Antioxidant Activity and HPLC Analysis of *Zanthozylum zanthoxyloide*

[1]Adekunle A.S., [2]Kamdem J.P., [2]Rocha J.B.T

[1] Department of Biochemistry, Ladoke Akintola University of Technology, Nigeria.
[1&2] Departamento de Química, Bioquímica Toxicológica, Universidade Federal de Santa Maria, Santa Maria, RS 97105-900, Brazil.
kunleniran@yahoo.com.

Abstract: Acceptance of medicinal plants in the treatment of ailments is increasing around the globe. *Zanthoxylum zanthoxyloides* was assessed for its antioxidant activity and presence of phenolic and flavonoid contents. *Z. Zanthoxyloide* demonstrated antioxidant ability by scavenging DPPH radicals and chelating iron. It reduces generation of reactive oxygen species in isolated mitochondria in the presence or absence of hydrogen peroxide. These activities were dose-dependent. High performance liquid chromatographic analyses revealed presence of phenolic acids such as chlorogenic and caffeic acids and flavonoid compounds such as quercetin, rutin and kaempferol. In conclusion, *Z. Zanthoxyloide* demonstrated high antioxidant activity which may be due to the presence of phenolic and flavonoid compounds.

[Adekunle Adeniran Sanmi, Kamdem Jean Paul, Rocha Joao Baptista Texeira. **Antioxidant Activity and HPLC Analysis of *Zanthozylum zanthoxyloide.*** Report and Opinion 2012;4(3):6-13]. (ISSN: 1553-9873). http://www.sciencepub.net/report. 2

Key words: Z.zanthoxyloides, medicinal plants, antioxidants, free radicals

1.0 Introduction

The use of medicinal plants for the treatments of different ailments is very common in Nigeria. Many of them are used to treat infectious diseases, which may be due to poor hygienic conditions or contacted from sexually-related route. Many are used for the treatment of other diseases such as malaria, hypertension, stroke etc. Various studies have shown that several medicinal plants have significant antifungal activity on a wide range of microorganisms (Odebiyi and Sofowora, 1979; Awuah, 1989; Rojas et al., 1992; Irobi et al., 1994; Larhsini et al., 1996; Amvam Zollo et al., 1998).

The Nigerian *Z. zanthoxyloides* is a common component of the rain forest vegetation of Southern Nigeria and is also widely distributed in African countries. It is well known for its varied uses in traditional medicinal practices. The root-bark extract is used in treating elephantiasis, toothache, sexual impotence, gonorrhoea, malaria, dysmenorrhoea and abdominal pain (Amvam et al., 1998; Odebiyi and Sofowora, 1979; Awuah, 1989). Workers in West Africa have reported the anti-sickling and antimicrobial activities of the extracts of the plant (Irobi et al., 1994). In Nigeria, *Z. zanthoxyloides* is used as a chewing stick. Water extracts from the plant showed activities against bacteria significant to periodontal disease (Larhsini et al., 1996; Rojas et al., 1992). The antihelmintic activity of the methanolic extract of the root-bark of *Z. zanthoxyloides* was also reported (Ajanohoun et al., 1993), and it is a very popular antihelmintic amongst the various tribes in Uganda. It has also been found that the alcoholic extracts of the root-bark possesses considerable antibacterial activity (Anokbongo et al., 1990). An anti-sickling agent (Watt et al., 1962) and an anti-inflammatory amide were isolated from the plant (Sofowora, 1993). *Z. zanthoxyloides*, is also commonly used in Cameroon for the traditional treatment of certain infectious diseases such as skin infections, gonococcies, urinary infections, dysentery (Noumi, 1984). Few reports are available on the antioxidative activity of *Z. zanthoxyloides* and its phenolic acid and flavonoid constituents. In the light of this, this study was undertaken to evaluate in vitro antioxidative properties using known methods and also determine phenolic acid and flavonoid constituents using high performance liquid chromatographic technique.

2.0 Materials and Methology

2.1 Chemicals

All chemicals used including solvents, were of analytical grade. 1,1-diphenyl-2-picryl hydrazyl (DPPH), Folin Ciocalteu´s phenol reagent, malonaldehyde bis-(dimethyl acetal) (MDA), Thiobarbituric acid, sodium dodecyl sulfate, ascorbic acid, 2′,7′- dichlorofluorescein diacetate (DCFH-DA), Tris-HCl, ethylene glycol tetraacetic acid (EGTA), quercetin, rutin, chlorogenic acid and gallic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES), ferrous sulfate, mannitol, sucrose were obtained from Vetec (Rio de Janeiro, RJ, Brazil).

2.2 Plant extract

Stem of Z. zanthoxyloide was obtained from Ogbomoso, Nigeria in 2011 and was identified by Dr.

Ogunkunle of the Botany Unit, Department of Pure and Applied Biology and were confirmed with a plant name index. The stem was grinded into a powdery form and the powder of Z. zanthoxyloide (100 g) was macerated at room temperature with ethanol (70%) and extracted for 72 hours at the Biochemical Toxicological Unit, Department of Biological sciences, Federal University of Santa Maria, Santa Maria RS, Brazil. On the third day, the combined ethanolic extract was filtered and the solvent was fully evaporated under reduced pressure to give a yellow solid. The ethanolic extract was then diluted in ethanol in order to prepare different concentrations (10, 50, 100 and 250 µg/mL).

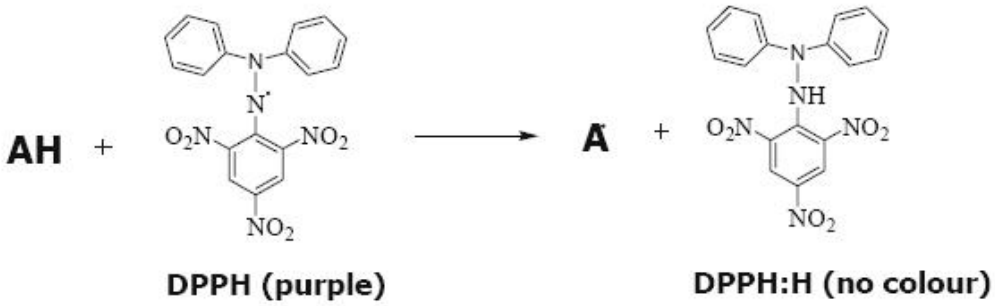
The free radical scavenging activity of Z. zanthoxyloide extract was measured with the stable radical 1,1-diphenyl-2-picryl hydrazyl (DPPH) Bandoniene and Murkovic, (2002). in terms of hydrogen-donating or radicals scavenging activity. A solution of DPPH (0.3 mM) in ethanol was prepared, and 100 µL of this solution was added to 20 µL aquoeus extract at different concentrations (10, 50, 100 and 250 µg/mL). Ascorbic acid at the same concentrations used for aqueous extract was used as positive control. After 30 minutes, absorbances were measured at 548 nm in ELISA plate reader (TP-Reader, Brazil).

2.3 DPPH radical scavenging activity

Table 1: Ethnobotanical data of plant studied

Species	Part used	Common name (tribes)	Popular uses	Form of administration (directions for use)
Z. zanthoxyloide	Leaves		E: For scaring, antiseptic, astringent. I: Laxative (Kerharo and Adam, 1974; Noumi, 1984)	Local and oral routes (macerated or decocted powder, paste)
	Roots		Antiseptic, anti-sickler, digestive aid, parasiticide (Kerharo and Adam, 1974; Sofowora et al., 1975; Noumi, 1984; Comoe, 1987)	Local and oral route: rectal and vaginal injection (macerated or decocted powder, paste, stick)
	Stem barks		E: antiseptic, antirheumatic, anti-odontalgic, Local and oral routes; (macerated or (Haoussa) I: diurectic, urinary antiseptic, digestive aid, decocted powder, paste, sticks) Parasiticide (Kerharo and Adam, 1974; Noumi, 1984; Comoe 1987)	Local and oral routes; (macerated or decocted powder, paste, sticks)

E, external use; I, internal uses



AH = antioxidant compound
DPPH = 1,1-diphenyl-2-picrylhydrazyl

Fig. 1. The In Vitro chemical representation of DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay.

The free radical scarvenging capacity of the extract is calculated with the equation;
% inhibition = [(Control Absorbance – Test Absorbance)/Control Absorbance] ×100

2.4 Total phenolic content

The total phenolic content of *Z. zanthoxyloide* was estimated according to the method of Makkar et al. [21] with minor modifications. Samples of the extract (10 - 250 µg/mL) were added to a test tube and the volume was adjusted to 1.4 mL with distilled water. Then, 0.2 mL of Folin-Ciocalteu reagent (diluted 1:1 with water) and 0.4 mL of sodium carbonate solution (7.5%) were added sequentially to the test tube. The tubes were then incubated for 40 min at 45°C and the absorbance was

measured at 725 nm in a spectrophotometer (SP-2000UV). The standard curve was prepared using 0, 1, 2.5, 5, 10 and 15 µg/mL solutions of gallic acid (0.1 mg/mL). Total phenol value was calculated and expressed as microgram gallic acid equivalent (µg GAE)/g of dry extract with the equation:

Total Phenol= [(CF x Abs (each)/Vol (extract in ml)] x2ml

Where CF is the quantity of garlic acid in µg divided by its absorbance

2 ml was the total volume of the reaction.

2.5 Iron-chelating activity.

The method described by Benzie and Strain (1996) was was adopted for the assay. The principle is based on the formation of O-Phenanthroline-Fe2+ complex and its distruption in the presence of chelating agents. The reaction containing 40 ul of ferric chloride (3.12 mM), 740 ul of Tris-HCl (0.1 M) and different volumes of different concentrations of extract was made up to 2ml with distilled water and was incubated for 5 minutes. The mixture was used to zero the wavelenght of the spectrophotometer. 25 ul of phenanthroline was thereafter added to the mixture in the cuvette and absorbance taken at 510nm. The Fe^{2+} chelating capacity was calcuted thus:

Fe^{2+} chelating activity (%) = {(Ac – As)/ Ac} x 100

2.6 Animals

Male Wistar rats weighing 270-320 g and with age from 2.5 to 3.5 months from breeding colony (Animal House-holding, UFSM, Brazil) were used for the determination of reactive oxygen species. They were kept in cages with free access to foods and water in a room with controlled temperature (22 ± 3°C) and in 12 h light/dark cycle. The protocol has been approved by the guidelines of the Brazilian association for laboratory animal science (COBEA).

2.7 Quantification of phenolics and flavonoids compounds by HPLC-DAD

Free phenolic and flavonoid contents were determined using High performance liquid chromatography (HPLC-DAD). This was performed with the HPLC system (Shimadzu, Kyoto, Japan), Prominence Auto Sampler (SIL-20A), equipped with Shimadzu LC-20AT reciprocating pumps connected to the degasser DGU 20A5 with integrator CBM 20A, UV-VIS detector DAD (diode) SPD-M20A and Software LC solution 1.22 SP1. Briefly, reverse phase chromatographic analyses were carried out under gradient conditions using C_{18} column (4.6 mm x 250 mm) packed with 5µm diameter particles; the mobile phase was water containing 2% acetic acid (A) and methanol (B), and the composition gradient was: 5% of B until 2 min and changed to obtain 25%, 40%, 50%, 60%, 70% and 100% B at 10, 20, 30, 40, 50 and 65 min, respectively, following the method described by Laghari et al. (2011) with slight modifications. The extracts of the MO, TG and ZZ were analyzed, at a concentration of 5 mg/mL. The presence of six phenolics compounds was investigated, namely, gallic, chlorogenic and caffeic acids and the flavonoids quercetin, rutin and kaempferol. Identification of these compounds was performed by comparing their retention time and UV absorption spectrum with those of the commercial standards. The flow rate was 0.6 ml/min, injection volume 40 µl and the wavelength were 254 nm for gallic acid, 325 nm for caffeic and chlorogenic acids, and 365 nm for quercetin, rutin and kaempferol. All the samples and mobile phase were filtered through 0.45 µm membrane filter (Millipore) and then degassed by ultrasonic bath prior to use. Stock solutions of standards references were prepared in the HPLC mobile phase at a concentration range of 0.031 – 0.250 mg/ml for kaempferol, quercetin and rutin; and 0.006 – 0.250 mg/ml for gallic, caffeic and chlorogenic acids. The chromatography peaks were confirmed by comparing its retention time with those of reference standards and by DAD spectra (200 to 500 nm). Calibration curve for gallic acid: Y = 11611x + 1468.8 (r = 0.9999); chlorogenic acid: Y = 14762x + 1257.5 (r = 0.9997); caffeic acid: Y = 11526x + 1293.1 (r = 0.9995); rutin: Y = 13035x – 1045.9 (r = 0.9998); quercetin: Y = 15105x – 1192.3 (r =0.9998) and kaempferol: Y = 15223x – 1303.9 (r = 0.9999). All chromatography operations were carried out at ambient temperature and in triplicate.

2.8 Isolation of rat liver mitochondrial

Rat liver mitochondrial was isolated as previously described by Puntel et al. [24] with some modifications. The livers were rapidly removed (within 1 min) and immersed in ice-cold `` isolation buffer I´´ containing 225 mM manitol, 75 mM sucrose, 1 mM K^+-EGTA and 10 mM K^+-HEPES, pH 7.2. The tissue was minced using surgical scissors and then extensively washed. The tissue was then homogenized in a power-driven, tight-fitting Potter-Elvehjem homogenizer with Teflon pestle. The resulting suspension was centrifuged, for 7 min at 2000 g in Hitachi CR 21E centrifuge. After centrifugation, the supernatant was centrifuged for 10 min at 12000g. The pellet was resuspended in ´´isolation buffer II´´ containing 225 mM manitol, 75 mM sucrose, 1 mM K^+-EGTA, and 10mM K^+-HEPES, pH 7.2, and recentrifuged at 12,000g for 10min. The supernatant was decanted, and the final pellet was gently washed and resuspended in respiration buffer containing 100 mM sucrose, 65 mM KCl, 10 mM K^+-HEPES and 50 µM EGTA, pH 7.2, to a protein concentration of 0.6 mg/mL.

2.9 Generation and measurement of reactive oxygen species (ROS)

ROS production in isolated mitochondria was measured using a 2′,7′-dichlorofluorescein diacetate (DCFH-DA) fluorescence probe. Mitochondrial suspensions (0.25 mg/mL) in respiration buffer containing 100 mM sucrose, 65 mM KCl, 10 mM K^+-HEPES and 50 µM EGTA, pH 7.2 were incubated with different concentrations of the extract (10-250 µg/mL) in the presence or absence of 12mM of H_2O_2. Then, 3.33 µM of DCFH-DA; then, 10, 50, 100, 250 µg/mL of ethanolic extract [25, 26, 27] were added to the solution. The formation of the oxidized fluorescent derivative (DCF) was monitored using a spectrofluorimeter (Shimadzu RF-5301) with excitation and emission wavelengths of 488 and 525 nm respectively and with slit widths of 1.5 nm.

2.1.0 Statistical analysis

Values were expressed as mean ± SEM (standard error of mean). Statistical analyses were performed by one way ANOVA, followed by Duncan's multiple range tests. The results were considered statistically significant for $p < 0.05$.

3.0 RESULTS

3.1 Iron- chelating capacity

Iron chelating activity of ethanolic extract of *Z. zanthoxyloide* at different concentrations was determined and the values were summarized in the table 1. Maximum chelation of metal ion was observed at 20µg/ml.

Table 2: Metal chelating activity of ethanolic extract of *Z. zanthoxyloide*

Concentrations	5µg/ml	10µg/ml	20µg/ml
% iron chelated	83.86±0.74	92.61±0.52	97.33±0.28

3.2 Determination of free radical scarvenging capacity using DPPH

Free radical inhibitions of different concentrations of ascorbic acid (standard antioxidant) increased in the order of 10ug (75.35%) <50ug (78.43%) <100ug (80.52%) <250ug (80.76%), while that of Z. zanthoxyloide increased in the order of 10ug (22.86%) <50ug (31.29%) < 100ug (37.94%) < 250ug (56.03%). IC50 for ascorbic acid was 6.63µg/ml as compared with IC50 38.58µg/ml for Z. zanthoxyloide.

Table 3: Percentage free radical inhibition by the ethanolic extract of *Z. zanthoxyloide*.

Concentrations	10µg/ml	50µg/ml	100µg/ml	2500µg/ml
Ascorbic Acid	75.35±3.18%	78.43±2.48%	80.40±2.22%	80.76±2.40%
Z.zanthoxyloide	22.86±6.17%	31.29±5.89%	37.94±5.96%	56.03±4.48%

3.4 Total phenolics

The amount of total phenolic of ethanolic extract of leaves of *Z. zanthoxyloide* estimated by Folin-Ciocalteau method was 2.46 ± 0.70 mgGAE/g of dry extract.

3.5 Determination of phenolic acids and flavonoid contents of Z. zanthoxyloide

HPLC fingerprinting of extract revealed the presence of the phenolic acids such as chlorogenic acid (t_R = 21.58 min) and caffeic acid (t_R = 24.97 min), while the flavonoids included rutin (t_R = 38.03 min), quercetin (t_R = 45.11 min) and kaempferol (t_R = 54.06 min) (Fig. 2 and Table 4). The highest of the estimated phenolic acids in the ethanolic extract of T. globiferus was chlorogenic acid (5.81±0.05) while the least was caffeic acid (1.77±0.01). The predominant of the estimated flavonoid contents is rutin (8.12±0.09).

Table 4 – Free phenolic acid and flavonoid composition of Zanthoxylum zanthoxyloide extracts by HPLC/DAD

	Z. zanthoxyloide	
	mg/g	%
Gallic acid	-	-
Chlorogenic acid	4.19 ± 0.01 a	0.42
Caffeic acid	8.11 ± 0.05 b	0.81
Rutin	**Compounds**	1.96
Quercetin	48.07 ± 0.02 d	4.81
Kaempferol	25.89 ± 0.06 e	2.599

Results are expressed as mean ± standard deviations (SD) of three determinations. Averages followed by different letters in the column differ by Tukey test at $p < 0.05$.

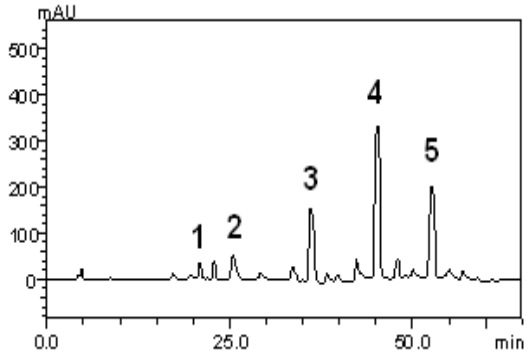


Figure 4 – Representative high performance liquid chromatography profile of *Zanthoxylum zanthoxyloide* extract. Chlorogenic acid (peak 1), caffeic acid (peak 2), rutin (peak 3), quercetin (peak 4) and kaempferol (peak 5).

3.6 Measurement of reactive oxygen species (ROS)

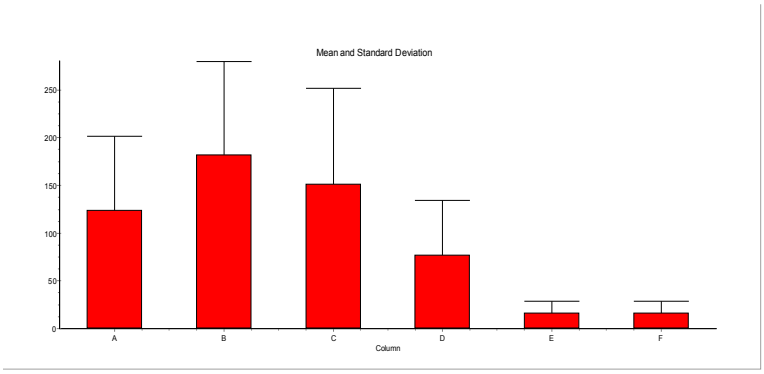


Fig 5: Graph showing the dose-dependent ROS production inhibitory ability of Z.zanthoxyloide
A & B= +ve & -ve controls respectively; C, D, E & F = ROS production at concentrations 10µg/ml, 50µg/ml, 100µg/ml, & 250µg/ml respectively.

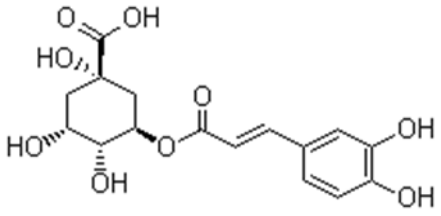
4.0 DISCUSSION

1,1-diphenyl-2-picrylhydrazyl radical (DPPH) is a stable free radical and accepts an electron or hydrogen radical to be a stable diamagnetic molecule. The reduction capability of DPPH is determined by the decrease in absorbance at 517nm induced by antioxidant. Data indicated ethanolic extract of the *Z. zanthoxyloide* is able to reduce the stable radical DPPH to the yellow-coloured diphenylpicrylhydrazine (Table 3). The scavenging effect of *Z. zanthoxyloide* at high concentration (250µg/ml) and ascorbic acid standard solution with the DPPH radical was in the following order Z.z. (56.03%) < A.A. (80.76%).Furthermore the IC50 for ascorbic acid was 6.63µg/ml as compared with IC50 38.58µg/ml for *Z. zanthoxyloide*. This indicates lower scavenging capacity of the ethanolic extract of *Z. zanthoxyloide* when compared with ascorbic acid. Data also revealed that the scarvenging activity of Z.zanthoxyloide was increased with increasing concentration of extract with IC50 at 227.35µg/ml. The findings here indicated that *Z. zanthoxyloide* demonstrated good antioxidant potential. The antioxidant potential exhibited by *Z. zanthoxyloide* can be associated with high levels of phenolic and flavonoid contents such as caffeic acid, chlorogenic acid, quercetin, rutin and kaempferol. Fahrat et al., (2010) reported similar observation of high antioxidative property of specie of *Zanthozylum* i.e. *Z. Alatum*.

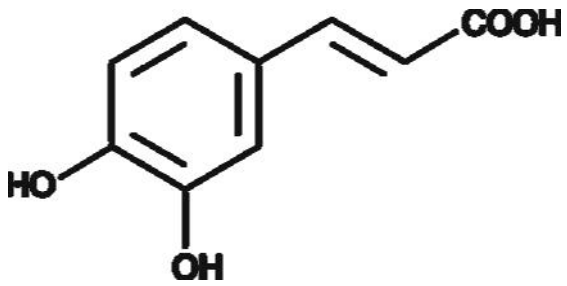
Among the transition metals, iron is known as the most important lipid pro-oxidant due to its high reactivity. The ferrous state of iron accelerates lipid oxidation by breaking down hydrogen and lipid peroxides to reactive free radicals via the Fenton reaction. The agents that can attenuate the action of these bivalent metal ions have been classified as secondary antioxidants which retard the rate of radical initiation reaction by the elimination of initiators (Vaya and Aviram, 2001). 1, 10-phenanthroline forms a complex with free Fe2+ but the extent of the complex formation is reduced when the Fe2+ is less available by being bound onto the plant extracts (or a chelating agent) for example. In the presence of chelating agents, the complex formation of ferrous ion and 1, 10-phenanthroline is altered and this can be monitored by decrease in the absorbance at 510nm. Benherlal and

Arumughan (2008) reported that phytochemicals/extracts with high antioxidant activity but without iron chelation capacity failed to protect DNA in Fenton's system, suggesting that iron chelation was an essential requirement for extracts studied here to retard HO• generation by Fenton's reaction. In this study, *Z. zanthoxyloide* demonstrated high iron-chelating capability as shown by the data (table 3). The capability *Z. zanthoxyloide* to chelate iron II ion increases with concentration i.e. a dose-dependent manner.

High performance liquid chromatographic determination of bioconstituents revealed presence of phenolic acids such as chlorogenic acids, caffeic acid, and flavonoids such as rutin, quercetin and kaempferol in the ethanolic extract of *Z. zanthoxyloide*.



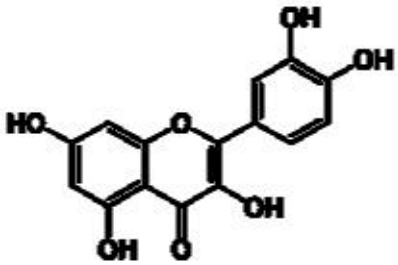
Chlorogenic acid



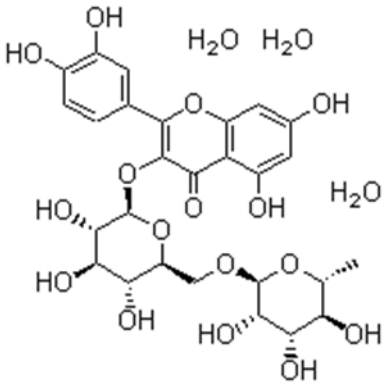
Caffeic acid

Predominant among the flavonoid and indeed among the estimated bioconstituents of *Z. zanthoxyloid* determined was quercetin (48.07±0.02). Quercetin is a flavonoid and, to be more specific, a flavonol. It is the aglycone form of a number of other flavonoid glycosides, such as rutin and quercitrin, found in citrus fruit, buckwheat and onions (Micheal et al., 2007). Quercetin forms the glycosides quercitrin and rutin together with rhamnose and rutinose, respectively. It is also found in many dietary supplements. Quercetin is found to be the most active of the flavonoids in studies, and many medicinal plants owe much of their activity to their high quercetin content. Quercetin has demonstrated significant anti-inflammatory activity because of direct inhibition of several initial processes of inflammation (Mark et al., 2009; Laura et al., 2008). For example, it inhibits both the manufacture and release of histamine and other allergic/inflammatory mediators. In addition, it exerts potent antioxidant activity and vitamin C-sparing action. Quercetin may have positive effects in combating or helping to prevent cancer, prostatitis, heart disease, cataracts, allergies/inflammations, and respiratory diseases such as bronchitis and asthma Nöthlings et al., (2007).

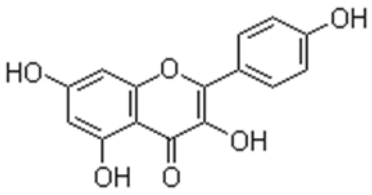
Flavonols, quercetin and kaempferol have a hydroxyl group at position 3 (Kim and Lee, 2004), which suggests a structurally important role of the 3-OH group of the chroman ring responsible for enhancement of antioxidant activity. In our research, *Z. zanthoxyloide* demonstrated high antioxidant activity which may be due to presence of high concentrations flavonoid contents of rutin, quercetin and kaempferol.



quercetin



Rutin



Kaempferol

Under normal physiological conditions there is equilibrium between reactive oxygen species (ROS) generated and antioxidants presents. The ROS generated is kept in check by antioxidant defense cascade consisting of enzymatic and non-enzymatic components. One specific ROS, hydrogen peroxide (H_2O_2), which is generated by mitochondrial respiration through a specialized enzyme, is a potent inducer of oxidative damage and mediators of ageing. Here, oxidative damage was stimulated by H_2O_2. DCFH-DA was first described as a probe to evaluate H_2O_2 (Keston and Brandt, 1965); subsequently, it has been suggested that increases in DCF fluorescence actually reflect the overall cellular oxidative stress (Wang and Joseph, 1999), since others forms of free radicals such as peroxyl radical, peroxinitrite, nitric oxide can oxidize DCFH. Our results indicated that H_2O_2 caused a significant increase in ROS production and that ethanolic extract of *Z. zanthoxyloide* was able to prevent significantly ROS production stimulated by H_2O_2 in a concentration-dependent manner. This effect may be attributed to the activities of quercetin, kaemferol and rutin found in plant extract. In fact, recently, our laboratory have reported that quercetin and its glycoside analog, rutin, prevents against methylmercury-induced ROS production in rat brain slices (Wagner et al., 2010).

In summary, ethanolic extracts from the stem *Z. zanthoxyloides* have *in vitro* antioxidant activity in different chemical and biological models. These effects can be attributed mainly to flavonoids and phenolic compounds present in the plant extracts. These results indicate that *Z. zanthoxyloides* has promising compounds to be tested as potential antioxidant drugs for the treatment of diseases resulting from oxidative stress and also for the use in different fields such as pharmaceuticals etc. But, further investigations are needed to understand the exact compounds eliciting these effects.

Correspondence to
Adekunle Adeniran Sanmi
P.O.Box 140066,
U.I., Ibadan,
Oyo State, Nigeria.
Cellular Phone: +234-8060967829
Email: kunleniran@yahoo.com

REFERENCES

1. Odebiyi, O.O., Sofowora, E.A., 1979. Antimicrobial alkaloids from a Nigerian chewing stick (*Fagara xanthoxyloides*). Planta Medica 36, 204–207.
2. Amvam Zollo, P.H., Biyiti, L., Tchoumbougnang, F., Menut, C., Lamaty, G., Bouchet, Ph.,1998. Aromatic plants of tropical Central Africa. Part XXXII. Chemical composition and antifungal activity of thirteen essential oils from aromatic plants of Cameroon. Flavour and Fragrance Journal 13, 107–114.
3. Awuah, R.T., 1989. Fungitoxic effects of extracts from some West African plants. Annales of Applied Biology 115, 451–453.
4. Irobi, O.N., Moo-Young, M., Anderson, W.A., Daramola, S.O., 1994. Antimicrobial activity of bark extracts of *Bridelia ferruginea* (Euphorbiaceae). Journal of Ethnopharmacology 43, 185–190.
5. Larhsini, M., Lazrek, H.B., Amarouch, H., Jana, M., 1996. Investigation of antifungal and analgesic activities of extracts from *Sium nodiflorum*. Journal of Ethnopharmacology 53, 105–110.
6. Rojas, A., Hernandez, L., Pereda-Miranda, R., Mata, R., 1992. Screening for antimicrobial activity of crude drug extracts and pure natural products from Mexican medicinal plants. Journal of Ethnopharmacology 35, 275– 283.
7. Ajanohoun J.E, Ahyi M.R.A, Ake-Assi L, et al. Traditional Pharmacopoeia 1993: Contribution to Ethnobotanical and Floristic Studies in Uganda. OAU/STRC. Lagos 1993.
8. Anokbonggo, W.W., Odoi-Adome, R. and Oluju, P.M. 1990: Traditional methods of diarrhoeal management in Uganda. Bulletin of the World Health Organisation; 68, 359-363.
9. Watt J.M. and Breyer-Brandiwijk M.G. 1962: Medicinal and Poisonous Plants of Southern and Eastern Africa. 2nd Edition. E and S Livingstone, London.
10. Sofowora E.A. Medicinal Plants and Traditional Medicine in Africa, 1993; 159-176; 179-189; 195-238. Spectrum Books Limited, Ibadan – Oweri – Kaduna – Lagos.
11. Taiwo O., Xu H. X., and Lee S.F. Antibacterial activities of extracts from Nigerian chewing sticks. Phytother. 1999; Res. 13 (8): 675 – 9.
12. Rotimi V.O., Laughon B.E., Bartlett J.G. et al Activities of Naigerian chewing stick extracts against Bacteroides gingivalis and Bacteroides melaninogenicus. Antibacterial Agents and Chemotherapy 1998; 32 (4): 598 – 600.
13. Ogwal-Okeng, J.W. The *in vitro* Study of the Anhelmintic Activity of Crude Extracts of two Ugandan Medicinal Plants: *Cissus adenocaulis* and *Fagara zanthoxyloides*. M.Sc. Thesis, Makerere University 1990.
14. El-Said, F., Fadulu, S.O., Kuye, J.O. and Sofowora, A. Native Cures in Nigeria; Part II: The antimicrobial properties of the buffered extracts of chewing sticks. *Lloydia* 1971; 34, 172-175.

15. Sofowora, E. A.,Isaac-Sodeye, W.A. and Ogunkoya, L.O. Isolation and characterisation of an anti-sickling agent from *Fagara zanthoxyloides* root. *Lloydia* 1975; 38, 169-171.
16. Oriowo M.A. Anti-inflammatory activity of piperonyl-4-acrylic isobutyl amide, an extractive from Zanthoxylum zanthoxyloides. *Planta Medica* 1982; 44 (1): 54-6.
17. Laghari, A.H, Memon, S., Nelofar, A., Khan, K.M., Yasmin, A., 2011. Determination of free phenolic acids and antioxidant activity of methanolic extracts obtained from fruits and leaves of *Chenopodium album*. Food Chem. 126, 1850–1855.
18. Kim, D.-O., & Lee, C. Y. (2004). Comprehensive study on vitamin C equivalent antioxidant capacity (VCEAC) of various polyphenolics in scavenging a free radical and its structural relationship. Critical Review Food Science and Nutrition, 44(4), 253–273.
19. U.S. Department of Agriculture (2003): USDA Database for the Flavonoid Content of Selected Foods. 1-75. Web site: http://www.nal.usda.gov/fnic/foodcomp.

A. E. Mitchell, Y. J. Hong, E. Koh, D. M. Barrett, D. E. Bryant, R. F. Denison and S. Kaffka (2007). "Ten-Year Comparison of the Influence of Organic and Conventional Crop Management Practices on the Content of Flavonoids in Tomatoes". *Journal of Agricultural and Food Chemistry* **55** (15): 6154–9. doi:10.1021/jf070344. PMID 17590007.

20. Nöthlings U., Suzanne P. M., Lynne R. W., Brian E. H., Laurence N. K.(2007). Flavonols and pancreatic cancer risk. *American Journal of Epidemiology* **166** (8): 924–931. doi:10.1093/aje/kwm172. PMID 17690219.
21. Laura K. Stewart, Jeff L. Soileau, David Ribnicky, Zhong Q. Wang, Ilya Raskin, Alexander Poulev, Martin Majewski, William T. Cefalu, and Thomas W. Gettys (2008). "Quercetin transiently increases energy expenditure but persistently decreases circulating markers of inflammation in C57BL/6J mice fed a high-fat diet". *Metabolism* **57**.
22. J. Mark Davis, E. Angela Murphy, Martin D. Carmichael, and Ben Davis (2009), Quercetin increases brain and muscle mitochondrial biogenesis and exercise tolerance", *Am J Physiol Regul Integr Comp Physiol* **296.**
23. Comoe, L., 1987. Apports de la me´decine traditionnelle africaine a` la chimiothe´rapie anticance´reuse: Activite´ antitumorale et diffe´renciante de la Fagaronine (*Fagara xanthoxyloides* Lam.). The`se de Doctorat d'Etat e`s-Sciences pharmaceutiques, Universite´ de Reims, pp. 26–52.
24. Kerharo, J., Adam, J.G., 1974. La pharmacope´e se´ne´galaise traditionnelle: Plantes me´dicinales et toxiques. Edition Vigot-fre`res, Paris, p. 712.
25. Noumi, E., 1984. Les plantes a` e´pices, a` condiments et a` aromates du Cameroun. The`se de Doctorat en Sciences biologiques, Universite´ de Yaounde´, p. 117.
26. Sofowora, E.A., Isaac-Sodeye, W.A., Ogunkoya, L.O., 1975. Isolation and characterisation of an antisickling agent from *Fagara zanthoxyloides* root. Lloydia 38 (2), 169–171.
27. Wagner C, Vargas AP, Roos DH, Morel AF, Farina M, Nogueira CW, Aschner M, Rocha JB (2010) Comparative study of quercetin and its two glycoside derivatives quercitrin and rutin against methylmercury (MeHg)-induced ROS production in rat brain slices. Arch Toxicol 84:89-97.
28. Wang H, Joseph JA. (1999). Quantifying cellular oxidative stress by a dichlorofluorescein assay using microplate reader. Free Radical Biology and Medicine 27, 612-616.
29. Roos DH, Puntel RL, Farina M, Aschner M, Bohrer D, Rocha JBT, de Vargas Barbosa NB (2011) Modulation of methylmercury uptake by methionine: Prevention of mitochondrial dysfunction in rat liver slices by a mimicry mechanism. Toxicol Appl Pharm 252:28-35.
30. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193:265-275.
31. Bravo, L., 1998. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutr. Rev. 56, 317–333.
32. Ketson AS, Brandt R. (1965). The fluorometric analysis of ultra micro quantities of hydrogen peroxide. Analytical Biochemistry 11, 1-5.

2/1/2012