PHYTOCHEMICAL, ANTI-OXIDANT PROPERTIES AND LIPID LOWERING EFFECT OF THE AQUEOUS EXTRACT OF *Dennettiatripetala* FRUITS ON WISTAR RATS

Supported by tetfund TERTIARY EDUCATION TRUST FUND

E. Ojogbane[1]*, J. E. Olajide[1], O. E. Yakubu[2], D. A. Musa[3] and P. I. Awodi[1]
[1]Department of Biochemistry, Kogi State University, Anyigba, Nigeria
[2]Department of Biochemistry, Federal University Wukari, Taraba State, Nigeria
[3]Department of Biochemistry, Ibrahim BadamasiBabangida University, Niger State, Nigeria
Corresponding author: elgbane@yahoo.com

Received: October 12, 2016 **Accepted:** July 10, 2017

Abstract: *Dennettiatripetala* belongs to the family *Annonaceae*. The aqueous and n-Hexane extract were screened for the presence of phenols, flavonoids and vitamin C and the 2,2-diphenyl-picrlhydrazyl (DPPH) radical scavenging activities of the extracts were also determined. Twenty five Wistar rats of either sex, weighing 130-140g, were used for this study. The animals were grouped into five Groups: A, B, C, D and E of five animals each. The control group animals were served single oral daily doses of normal saline, while the experimental groups B, C, D and E received 1.0, 1.5, 2.0 and 2.5 mg/kg b.w of the aqueous extract for 10 days, respectively. Twenty four hours after the last administration, the animals were sacrificed under chloroform anaesthesia. Determination of the concentrations of total proteins, cholesterol, LDL, TAG and HDL were by standard methods. The results showed that the extract caused a dose dependent significant ($p < 0.05$) increase in the body weight of the test animals when compared with the control group A, but the weight of the liver and kidney did not increase or decrease significantly ($p > 0.05$). Both extracts contain phenols, flavonoids and vitamin C. However, the concentration of these phytochemicals were more in the aqueous extract, also the aqueous extract exhibited radical scavenging activity than the n-Hexane extract. The treatment of the rats with the aqueous extract produced a dose dependent significant ($p < 0.05$) increase in the concentration of total proteins and HDL. The treatment significantly ($p < 0.05$) and in a dose dependent manner decreased the concentration of cholesterol, TAG and LDL. The phenolic content of the extract could be responsible for the radical scavenging activity; extract has lipid lowering properties, which may be enhanced in the management of lipid related disorders.

Keywords: *Dannettiatripetala*, 2,2-diphenyl-picrlhydrazyl (DPPH), scavenging activities, lipid profile

Introduction

Dennettiatripetela fruit is a famous forest spicy indigenous medicinal plant which belongs to the family of *Annonaceae*. It is widely distributed/domesticated in West African countries such as Nigeria, Ghana, Niger Republic, Benin Republic. It is either reddish (ripped form) or greenish (unripped form); however, it can be eaten in both forms (Idoko and Adebayo, 2011).

The fruit of*Dennettiatripetala* is sometimes taken with kolanut, garden egg and palm wine especially in the southern part of Nigeria where it also serves for cultural entertainment of guest during coronation, new yam festival, wedding and marriage ceremonies. Adedayo*et al.* (2010) reported that they are used as spices in flavouring food and as seasoning which are added to prepared food, and vegetables. *Dennettiatripetela* fruits contain essential oils, phenolic compounds, alkaloids, ethyl acetate, flavonoids, vitamin C, tannins and glycosides (Okwe*et al*., 2005). It is a masticator which has insecticidal activity (Ejechi*et al.*, 1999), anti-fungal and anti-bacteria activities (Okwu and Morah, 2014), It also aids uterian contraction (Adebayo *et al.*, 2010). Studies by Blake *et al.* (2007) recorded that the essential oil of *Dennettiatripetela* fruit has analgesic and anti-inflammatory properties.

Claims from recent studies by Head and Kathleen, (2011) observed that *Dennettiatripetela* fruit decreases the risk of optic nerve damage by stabilizing the intra ocular pressure of the eye known as glaucoma, patients with diabetes/hypertension subsequently will almost present with glaucoma (Idoko and Adebayo, 2011). However, cellular damage arising from free radical is the fundamental mechanism underlying a number of human neurodegenerative disorders such as diabetes mellitus, inflammation, viral infection, auto immune pathologies and so on. It is against this background that this study was carried out to accessing the phytochemical, antioxidant properties and the effect of the aqueous extract of *Dennettiatripetela* fruit on total protein, cholesterol, low density lipoproteins, triacylglycerols and high density lipoproteins, respectively.

Materials and Methods

Materials

Ripped *Dennettiatripetala* fruits were purchased from Anyigba Central Market on April 2015, in Dekina Local Government Area of Kogi State. They were rinsed, dried at room temperature and pulverized with Creson high speed milling machine into a coarse powder.

Determination of the concentration of phenol and Vitamin C in extract was by the method of singleton *et al*. (1999). While the concentration of Total proteins in plasma was by Biuret method, (1978), triacylglycerols, low density lipoproteins and Cholesterol were by the method of Tietz, (1990), high density lipoproteins concentration was by Groove (1989).

Chemicals and reagents

n-Hexane and 2,2-dipheny-picrylhydrazyl (DPPH) were products of British Drug House (BDH) and Sigma. Commercially prepared reagent from Randox Laboratories Ltd, 54 Diamond Road, Crumlim, Co. Antrim, United Kingdom. They were used to determine the concentration of Total proteins, cholesterol, high density lipoproteins, low density lipoproteins and triacylglycerols.

Blood samples

Blood samples were collected via cardiac puncture into heparinized centrifuge tubes and spun at 1000 rpm for 5 min to separate the plasma from the cells.

Methods

n-Hexane extraction

A 200 g quantity of pulverized dried fruits of *Dennettiatripetala* was soaked in 1000 ml of n-Hexane for 18 hours and filtered. The n-Hexane was separated from the filtrate using a rotary evaporator and the filtrate was heated in a water bath at 60^0C to get the oil extract.

Aqueous extraction

A 200g quantity of pulverized dried fruits of *Dennettiatripetala* was soaked in 1000 ml of distilled water for 18 hours and then filtered. The filtrate was evaporated in a water bath at 100ºC to get the dried residue. The percentage yield for both extractions was calculated.

Experimental design

Twenty five (25) Wistar rats (130-140 g) of either sex which were purchased from the Animal House of the Department of Biochemistry, Kogi State University Anyigba, Kogi State, Nigeria were randomly assigned to five groups A, B, C, D and E and maintained under standard laboratory conditions. The rats were fed with their normal diet (Top Feed Nig. Ltd) and allowed water *ad libitum* throughout the period of the experiment. Group A served as control and received normal saline (0.85 g NaCl; 5 ml/kg). Groups B, C, D and E were served single oral daily doses of 1.0, 1.5, 2.0, and 2.5 mg/kg. b.w of the aqueous extract using stomach tubes for 10 days, respectively. Animals were sacrificed under chloroform anesthesia, 24 h after the last administration and the blood samples were collected into heparinized centrifuge tubes.

Statistical analysis

Data collected were subjected to Analysis of Variance (ANOVA) and Student T-test and presented as Mean ± Standard deviation ($p < 0.05$) was accepted as the level of significance.

Results and Discussion

Percentage yield

The result of this study showed that n-Hexane and aqueous extract yielded 6.08 and 16.57%, respectively.

Table 1 showed that both extracts contain phenols, flavonoids and Vitamin C. However, the aqueous extract shows significant increases ($p < 0.05$) in the concentration of phenols by 40.07 mg/g, flavonoids by 4.91 mg/g and vitamin C by 18.00 mg/dl when compared with the n-Hexane extract.

Table 1: Phenolic, flavonoid and vitamin content of extracts

Phytochemical	n-Hexance extract (mg/g)	Aqueous extract (mg/g)
Phenols	20.04	60.11
Flavonoids	6.17	11.08
Vitamin C	6.00	24.00

Inhibition Capacity (IC_{50}) of the aqueous extract is 436.5 mg/ml while that of hexane extract is 602.6 mg/ml respectively. Table 2, shows significant($p < 0.05$) dose dependent increases in anti-oxidant capacity for both extracts. Also, there were significant ($p<0.05$) dose dependent increases in antioxidant capacity between the aqueous and n-Hexane extract by 3, 4, 5, 2 and 9% at concentrations of 1000, 500, 250, 125, 62.5 mg/ml. A lower inhibition capacity is an index of higher antioxidant activity.

Table 2a: Extract induced increases in anti-oxidant activity

Extract Conc. (mg/ml)	Anti-oxidant capacity (%)		IC_{50} (mg/ml)	
	aqueous	n-heaxane	Aqueous	n-hexane
1000	59	56		
500	50	46		
250	46	41	436.5	602.6
125	35	33		
62.5	21	22		

Table 2b shows that the vitamin C standard has more anti-oxidant capacity in their DPPH radical scavenging ability than the test samples (aqueous extract and n-hexane extract). However, the aqueous vitamin C has significant ($p<0.05$) higher anti-oxidant capacity than the n-hexane vitamin C standard. IC_{50} of the aqueous vitamin C is 128.8 mg/ml while that of hexane extract is 141.3 mg/ml, respectively.

Table 2b: Increases in anti-oxidant activity of standard aqueous vitamin C and n-Hexane vitamin C.

Extract Conc. (mg/ml)	Anti-oxidant capacity (%)		IC_{50} (mg/ml)	
	Aqueous vitamin C	n-heaxane extract	Aqueous vitamin C	n-heaxane extract
1000	92	86		
500	82	73		
250	63	59	128.8	141.3
125	48	50		
62.5	29	38		

Table 3 shows dose dependent significant ($p<0.05$) increases in the body weight, of rats by 3.51, 5.01, 7.00 and 14.64 g in Groups B, C, D and E respectively which were served doses of 1.0, 1.5, 2.0 and 2.5 mg/kg b.w of the extract compared with an increase of 2.64g of animals which received normal saline (Group A).Table 4shows that there is no significant ($p > 0.05$) effect on the weight of both liver and kidney of Wistar rats in the experimental groups B, C, D, and E when compared with the control group A.

Table 3: Body weight of Wistar rats given extract

Group	Treatment (mg/kg)	Weight (g) before administration	Weight (g) after administration
A	Normal saline 5 ml/kg	149.44 ± 11.00	152.08 ± 9.13
B	1.0	153.71 ± 7.61	157.22 ± 7.63
C	1.5	143. 92 ± 9.08	148.93 ± 9.26
D	2.0	146.40 ± 9.39	153.40 ± 13.80
E	2.5	144.92 ± 13.20	159.56 ± 11.70

Table 4: Liver and kidney weights of Wistar rats exposed to extract

Group	Treatment (mg/kg)	Weight of organs (g)	
		Liver	Kidney
A	Normal saline 5 ml/kg	2.79 ± 0.77	0.87 ± 0.10
B	1.0	2.78 ± 0.47	0.88 ± 0.11
C	1.5	2.79 ± 0.69	0.86 ± 0.08
D	2.0	2.76 ± 0.79	0.85 ± 0.17
E	2.5	2.79 ± 0.54	0.89 ± 0.09

Table 5shows that the extract induced significant ($p<0.05$) dose dependent increases in the concentration of total proteins in plasma by 0.71, 0.97, 1.28 and 1.58 mg/dl of rats which received 1.0, 1.5, 2.0 and 2.5 mg/kg b.w of extract from the control value of 5.57 ± 0.22 in group (A, control). Similarly, there were significant ($p<0.05$) dose dependent increases in the concentration of

HDL by 3.51, 5.38 and 6.57 mg/dl in Groups B, C, D and E from the control value of 16.88 ± 1.0

mg/dl in Group A. On the contrary, the same treatment caused significant ($p<0.05$) dose dependent decreases of 9.02, 13.65, 16.95 and 22.48 mg/dl in the concentration of LDL from the control value of 110.40 ± 4.51 mg/dl in Group A. In the same vein, there were dose dependent significant ($p<0.05$) decreases in the concentration of TAG by 2.15, 2.98, 4.96 and 8.96 mg/dl in Groups B, C, D and E from the control value of 142.49 ± 2.25 in Group A. The treatment also induced significant ($p<0.05$) dose dependent decreases in the concentration of total cholesterol by 5.11, 6.81, 9.57 and 13.95 mg/dl of animals in Groups B, C, D and E, respectively from the control value of 156.78 ± 4.92 mg/dl.

Table 5: Effect of extract on total proteins and lipid profile

Group	Treatment	Total protein (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Triacylglycerol (mg/dl)	Total cholesterol (mg/dl)
A	Normal saline 5 ml/kg	5.57 ± 0.22	16.88 ± 1.03	110.40 ± 4.57	142.49 ± 2.25	156.78 ± 4.92
B	1.0 mg/kg	6.28 ± 0.72	20.39 ± 2.72	101.38 ± 3.32	140.34 ± 5.00	151.67 ± 2.71
C	1.5 mg/kg	6.54 ± 0.85	22.18 ± 2.58	97.73 ± 6.18	139.51 ± 5.00	149.97 ± 2.56
D	2.0 mg/kg	6.84 ± 0.75	22.26 ± 2.11	93.45 ±6.75	137.53 ± 5.29	147.21 ± 6.44
E	2.5 mg/kg	7.15 ± 0.37	23.45 ± 2.27	87.92 ± 6.41	133.53 ± 4.58	142.83 ±3.82

The aqueous and n-Hexane extracts of *Dennettiatripetala* fruits yielded 16.57 and 6.08%, respectively.

Higher concentration of the phenols, flavonoids and vitamin C was contained in the aqueous extract than the n-hexane extract. These phytochemicals are more soluble in water than in organic solvents. Phenolic compounds are classes of anti-oxidant which act as free radical scavengers aided by the presence of hydroxyl groups in their structures (Adedayo*et al*., 2010). Flavonoids are a class of widely distributed phytochemicals with anti-oxidant properties (Donald, 2007). Their biological activity has been implicated in the management of several disorders such as fever, cough, toothache and in terminal disease such as cancers (Okwu*et al*., 2005).

Accordingly, McGregor and Biesalski, (2006) had reported that Vitamin C is one of the most abundant water soluble anti-oxidant. It plays a functional role in combatting free radical formation and inhibits oxidation. This result is in agreement with the study of Daniel and Clement (2008) who reported that anti-oxidants are useful in the management of various disease state and disorders. The anti-oxidant components of *Dennettiatripetala* fruits could be enhanced in the management of degenerative disease like glaucoma.

The extract exhibited significant ($p<0.05$) dose dependent increases in the percentage anti-oxidant activity vide the hydrated form of 2,2-diphenyl-picrlhydrazyl (DPPH), a phenolic compound which inhibits lipo-oxygenase and scavenges free radicals (Hu *et al.*, 2005). This anti-oxidant activity may be consequent on the presence of flavonoids, vitamin C and some other bioactive agents which may be resident in the extract. Treatment with the extract improved on the body weight of the animals and had no adverse effect on the organ weights.

Proteins play important roles in the maturation of the immune system (Copper, 2009). From the present result, treatment with *Dennettiatripetala* fruits extract increased significantly ($p<0.05$) in a dose dependent manner the concentration of proteins in the test animals when compared with the control animals. The observation that the extract significantly ($p<0.05$) increase the concentration of HDL-cholesterol is beneficial in maintaining cholesterol levels. HDL is involved in the clearance of cholesterol from the circulation back to the liver. Such increase promotes the removal of the excess cholesterol in the plasma to the liver for excretion.

The extract significantly ($p<0.05$) decreased the concentration of LDL-cholesterol which is capable of depositing cholesterol in the arterial wall and signal medical conditions like cardiovascular ill health. The decrease in LDL-cholesterol may serve a protective index against such disorders. This result, buttresses the claim of Idoko and Adebayo (2011) that *Dennettiatripetala* fruit extract may be a useful therapy in reducing the risk of cardiovascular disease and some other degenerative systemic disorder. The extract also caused a dose dependent significant ($p<0.05$) decrease in the concentration of both triacylglycerol and total cholesterol. Evidence exist Despress (2009), that high concentration of triacylglycerol and cholesterol in the blood stream is linked to atherosclerosis, excess triacylglycerol in the plasma could be minimized by this extract. The triacylglycerol lowering effect of the extract is a two pronged attack on both triacylglycerol and cholesterol bioavailability in the body (Head and Kathleen, 2011).

Peroxidation of lipids can disturb the assembly of the membrane causing alteration of ion transport and inhibition of some metabolic processes (Laguerre, 2007). *Dennettiatripetala* fruit extract has lipid lowering effect and may tamper and manage cellular damage arising from lipid peroxidation and oxidative injury arising from reactive oxygen species, as they have been implicated by Daniel and Clement (2008) as the fundamental mechanism underlying a number of human neurodegenerative disorders.

Conclusion

The fruit extract of *Dennettiatripetala* has anti-oxidant properties and lipid lowering effects, which can be enhanced in the management of lipid related and neuro degenerative disorder. Impaired immune responses which is consequent on decreased synthesis of protein could be managed by the extract.

References

Adedayo BC, Oboh G &Akindahunsi AA 2010.Changes in the total phenol content and anti-oxidant properties of pepper fruit (*Dennettiatripetala*) with ripening.*Afr. J. Food Sci.*, 4(6): 403 – 409.

Block G, Jensun CD, Norkus EP, Dalvi TB, Wang LG, McmanusJF&Hudes ML 2007. Usage patterns, health and nutritional status of long term multiple details, supplement users: a cross sectional study. *Nutritional Journal*, 6: 30-40.

Copper R 2009. Hyperaldosteronism. Medline plus available at http://www.nlm.nil.gov/medlineplus/ency/article/000330.htm

Daniel EI& Clement ON 2008.Effect of ethanolic extract of *Dennettiatripetala* fruit on haematological parameters in albino Wistar rats.*Nig. J. Physiol. Sci.*, 23(1-2): 13-17.

Despress JP 2009. The atherogenic triad of metabolic risk factors: importance of waist and fasting triglyceride as screening tool. *Amer. Diabettes Assoc. J.*, 32(10):12-7.

Groove TH 1989. Determination of HDL by precipitation with phosphotunstate magnesium.*Clinical chemistry*, 41: 91-103.

Head I& Kathleen C 2011. Natural therapies for Ocular disorder, part two: cataract and glaucoma. *Alternative Review*, 6(2): 141-166.

Hu S, Nlederau C, KlonowskiSH, Halank W &Lutlen B 2005.Oxidative stress in acute pancreatic hepato-gastroenterology.*Journal of physiology*, 46: 2736-2750.

Idoko JE & Adebayo RA 2011. Effect of *Dennettiatripetala* powder and reduced rates of pirimiphos-methyl singly and

combined in management of *callosobruchusmaculatus* (F) (Coleoptera: Bruchidae). *Intern. J. Bio*., 3(2): 174-178

Laguerre M 2007. Lipid peroxidation.*Progress in Lipid Research*, 36: 244-282.

McGregor GP &Biesalski K 2006.Rationale and impact of vitamin C in clinical nutrition.*Current Opinion in Clinical Nutrition & Metabolic Care*, 9(6): 697-703.

Meda A, Lamien CE, Romito M, Millogo J &NacoolmaOG 2005. Determination of the total phenolic, flavonoid and proline contents in Burkina fasan Honey as well as their radical scavenging activity. *J. Food Chem.*, 91: 571-577.

Okwu DE &MorahFNI 2004. Mineral and nutritive value of *Dennettiatripetala*fruits. *J. Medical & Aromatic Plant Sci.*, 59: 437-442.

Okwu DE, MorahFNI& Anan EM 2005.Isolation and characterization of phenanthrenic alkaloid Uvariopsine from *Dennettiatripetala* fruits.*J. Medical & Aromatic Plant Sci.*, 27: 496-498.

Singleton VL, Orthofer R &Lamuela–RaventosRM 1999.Analysis of total phenols and other oxidation substrates and anti-oxidants by means of Folin-ciocalteau reagent.*Methods in Enzymology*, 299: 152-178.

Tietz NW 1990. *Clinical Guide to Laboratory Test*. 2nd edn W.B Saunders Company Philadelphia USA, pp. 554-554.