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Tetracarpidium conophorum (African walnut) seed phenolic extract modulates the activities of selected enzymes of cardiovascular and neurological relevance

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Abstract

Background: African walnut (*Tetracarpidium conophorum*) root, leaf and seed are used to treat variety of ailments in ethno-medicine. The objective of this study was to evaluate effects of phenolic extract of *T. conophorum* seed on some enzymes of cardiovascular and neurological significance.

Materials and Methods: Pulverized seed (400g) of *T. conophorum* was extracted with methanol-acetone using standard extraction procedures. Total phenolic content (TPC) and Gas Chromatography-Mass Spectrophotometry analysis of free phenolic of *T. conophorum* seed (FPTC) extract were determined. Fifteen albino rats were sacrificed by cervical dislocation. Lung, heart and muscle, and brain tissues were freshly harvested, homogenized using standard laboratory procedures and used as enzyme extracts for angiotensin-1 converting enzyme (ACE), lactate dehydrogenase (LDH), sodium-potassium (Na^+/K^+)-ATPase, and acetylcholinesterase, AChE assays respectively. Effects of FPTC on ACE, LDH, Na^+/K^+-ATPase and AChE activities were assessed ex-vivo.

Results and Conclusion: TPC of FPTC was 86.99 mg/GAE/g dry weight. FPTC demonstrated significant dose-dependent inhibition of ACE. IC_{50} of ACE by FPTC was 7.0 µg/ml when compared with that of lisinopril (IC_{50} 0.15 µg/ml). Also, FPTC significantly ($p < 0.05$). Increase activities of tissue LDH, Na^+/K^+-ATPase and AChE in dose-dependent manner. This study suggested that the folklore benefits of the nut in treating hypertension and neurological-related diseases could be due to its effects on ACE, LDH, Na^+/K^+-ATPase and AChE.

Keywords: *Tetracarpidium conophorum*, phenolics, diseases, cardio-protection, neuroprotection.

Introduction

The use of plant-based antioxidants is on the increase of recent (1). Nut-containing diets were shown to possess beneficial effects on plasma lipoproteins (2) and reduce the possibility of developing heart-related diseases (3). Low rate of obesity and coronary artery disease are associated with increased eating of nuts like walnuts and peanuts (4, 5).

Polyphenols are antioxidants that reduce oxidative stress in the body (6). Phenolic compounds in plant extracts possess potentials to inhibit angiotensin-1-converting enzyme, ACE (6 -8) and lower incidence of neurological related diseases (9). Acetylcholinesterase (AChE) and sodium-potassium ATPase (Na^+/K^+-ATPase) are key enzymes in neurons physiology (6). Lactate dehydrogenase, LDH is found and expressed in nearly all cells of the body. The enzyme is responsible for the conversion of pyruvate to lactate in anaerobic conditions or in cells like erythrocytes which have no mitochondria. LDH is released during tissue damage and its serum level

is usually being used as a marker of tissue injury (10). In clinical conditions like rhabdomyolysis, lactic acidosis, muscular fatigue, muscle pain, and cramps whereby there is oxygen insufficiency, lactate cannot be converted to pyruvate to generate ATP for metabolic processes.

Tetracarpidium conophorum known as African walnut is called "asala" by Yoruba people in South-Western Nigeria and "ukpa" by Igbo ethnic group in South-Eastern Nigeria (11). The root, leaf and seed of the plant are being used to treat arrays of conditions like diabetes, bacterial and fungal infections, infertility, fever, diarrhoea, cancer, high blood pressure etc in traditional medicine (11-14) possibly due to high bioactive compounds such as ascorbic acids, omega-3-fatty acids, vitamins, oxalates, phytates, tannins, saponins and alkaloids contents (15). Little or no information on the phenolic components of *T. conophorum* seed is available in the literatures. The aim of the present study was to identify the phenolic compounds in the plant seed extract, and evaluate *ex-vivo* the biochemical effects of the phenolics on selected enzymes of cardiovascular and neurological disorders.

Materials and Methods

Chemicals

Acetylcholine iodide, sodium barbital, gallic acid, N-hippuryl-His-Leu hydrate, quercetin and Folin-Ciocalteu reagent were purchased from Sigma-Aldrich chemical company, U.S.A. Sodium chloride, lactic acid, Tween20 and hydrochloric acid were purchased from British Drug House (BDH) chemical Ltd., England. Mercapto ethanol, tris-aminomethane, ethylene diamine-tetraacetic acid were purchased from Scharlab S.L, Spain. Nicotinamide adenine dinucleotide, acetone, methanol and ethylacetate were purchased from Gungdong, Gunghun chemical factory, China while lisinopril was from MA Holder: TEVA UK Ltd. Digoxin was gotten from NEM Laboratories PVT. Ltd, India, and commercial total protein diagnostic kit was purchased from RANDOX Laboratories Ltd., County Antrim, UK.

Animals

Fifteen albino rats weighing between 180 and 200 g were used for this study. The animals were kept in plastic cages littered with saw dust and provided rat pellet and water ad libitum and maintained in room temperature with 12-hour light/ dark cycle. Experimental animals were used in accordance with the institution guidelines on the use animals for experimental and scientific purposes.

Plant Materials

Tetracarpidium conophorum nuts were bought from a local market in Osogbo, Nigeria. The plant was identified at IFE HERBARIUM, Obafemi Awolowo University, Ile-Ife, Nigeria. Herbarium identification number allocated was 17713. The shells were removed, and seeds were shade dried for 4 weeks at room temperature and pulverised into fine powder using electric blender.

Extraction of *Tetracarpidium conophorum* Phenolics

The pulverized seed (400g) was macerated with 4L of methanol (80 %) for two days at room temperature, with frequent agitation. The suspension was filtered with muslin cloth. The filtrate was concentrated by rotatory evaporator, re-extracted with 80% acetone for ten hours at room temperature (6), and the final filtrate concentrated.

Estimation of Total Phenolic Content

The total phenolic content, TPC, of the extract was determined by Folin-Coicalteu method of Demiray *et al.* (2009). Briefly, 50 mg/ml of the extract was mixed with 5 ml of Folin-Ciocalteu Reagent previously diluted with distilled water (1:10 v/v) and 4 ml of 7.5% Na2CO3 in test tubes. The tubes were vortexed for few seconds and

incubated for 30 minutes at room temperature for colour development. Absorbance of standard and samples were measured spectrophotometrically against the blank at 765nm, and the TPC was expressed as gallic acid equivalent (g/100g dry weight).

Sample Preparation for GCMS Analysis

About 5g of the phenolic extract of *Tetracarpidium conophorum* seed was soaked in 25 ml of 100 % methanol in a glass container with lid for seven days. The mixture was filtered using whattman filter paper. The filtrate obtained was evaporated and used for GCMS analysis.

GC-MS Analysis of *Tetracarpidium conophorum* Seed Phenolic Extract

GC-MS was utilized to identify compounds in the phenolic extract of the plant using standard procedure. Briefly, the oven temperature was programmed at 70 °C with an increase of 4 °C /min until 280 °C and maintained for 15 min. The carrier gas was helium, with a constant flow of 1.4 mL/min. The temperature of the ionization source was maintained at 280 °C, the ionization energy at 70 eV, and the ionization current at 0.7 kV. Mass spectra were recorded from 300 m/z to 450 m/z. Individual components were identified by matching their 70 eV mass spectra with those of the spectrometer data base using the Wiley L-Built library, and by comparing their retention indices with those of NIST computer MS library and with the fragmentation patterns of the mass spectra with those reported in the literature.

Sample Collection

The animals were sacrificed and fresh lung was removed. The lung was homogenized in cold 125mM Tris-HCl buffer, pH 8.3**,** centrifuged at 4,000 xg for 10 minutes at 4℃. The supernatant collected was used as enzyme extract for angiotensin-1-converting enzyme assay [16, 17]. Fresh heart and muscle tissues were collected, sliced into pieces and homogenized (50g) with 100ml cold extraction buffer (Tris-HCl buffer 50mM pH 7.5, EDTA 1mM and 1mM Mercapto ethanol), centrifuged for 15minutes at 15,000 x g and 4℃. The supernatant collected was used as enzyme extract for lactate dehydrogenase assay. For acetylcholinesterase (AChE) and Sodium/Potassium (Na^+/K^+)-ATPase assays, fresh whole brain was collected and homogenized in cold Tris-HCl, 60mM pH 7.4 (1:10), and then centrifuged for 10 minutes at 3000 x g. The supernatant collected was used as enzyme extract for AChE and Na^+/K^+-ATPase assays.

Assay for Angiotensin-1-converting Enzyme (ACE) Inhibition

The principle for this assay is based on hydrolysis of N-hippuryl-His-Leu hydrate by angiotensi-1-converting enzyme (ACE). Inhibition of ACE by phenolic extract of *T. conophorum* was estimated spectrophotometrically according to the method described by Cushman and Cheung (18) as shown in the Table 1.

Table 1: Protocol for Angiotensin-1-converting Enzyme Assay

Assay mixture	Treatment groups		
	Normal control	FPTC	Lisinopril
125 mM Tris-HCl pH 8.3	-	40 µl	40 µl
ACE extract	50 µl	50 µl	50 µl
FPTC extract (10-50 mg/ml)	------	50 µl	-----
Lisinopril (0.1-1.0 µM)	-----	-----	50 µl

FPTC = Free phenolic extract of *Tetracarpidium conophorum*.

The reaction mixtures were incubated at 37 °C for 15 minutes. Then, ACE substrate, N-hippuryl-His-Leu hydrate (8.3 mM; 50µl) was added and the assay mixtures were further incubated for 30 minutes at 37^{0}C in an orbital shaker (UNISCOPE Surgifriend Medicals, England) at 100 rpm, and the reaction was terminated with 1M HCl (1ml). The product was then extracted by vortex mixing with acidified ethyl acetate (1 to 2ml) for 1

minute. The aliquot obtained was centrifuged at 3,000 x g for 5 minutes. Ethylacetate fraction was evaporated at 100 C while the product obtained (hippuric acid) was re-dissolved in distilled water (1ml), and the absorbance was read using spectrophotometer at 417nm. All measurements were done in triplicates. The percentage ACE inhibition was calculated as follows:

$$\frac{\text{(Hippuric acid concentration in normal control) – (Hippuric acid concentration in test group)}}{\text{(Hippuric acid concentration in normal control) X 100}}$$

Lactate dehydrogenase Assay

Lactate dehydrogenase activities in heart and muscle were determined using lactate as substrate according to the method of Karamanos (19). The reaction mixture contained 0.01M lactic acid (0.1 ml), 1.8ml Tris-HCl buffer (100mM, pH 8.8), 0.1ml NAD (150mM) and phenolic extract of *T. Conophorum* (5, 10, 25, 50 mg/ml) in different sets of test tubes. In another set of tubes, the reaction mixtures contained all (above) except phenolic extract. Enzyme extract (0.1 ml) was later added to initiate the reaction, and reduction of NAD was measured spectrophotometrically at 340 nm over 3 minutes. All tests were carried out in triplicates.

Acetylcholinesterase Assay

Acetylcholinesterase activity in the brain was determined according to the electrometric method described by Michell *et al* (20) with modifications by Ajilore *et al* (21) as shown in Table 2.

Table 2: Protocol for Acetylcholinesterase Assay

Assay mixture	Treatment groups				
		FPTC Extract			
	Normal control	5µg/ml	10µg/ml	25µg/ml	50µg/ml
AChE enzyme extract	0.2 ml	0.2 ml	0.2 ml	0.2 ml	0.2 ml
Distilled water	3 ml	3 ml	3 ml	3 ml	3 ml
Barbital PO4 buffer (pH 8.1)	3 ml	3 ml	3 ml	3 ml	3 ml
1N HCl (to adjust pH to 8.1)	Drops	Drops	Drops	Drops	Drops
FPTC extract	-----	0.1 ml	0.1 ml	0.1 ml	0.1 ml

FPTC= Free Phenolics of *Tetracarpidium conophorum*
AChE = Acetylcholinesterase

The pH of reaction mixtures was measured by pH meter (JENWAY 3520, Bibby Scientific Ltd., Essex, UK) as pH1, and later incubated at 37°C for 20 minutes following addition of 0.1 mL 75 mmol/L acetylcholine iodide before measuring the pH again as pH2. The measurements were done in triplicates. AChE activity was determined as follows:

$$\text{AChE activity } (\Delta pH/20 \text{ min}) = (pH1 - pH2) - pH \text{ blank}$$

Mixture of water and barbital phosphate buffer was used as blank. AChE enzyme inhibition in percentage was calculated as follows:

$$\frac{\text{(AChE activity in normal control) – (AChE in FPTC group)}}{\text{(AChE activity in normal control)}} \times 100$$

The 50% inhibitory concentration (IC_{50}) was calculated.

Assay for Sodium/Potassium (Na^+/K^+)-ATPase

Activity of sodium/potassium (Na^+/K^+)-ATPase was evaluated using the method described by Bonting (22) with modifications by Ajilore *et al* (21). The enzyme extract was incubated with free phenolic extract of *T. conophorum* (FPTC) and digoxin as described in Table 3:

Table 3: Protocol for Na/K-ATPase Assay

Assay mixture	Treatment groups				
		FPTC Extract			
	Normal control	1 mM Digoxin	10µg/ml	25µg/ml	50µg/ml
Na^+/K^+ - ATPase extract	0.2 ml	0.2 ml	0.2 ml	0.2 ml	0.2 ml
FPTC extract	-------	------	0.1 ml	0.1 ml	0.1 ml
1 mM Digoxin	-------	0.2 ml	-------	-------	-------
10 mm Tris-HCl pH 7.4	1 ml	1 ml	1 ml	1 ml	1 ml
50 mM $MgCl_2$	0.2 ml	0.2 ml	0.2 ml	0.2 ml	0.2 ml
50 mM KCl	0.2 ml	0.2 ml	0.2 ml	0.2 ml	0.2 ml
600 mM NaCl	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml
1 mM EDTA	0.2ml	0.2ml	0.2ml	0.2ml	0.2ml
10 mM ATP	0.2ml	0.2ml	0.2ml	0.2ml	0.2ml

FPTC= Free Phenolics of *Tetracarpidium conophorum*
Na/K-ATPase = Sodium/potassium-ATPase

ATP was added to the assay mixture to initiate the reaction, and the mixture was incubated for 30 minutes at 37 °C before terminating the reaction with ice-cold 5 % TCA (5 ml). The inorganic phosphate (Pi) liberated was estimated according to the modified method of Fiske and Subbarow (23) at 680 nm. The specific Na^{+}/K^{+}-ATPase activity (µg Pi liberated/mg protein/min) was calculated by subtracting the digoxin-insensitive activity from the overall activity.

Total Protein Estimation

The amount of total protein in the enzyme extracts was determined as described by Gornal *et al*. (24) using Randox diagnostic kit using the protocol in Table 4:

Table 4: Protocol for Total Protein Assay

	Reagent blank	Standard	Sample
Distilled water	0.02 ml	–	–
Standard	–	0.02 ml	–
Sample	–	–	0.02 ml
Biuret reagent (serially diluted)	1.00 ml	1.00 ml	1.00 ml

The assay mixture was thoroughly mixed and incubated for 30 minutes at room temperature for maximum colour development after which the absorbance was read at 570nm against reagent blank.

Total protein concentration =

$$\frac{\text{Absorbance of sample x standard concentration}}{\text{Absorbance of standard}}$$

Statistical Analysis

One-Way Analysis of Variance (SPSS version 20.0) was used for statistical analysis. Homogeneity of variance was assessed using Levene statistics while multiple comparisons and homogenous subsets were identified by using Duncan. Results obtained were statistically significant at $P < 0.05$.

Results

Total Phenolic Content and GC-MS Spectral Analysis of *Tetracarpidium conophorum* Phenolic Seed Extract

The total phenolic content of the plant extract was 86.99 mg/GAE/g dry weight. Three novel alcohol-phenol based derivatives were identified by GCMS from methanol-acetone extract of *Tetracarpidium conophorum* seed (Table 1, and figures 1 and 2).

Effects of Free Phenolic Extract of *Tetracarpidium conophorum* on Angiotensin-1-converting Enzyme and Lactate dehydrogenase Activities

Both Free Phenolics of *Tetracarpidium conophorum* (FPTC) and lisinopril showed dose-dependent significant ($p<0.05$) inhibition of angiotensin-1-converting enzyme activity in vitro (Table 2 and Figure 3). FPTC also showed a dose-dependent increase in lactate dehydrogenase

Table 5: GC-MS Spectral Analysis of Free Phenolic Extract of *Tetracarpidium conophorum* Seed.

Peak No.	Compounds	Retention time (min)	Corr. (%) max.	Area %	Molecular formula	Molecular mass
1	3-(1,3-Dihydroxyisopropyl)-1,5,8,11-tetraoxacyclotridecane	37.502	15.30	7.681	$C_{12}H_{24}O_6$	264.3 g/mol
2	3-(1,3-Dihydroxyisopropyl)-1,5,8,11,14,17-hexaoxacyclononadecane	38.741	15.70	7.880	$C_{16}H_{32}O_8$	352.42 g/mol
3	3-(1,3-Dihydroxyisopropyl)-1,5,8,11,14,17-hexaoxacyclononadecane	39.103	100.00	50.190	$C_{16}H_{32}O_8$	352.42 g/mol
4	Heptaethylene glycol monododecyl ether	39.816	4.25	2.131	$C_{26}H_{54}O_8$	494.7 g/mol
5	3-(1,3-Dihydroxyisopropyl)-1,5,8,11,14,17-hexaoxacyclononadecane	40.035	63.99	32.117	$C_{16}H_{32}O_8$	352.42 g/mol

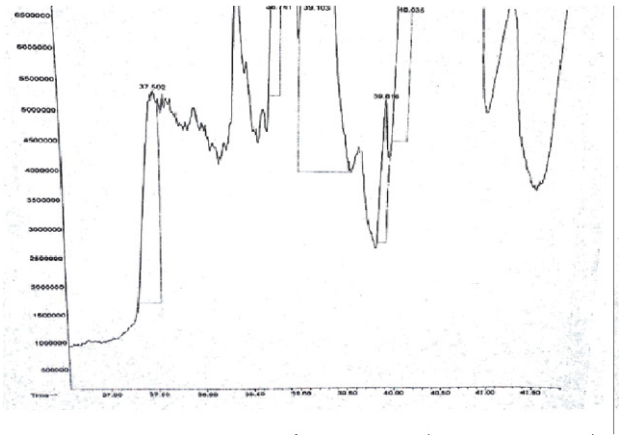

Figure 1: Representative Gas Chromatography-Mass Spectrometry profiles of free phenolic extract of *Tetracarpidium conophorum* seed.

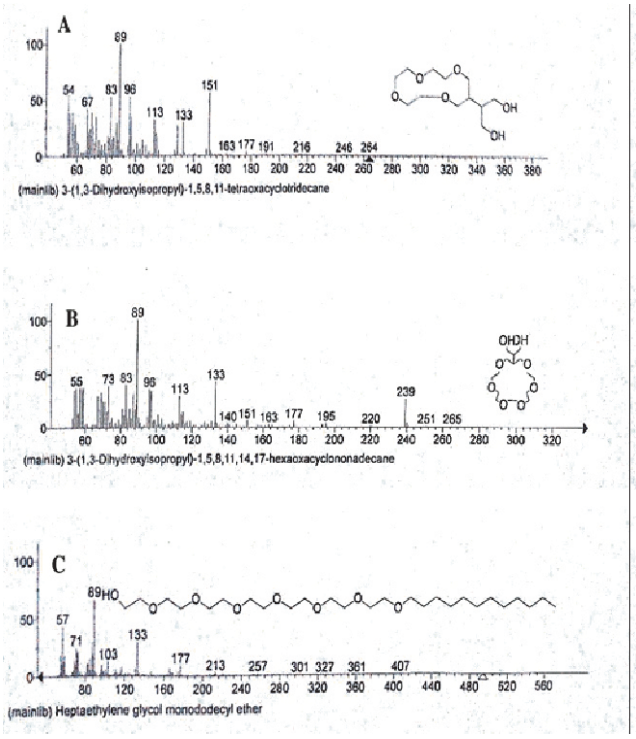

Figure 2: Phenolic Compounds identified from *Tetracarpidium conophorum* Seed Extract.

A. 3-(1,3-Dihydroxyisopropyl)-1,5,8,11- tetraox acyclotridecane

B. 3-(1,3-Dihydroxyisopropyl)-1,5,8,11,14,17-hexaoxacyclononadecane

C. Heptaethylene glycol monododecyl ether

activities in both the heart and skeletal muscle (Figure 4). The induction of LDH activities was higher in heart (though not significant) at 5 µg/ml and 10 µg/ml FPTC. At 50 µg/ml, LDH activities was higher in skeletal muscle.

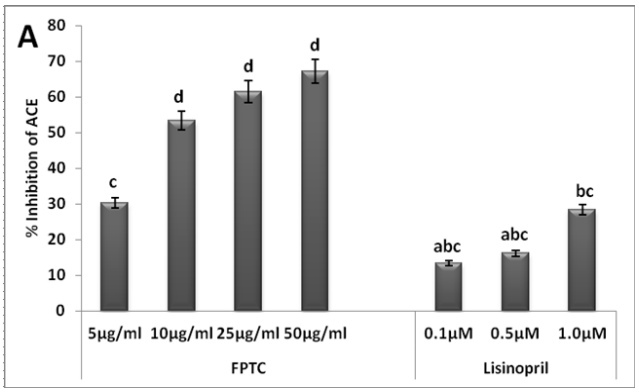

Figure 3: Inhibition of angiotensin-1 converting enzyme.

Values are expressed as mean ± SD (n=3). Duncan superscripts a, abc, c, d are significance homogenous subsets of means between groups. Bars with different superscripts are statistically significant at the 0.05 level.

FPTC: Free phenolic extract of Tetracarpidium conophorum. ACE: Angiotensin-1-converting enzyme.

Table 6: Ic_{50} Inhibition of Angiotensin-1 converting enzyme (ACE) *in vitro*

Sample	IC_{50} (µg/mL)
FPTC	7.00 ± 0.16
Lisinopril	0.15 ± 0.02

Results are mean ± SD (n = 3).
FPTC = Free phenolic extract of *Tetracarpidium conophorum*.

Effects of Free Phenolic Extract of *Tetracarpidium conophorum* on Sodium/Potassium-ATPase and Acetylcholinesterase Activities

Free Phenolic Extract of *Tetracarpidium conophorum* (FPTC) significantly ($p<0.05$) induced cerebral Na^+/K^+ ATPase activities in all the three doses (10 µg/ml, 25 µg/ml and 50 µg/ml) observed when compared with normal control (149.73 ± 11.37) and digoxin treatment group (133.93 ±6.69) as shown in Table 7.

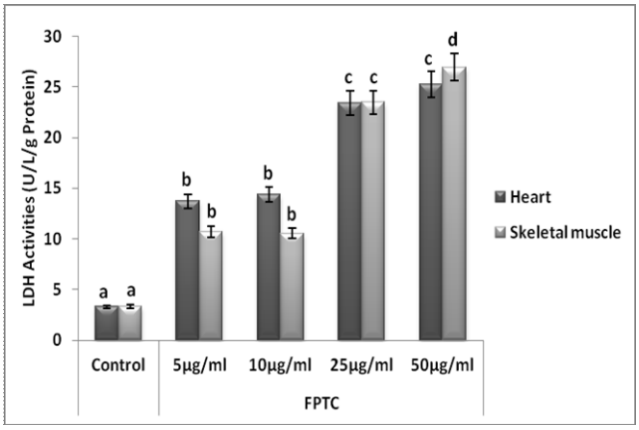

Figure 4: Lactate dehydrogenase (LDH) activities in heart and skeletal muscle.

Values are expressed as mean ± SD (n=3). Duncan superscripts a, b, c, d are significance homogenous subsets of means between groups. Bars of the same legend with different superscripts are statistically significant at the 0.05 level.
FPTC = Free phenolic extract of *Tetracarpidium conophorum.*

The highest cerebral Na^+/K^+ ATPase activity (168.55 ± 1.30) was observed at 10 µg/ml. At 25 µg/ml and 50 µg/ml, FPTC showed a significant ($p<0.05$) increase on cerebral acetylcholinesterase activity (0.24 ± 0.03 and 0.24 ± 0.02) but there was no significant difference at 5 µg/ml (0.18 ± 0.02) when compared with normal control (Table 8).

Table 7: Cerebral Na^+/K^+ ATPase Activities

Treatment groups	Amount of Pi liberated (mg/ml/min)	Na^+/K^+ ATPase Activities (µg Pi liberated/mg protein/min)
Normal Control	0.66 ± 0.05^{b}	149.73 ± 11.37^{b}
Digoxin (1 µM)	0.59 ± 0.03^{a}	133.93 ± 6.69^{a}
FPTC 10 µg/ml	0.75 ± 0.01^{c}	168.55 ± 1.30^{c}
FPTC 25 µg/ml	0.73 ± 0.03^{c}	161.78 ± 5.68^{bc}
FPTC 50 µg/ml	0.72 ± 0.03^{bc}	160.27 ± 9.03^{bc}

Values are expressed as mean ± SD (n=3). Duncan superscripts a, b, c, bc are significance homogenous subsets of means between groups. Means with different superscripts along the column are statistically significant at the 0.05 level. FPTC = Free phenolic extract of *Tetracarpidium conophorum*.

Table 8: Cerebral Acetylcholinesterase (AChE) Activities

Treatment groups	AChE Activities (? pH/20min)
Normal Control	0.17 ± 0.06^{a}
FPTC 5 µg/ml	0.18 ± 0.02^{a}
FPTC 10 µg/ml	0.21 ± 0.02^{ab}
FPTC 25 µg/ml	0.24 ± 0.03^{abc}
FPTC 50 µg/ml	0.24 ± 0.02^{abc}

Values are expressed as mean ± SD (n=3). Duncan superscripts a, ab, abc are significance homogenous subsets of means between groups. Means with different superscripts along the column are statistically significant at the 0.05 level. FPTC = Free phenolic extract of *Tetracarpidium conophorum*.

Discussion

The present study identified the phenolic compounds in the *Tetracarpidium conophorum* seed and evaluated the biochemical effects of the phenolics on selected enzymes of cardiovascular and neurological disorders. Plant phenolic compounds are one of the most studied families of natural products due to their wide range of biological significance. Three novel alcohol-phenol based derivatives were identified by GCMS from *T. conophorum* seed. 3-(1,3- Dihydroxy isopropyl)-1,5,8,11-tetraoxacyclotridecane and 3-(1,3-Dihydroxy-isopropyl)-1,5,8,11.14,17-hexaoxacyclononadecane are heterocycloalkanes. Cycloalkanes are central nervous system agents which are useful as dopaminergic agents, antipsychotics, and anti-hypertensives and in treating conditions associated with central nervous system disorders (25). 3-(1,3-Dihydroxyisopropyl)-1,5,8,11-tetraoxacyclotri-decane was reported by Abeer *et al*. (26) to also possess anti-cancer activity. The third identified compound, heptaethylene glycol monododecyl ether, is alkyl polyglycol ether of lauryl alcohol which has been reported to possess anaesthetic and sclerosing properties and is being used for the treatment of oesophageal and gastric varices, varicose veins, haemorrhoids and peptic ulcer disease (27).

Angiotensin converting enzyme (ACE) is respon-sible for the conversion of the inactive peptide angiotensin I into angiotensin II which increases blood pressure by promoting sodium and water

retention in the body (28). *T. conophorum* seed phenolics demonstrated a dose-dependent inhibition of angiotensin-1-converting enzyme as effective as lisinopril, a known inhibitor of the same enzyme. This is in support of previous studies that phenolic compounds from plant extract could inhibit ACE and be used as antihypertensives. The hydroxyl groups in the identified compounds (heterocycloalkanes) may be responsible for significant inhibition of ACE and previously mentioned antihypertensive claim (6, 25, 29-31). Lactate dehydrogenase (LDH) is found in most living tissues and it converts pyruvate, the end product of glycolysis, into lactic acid (vice-versa). This conversion is needed to continue making ATP for metabolic processes when a cell has little to no oxygen. In our study, the plant extract significantly increased the activities of LDH in both heart and skeletal muscles.

Acetylcholinesterase (AChE) and Na+/K+-ATPase enzymes activities are vital to normal neuronal function. Decreased Na+/K+-ATPase activity has been implicated in some neurodegenerative diseases [32, 33] while impairment of cholinergic neuronal system in brain as a result of reduced AChE activity was reported to be the underlying cause of cognitive impairment seen in some neurodegenerative diseases (34, 35). This is the first study to report the effects of the plant extract on AChE and Na+/K+-ATPase enzymes activities. Increase in the activities of the two enzymes by the plant extract could be responsible for its anti-depressant-like activity as previously reported (36), and this biological activity could be attributed to the hetero cyclo alkanes (25) identified in the plant extract.

Conclusion

Inhibition of ACE and cerebral modulation of AChE and Na+/K+-ATPase activities by *Tetracarpidium conophorum* seed phenolics are due to the presence of the hetero-cyclic derivatives present in the seed extract. The mechanism by which hetero alkanes exert their actions is presently unknown and it is subject to further studies. The findings on ACE inhibitory properties of phenolic extract contributed to the scientific basis for future development of nut-based nutritional supplements for controlling hypertension while the biochemical effects of walnut on AChE and Na+/K+-ATPase activities may be responsible for its folklore use in the management of neurological disorders.

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