

The extraction of oil from the African walnut (Tetracapidium Conophorum) samples was carried out using Rotary evaporator apparatus. After extraction, the solvent was removed yielding the oil. Any remaining solvent in the oil was removed by gentle evaporation over a water bath at 60 °C for about three weeks. The oil was stored in a solvent bottle and kept under a room temperature until used.

2.1.3. Tin-Layer Chromatography Separation of the sample

Tin-layer chromatography was carried out using Methanol and Dichloromethane in a ratio of 1:1(100mil methanol: 100mil dichloromethane) as mobile phase solvent gradient. The *Tetracarpidium conophorum* samples were spotted on the TLC plates then placed in the solvent gradient for 20min. Then the spotted TLC plate was viewed under UV lamp and a uniform single blue band was seen and then scrapped into a beaker. This shows a clear separation. The above procedure was carried out many times until a good amount was gotten. Then the scrapped band was mixed with methanol and filtered (This is done to remove the silica gel of the TLC place.

Then the filtrate was taken for a GC-MS active components.

2.2. The GC-MS analysis

The fatty acid composition was determined as methyl esters. Briefly, 100 µl oil plus 1 ml 10% potassium hydroxide in methanol were heated for 45 minutes at 85°C. Fatty acids were methylated with 1 ml boron trifluoride-methanol-complex (20% solution in methanol) plus 1 ml methanol for 45 min at 60°C and then extracted from the methanolic phase with petroleum ether. The analyte, 1 µl was injected in the column equipped with column oven temperature of 600 C and column flow of 0.99 ml/min. Dilutions of African walnut fatty acid methyl esters and standard mix C8 - C22 at 1:100, 1:50 and 1:20 were pre-analyzed using an Agilent 6890N Gas Chromatography device equipped with flame-ionization detector (GCFID) and a multi-chambered auto sampler 7683 series. For GCMS analysis, 100 Lof oil was dissolved in 2 ml of dichloromethane (DCM). The sample was analyzed on an Agilent technology 7890 GC system equipped with a Mass Spectrometric Detector (5975 MS model), the column used is HP-5MS Agilent technology, length of the column is 30 m, internal diameter 0.320 mm with thickness of 0.25 µm and helium as the carrier gas. One microlitre of the sample was injected using split less injection with injector temperature 300°C according to the following scheme: 50°C for 2 min with 10°C/min up to 300°C. The final temperature was held for 10 min. The total runtime for the sample was 37 min. For MS detection, electron ionization with 70 eV was applied and mass fragments were detected between 40 and 500 m/z. The ion source temperature and transfer line temperature were 200°C and 300°C, respectively.

2.3. Detection of Components

Analysis of mass spectrum GC-MS was conducted by the database of National Institute Standard and Technique (NIST) having more than 62,000 patterns. The spectrum of the unidentified component was compared with the spectrum of the identified components stored in the NIST library. The name, molecular weight, and structure of the components in the test material were ascertained

3. Results and discussion

The bioactive compounds present in the methanol extracts of raw Tetracapiduim conophorum (African walnut) with their molecular formulae, molecular weights, Retention Times (RT) and Peak areas (%), are shown in Fig 1. The GC-MS chromatograms detected 13 peaks of bioactive compounds for extract as shown in Fig 2.

The height of the peak indicates the relative concentration of the components present in the extracts. These compounds mainly comprised esters, alcohols, hydrocarbons and ketones. The major chemical constituents identified in the raw extract was 9,12,15-octadecatrienoic acid, (Z, Z,Z)- (67.61%), other compounds identified were n-hexadecanoic acid (4.92%); 9,12-octadecadienoic acid, methyl ester (0.27%); 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)- (1.38%); heptadecanoic acid, 16-methyl-, methyl ester (0.10%); 9,12,15-octadecatrienoic acid, ethyl ester, (Z,Z,Z)- (17.15%); ethyl 9,12,15- octadecatrienoate (5.21%); 9,12,15-octadecatrien1-ol, (Z,Z,Z)- (2.85%); 9,12-octadecadienoic acid (Z,Z)- (9.55%); methyl 8,11,14 heptadecatrienoate (4.35%); octadecanoic acid (3.87%); bis(2-ethylhexyl) phthalate (0.11%) and squalene (0.23%).

Gas chromatography coupled with mass spectrometry (GC-MS) is an established technique for reliable identification of bioactive compounds existing in medicinal plants including volatile matter, long chain and branched chain hydrocarbons, alcohols, acids, esters (Tchiegang *et al.,* 2001). Plants contain numerous phytochemical constituents, many of which are known to be biologically active compounds and are responsible for exhibiting diverse pharmacological activities (Tchiegang *et al.,* 2001).

Generally, the reliability of medicinal plants for use is evaluated by correlating the phytochemical compounds with their biological activities (Tchiegang *et al.,* 2001). From the GC-MS results of the methanol extract of raw *Tetracarpidium conophorum*, it is noted that there are 13 bioactive compounds. The compound with the highest peak value was 9, 12,

15-octadecatrienoic acid, (Z, Z, Z) (67.61%) but no activity has been reported for this compound. Other compounds identified are: 9, 12-octadecadienoic acid (Z, Z) (9.55%) having anti-inflammatory and antiarthritic properties (Tchiegang *et al.,* 2001). However, n-hexadecanoic acid (4.92%)has been reported to have antimicrobial properties (Tchiegang *et al.,* 2001); octadecanoic acid (3.87%) also has antifungal, antibacterial properties (Tchiegang *et al.,* 2001); while 9,12,15-octadecatrien-1-ol, (Z,Z,Z)- (2.85%) has antibacterial properties (Tchiegang *et al.,* 2001), 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)- (1.38%) also has anticancer, antibacterial, antioxidant, antipyretic, cardio protective, neural function, antiandrogenic (5-alpha reductase inhibitor), and antiarthritic properties (Tchiegang *et al.,* 2001). Squalene (0.23%), on the other hand, possessesantibacterial, antioxidant, antitumor, cancer preventive, chemo-preventive,immune stimulant, lipoxygenase-inhibitor, perfumery, pesticide and sunscreen properties (Tchiegang *et al.,* 2001).

SN	Retention time	Name of the compound	Molecular formulae	Molecular Weight (g/mol)	Peak area (%)	Reported activity
1.	16.888	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4241	4.92	Antimicrobial (Akpuaka, *et al.,* 2013)
2.	18.330	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294.4721	0.27	Antiinflammatory nematicide, antiacne, antihistaminic, insectifuge, anticancer, hypocholesterolemic, hepatoprotective, and antiarthritic (Ha *et al.,* 1990; Johnson *et al.,* 2011)
3.	18.450	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292.45618	1.38	Anticancer, antibacterial, antioxidant, antipyretic, cardioprotective, neural function, antiandrogenic (5-alpha reductase inhibitor), and antiarthritic properties. (Akpuaka *et al.,* 2013; Johnson 2011)
4.	18.811	Heptadecanoic acid, 16-methyl-, methyl ester	$C_{19}H_{38}O_2$	298.5038	0.10	Used against skin cancer protein.
5.	19.492	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	$C_{20}H_{34}O_2$	306.4828	17.15	No activity reported
6.	19.532	Ethyl 9,12,15-octadecatrienoate	$C_{20}H_{34}O_2$	306.4828	5.21	No activity reported
7.	19.640	9,12,15-Octadecatrienoic acid, (Z, Z,Z)-	$C_{18}H_{30}O_2$	278.436	67.61	No activity reported
8.	19.680	9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	$C_{18}H_{32}O$	264.4461	2.85	Antibacterial (Growther, *et al.,* 2012)
9.	19.846	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.4455	9.55	Anti-inflammatory and antiarthritic (Jones, 2002; Lalitharani, *et al.,* 2009).
10.	20.476	Methyl 8,11,14 heptadecatrienoate	$C_{15}H_{30}O_2$	278.430	4.35	No activity reported
11.	20.556	Octadecanoic acid	$C_{18}H_{36}O_2$	284.4772	3.87	Antifungal, antibacterial (Akpuaka *et al.,* 2013)
12.	25.071	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.23	0.11	Oral toxicity during pregnancy and suckling in the Long-Evans Rat (Arcadi *et al.,* 1998).
13.	28.006	Squalene	$C_{30}H_{50}$	410.73	0.23	Antibacterial, pesticide (Akpuaka *et al.,* 2013)

Figure 1 Components identified in the raw T. conophorum sample analysed by GC-MS

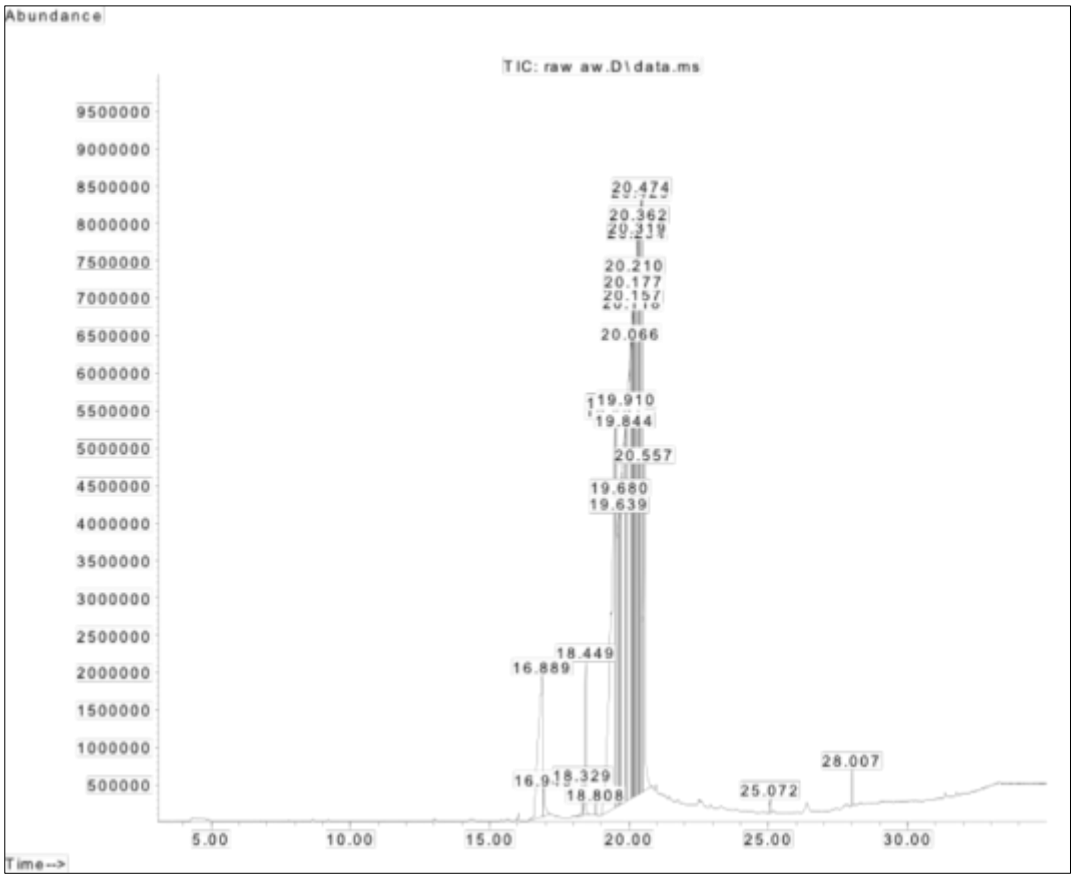

Figure 2 GC-MS chromatogram of raw African walnut extract

4. Conclusion

In conclusion, the proximate, vitamins, minerals and phytochemical compositions of *Tetracarpidium conophorum* (Nigerian walnut) seed. This partly shows the use of this seed in herbal medicine. As a rich source of alkaloids, coupled with the presence of the essential vitamins and minerals, *Tetracarpidium conophorum* can be seen as a potential source of useful food and drugs. The presence of tannin supports its anti-inflamatory property. This also proves that the seed may be helpful in asthma, rheumatoid and arthritis. High content of ascorbic acid also indicates that the plant can be used to prevent or at least minimize the formation of carcinogenic substances from dietary material. Further studies have to be carried out to isolate, characterize and elucidate the structure of the bioactive compounds from the seed for industrial drug formulation. More so, extensive works should be carried out to search for the effectiveness of the seed in male reproductive organ and also its cardiovascular functions.

Compliance with ethical standards

Disclosure of conflict of interest

There is no conflict of interest between the authors to be disclosed.

References

[1] Aiyeloja, A.A. & Bello, O.A., 2006, 'Ethnobotanical potentials of common herbs in Nigeria: A case study of Enugu State', Educational Research and Review 1(1), 16–22.

[2] Animashaun, T., Togun, R.A. & Hughes, R.C., 1994, 'Characterization of isolectins in Tetracarpidum conophorum seeds (Nigerian Walnut)', Glycoconj Journal 11(4), 299–303.

[3] Asaolu, S.S., 2009, 'Amino acid composition of *Tetracarpidium conophorum*', Oriental Journal of Chemistry 25(1), 63–66.

[4] Ayoola, P.B., Adeyeye, A., Onawumi, O.O. & Faboya, O.O.P., 2011, 'Phytochemical and Nutrient Evaluation of *Tetracarpidium conophorum* (Nigerian Walnut) Roo*t, International Journal of Research and Reviews in Applied Sciences* 7(2), 197–208

[5] Dada, A. & Aguda, O., 2015, 'Dietary effects of African walnut (*Tetracarpidium conophorum*) on the reproductive indices in male African catfish (Clarias gariepinus) broodstock', Journal of Coastal Life Medicine 3(6), 471–475.

[6] Dauda, A. O., Abiodun, O. A., Akintayo. O. A., Babayeju, A. A., Salami, K. O. and Oyekanmi, I. A. (2020). Influence of walnut on the nutritional and physicochemical properties of biscuits made from whole wheat Agrosearcch, 20(1): 45-58.

[7] Ekwe, C.C. & Ihemeje, A., 2013, 'Evaluation of physiochemical properties and preservation of African walnut (*Tetracarpidium conophorum*)', Academic Research International 4(6), 501–512.

[8] Enujiugha, V.N. & Ayodele, O.O., 2003, 'Evaluation of nutrients and some anti-nutrient in less known underutilized seed', International Journal of Food Science and Technology 38, 525–552.

[9] Enujiugha, V.N., 2003, 'Chemical and functional characteristics of conophor nut', Pakistan Journal of Nutrition 2(6), 335–338.

[10] Ikpeme, E.V., Ekaluo, U.B., Udensi, O., Ekerette, E.E., Ekpo, P.B. & Asuquo, B.O., 2014, 'Sperm quality and hormone profile of male albino rats fed with seeds of African walnut (*Tetracarpidium conophorum*, Mull)', Annual Research & Review in Biology 4(9), 1379–1386.

[11] Janick, J. & Paul, R.E., 2008, 'The encyclopedia of fruits and nuts, Cab International England, Oxfordshire.

[12] Kanu, A.M., Kalu, J.E., Okorie, A.C., Olabinri, B.M., Eniyansoro, O.O., Okoronkwo, C.O. et al., 2015, 'Evaluation of chelating ability of aqueous extract of *Tetracarpidium conophorum* (African walnut) in vitro', International Journal Applied Research in Natural Products 3(3), 1–13.

[13] Kanu, A.M., Kalu, J.E., Okorie, A.C., Olabinri, B.M., Eniyansoro, O.O. and Okoronkwo, C.O. 2015, 'Evaluation of chelating ability of aqueous extract of *Tetracarpidium conophorum* (African walnut) in vitro*. International Journal Applied Research in Natural Products* 3(3):1–13.

[14] Kim, Y.H. & Lee, J.H., 2002, 'Long-term stress and analysis and utilization of a drying oil from H. pylori infection independently induce gastric *Tetracarpidium conophorum*', Journal of Gastroenterology 37(11), 1259–1264.

[15] Negi, A.S., Luqma, S., Srivastava, V., Krishna, N. & Gupta, M.P.D., 2011, 'Antiproliferative and antioxidant activities of African walnut (Julgan regina L.) fruit extracts', Pharmaceutical Biology 49(6), 669–705.

[16] Nkwonta, C.G., 2015, 'Biochemical and biomedical studies on African walnut (*Tetracarpidium conophorum*-Mull. Arg.) – A postharvest perspective', PhD thesis, Cranfield University, pp. 1–171.

[17] Nwachoko, N. & Jack, I.R., 2015, 'Phytochemical screening and anti-diarrhea activities of *Tetracarpidium conophorum* induced in albino rats', Sky Journal of Biochemistry Research 4(4), 21–24.

[18] Nwaoguikpe, R.N., Ujowundu, C.O. & Wesley, B., 2012, 'Phytochemical and biochemical compositions of African walnut (*Tetracarpidium conophorum*)', Journal of Pharmaceutical and Biomedical Sciences 20(9), 1–5.

[19] Nwauzoma, A.B. & Dappa, M.S., 2013, 'Ethnobotanicals studies of Port Harcourt metropolis, Nigeria', ISRN Botany 1–5.

[20] Tchiegang, C., Kapseu, C. & Parmentier, M., 2001, 'Chemical composition of oil from *Tetracarpidium conophorum* (Müll. Arg.) Hutch.and Dalziel) nuts', Journal of Food Lipids 8(2), 95–102.