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IN VITRO ANTIOXIDANTS AND TOTAL PHENOLIC CONTENTS OF THREE MEDICINAL PLANTS COMMONLY USED IN NORTH - EAST NIGERIA

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Abstract: Crude aqueous and ethanolic extracts of three plants *Parkia bigbolosa, Kaya senegalensis,* and *Leptadenia hastata* were analysed for their antioxidant activities and total phenolic content. The antioxidant activities were measured as radical scavenging activity by 2, 2-Diphenyl-2-picrylhydrazyl DPPH, inhibition of lipid peroxidation by Ferric-thiocyanate method and metal chelating activity by ferrous ion chelating method and Folin-ciocalteau method was used to measure the total phenolic content expressed as mg/g GAE. The highest concentration of polyphenols was observed in extracts of *Kaya senegalensis* followed by *Parkia bigbolosa* and then *Leptadenia hastata*. For the radical scavenging assay by DPPH, both *Kaya senegalensis* and *Parkia bigbolosa* demonstrated very good ability of scavenging with SC_{50} value ranging between 0.025-0.027mg/ml when compared with 0.017mg/ml of ascorbic acid with no statistically significant difference ($p>0.05$) in terms of activity, but *Leptadenia hastata* showed a significantly weak scavenging activity with SC_{50} of 1.959mg/ml and 0.524mg/ml compared to the reference standard. *Kaya senegalensis* aqueous extract appeared to have better ability to inhibit lipid peroxidation having LP_{50} values of 0.257mg/ml with over five fold potency than that of the standard ($p<0.05$), *leptadenia hastata* aqueous and *kaya senegalensis* ethanolic extracts exhibited very poor inhibition activity ($p>0.05$) when compared with the reference standard Vitamin E which has LP_{50} of 1.440mg/ml. However, all of the extracts revealed a weak ability to chelating ferrous ion with MC_{50} values range of (3.837 to 13.838mg/ml) when compared with the standard EDTA that has MC_{50} of 0.450mg/ml. This indicates that these plants are good source of antioxidant for free radical scavenging and some having the ability to inhibit lipid peroxidation and thus, can be used in preventing, treating or managing oxidative stress related diseases.

Keywords: Anti Oxidants, Total Phenolic, Radical Scavenging, and Lipid Peroxidation.

INTRODUCTION

Generation of free radicals is an integral part of aerobic metabolism. Many biological processes as well as external factors contribute to generation of free radicals in the body, such as simple oxidation of macro nutrients, ultraviolet light, pesticides, cadmium, ionizing radiation, alcohol, cigarette smoke and pollution [3]. These radicals include hydroxyl radical ($^{\cdot}OH$), singlet oxygen, hydrogen peroxide (H_2O_2), ferric ion (Fe^{3+}), nitric oxide (NO) and superoxide anions ($O^{2\cdot}$). The body has the capacity of checkmating the oxidative action of these radicals by simply using the antioxidant components inherent. However, when these free radicals are produced in excess overwhelming the antioxidant systems of the body, leads to oxidative stress that can cause tissue injury. Free radicals have been implicated of causing ailments such as cancer, inflammation, diabetes, liver cirrhosis, cardio vascular disease, Alzheimer's, Aging and acquired immunodeficiency syndrome[22]. Some of the mechanism through which over production of free radicals can lead to oxidative damage include lipid peroxidation that causes breakdown or hardening of lipids on cell membranes and cell walls, as well as affecting other biological molecules including RNA, DNA and protein enzymes [22]. Increasing antioxidant intake can prevent diseases and lower the health problems. Research is increasingly showing that

antioxidant rich foods, and herbs reap health benefits. Fruits, vegetables and medicinal herbs are the richest sources of antioxidant compounds [24]. *Parkia biglobosa* is a dicotyledonous angiosperm belonging to the family Fabaceae – Mimosoideae. It is categorized under spermatophytes, vascular plants [26]. It is a deciduous perennial that grows to between 7 and 20 metres high. Indigenous healers in Africa use different parts of the locust bean tree for health benefits. In a survey conducted on healers in Togo, *Parkia biglobosa* was one of the highest cited plants used for treating hypertension [13]. In South-Western Nigeria the tree was found to have wound-healing properties influencing the proliferation of dermal fibroblasts significantly [1]. It is popularly called'*Rumo*' in Kanuri and '*daurawa'* in Hausa by the local people in North - East Nigeria, It is also used in treating snake bites in Nigeria due to its anti snake venom activity [10]. *Leptadenia hastata* (Pers.) Decne. (Asclepiadaceae) is edible non-domesticated vegetable and it is collected in the wild throughout Africa. *L. hastata* is a voluble herb with creeping latex stems, glabescent leaves, glomerulus and racemus flowers as well as follicle fruits. It is typically grown in tropical dry lands in sandy soil. Wild foods like *L. hastata* provide food security during seasonal changes and are used medicinally in many areas [25]. It is popularly called '*njara'* in Kanuri and '*yadiya'* in Hausa, the local people in North-East Nigeria use its root infusion to treat jaundice related ailments such as hepatitis, and in other parts of Nigeria, local healers use the plant for hypertension, catarrh and skin diseases[6]. Phenolic glycosides, tannins, flavonoids, proanthocyanidins, alkaloids and saponins were detected as part of it secondary metabolites of the leave extract [4]. *Khaya senegalensis* A. Juss (Meliaceae) or African mahogany in English is a deciduous evergreen tree that grows up to 15-30 m high, and up to 1 m in diameter. It is called 'Kagum' in Kanuri and 'Madachi' in Hausa of Northern Nigeria. It has so many medicinal values as it is believed that any bitter plant is a medicine. The very bitter bark has a considerable reputation in its natural range as a fever remedy. The bark is also used as a vermifuge, taenicide, depurative and for treating syphilis. Bark extract is used for treating jaundice, dermatoses, scorpion bite, allergies, infection of the gums, hookworm, bleeding wounds (disinfectant), and as a laxative. Seeds and leaves are used for treating fever, headache; roots against sterility, for the treatment of mental illness, syphilis, leprosy and as an aphrodisiac [18]. *Kaya senegalensis* contains scopoletin, scoparone, limonoid, bitter principle, tannins, saponins and sterol [8]. Since the above - mentioned plants have very wide medicinal uses, this study is aimed at determining the antioxidant capacity of the plants to further buttress some of the claims.

MATERIALS AND METHODS

Chemicals and Reagents

Folin-ciocalteau, Ascorbic acid, NH_4SCN, Iron II chloride were purchased from Loba Chemie India, 2,2-Diphenyl-2-picrylhydrazyl, Gallic acid monohydrate and alpha tocopherol were purchased from Sigma Aldrich. Dimethyl sulphoxide (DMSO) was purchased from Guangdong Guanghura China while 3-(2-pyridyl)-5,6-bis (4-sulfophenyl)-1,2,4-triazine disodium salt hydrate (ferrozine) was from Tokyo Chemical Industry Japan. All other reagents were of analytical grade.

Plant Collection and Preparation

The plants were collected at Konduga and Damboa towns of Borno state, Nigeria and identified by a plant taxonomist from the Department of Botany University of Maiduguri and voucher specimens were kept at the herbarium of Department of Biochemistry University of Maiduguri. The plants were washed and shade dried before milling into powdered form. Two

solvents were used for the extraction Water and ethanol. For the aqueous extraction 30g of the powdered sample were soaked in 300ml of distilled de-ionized water for 24 hours and then filtered through a cotton and finally through Whatman no 1 filter paper by the aid of suction pump. Another 20g of the powdered samples were extracted by soxhlet extractor for 2 hours with 200ml of 95% ethanol as the solvent of extraction. All the extracts were evaporated using rotary evaporator (Bucchii) to dryness. The semi dry extracts were kept at -20°C until used.

Radical scavenging activity

Free radical scavenging activity of the extracts was determined by a modified DPPH method as described by [28] with little modification. Briefly, 100µl of the sample at different concentration range of 0.0001, 0.001, 0.01, 0.1 1.0mg/ml and 100 µl of DPPH solution (0.1mg/ml in ethanol) were placed into each well of a 96-well micro plate. The plate was incubated at room temperature in the dark for 30min and absorbance was measured using micro plate reader (Glomax Promega multi detection system) at 560nm. Vitamin C was used as standard. Each of this was performed in quadruplet. The percentages of the DPPH radical scavenging activity was calculated as follows: Percentages of DPPH free radical scavenging activity (%) = $\{(A_o-A_1)/A_o\}$ x 100. Where A_o is the absorbance of the control and A_1 is the absorbance of the sample. The concentrations providing 50% scavenging (SC_{50}) was calculated from the graph plotted between the free radical scavenging percentages and the sample concentrations.

Inhibition of Lipid Peroxidation Activity

The lipid peroxidation activity of the extracts was assayed by the modified Ferric-thiocyanate method as described [11]. An amount of 50 µl of the samples at concentration range of 0.0001, 0.001, 0.01, 0.1 1.0mg/ml was added to 50 µl of linoleic acid in 50% DMSO, and then 50 µl, both of (5mM) NH_4SCN and (2mM) $FeCl_2$ were added and allowed to stay for 60min at room temperature, absorbance was measured at 490nm by a micro plate reader. Vitamin E was used as the standard. The experiment was done in quadruplicate. Percentage of lipid peroxidation inhibition of linoleic acid was calculated by the formulae: Percentages of lipid peroxidation inhibition. (%) = $\{(A_o-A_1)/A_o\}$ x 100. Where A_o is the absorbance of the control and A_1 is the absorbance of the sample. The concentrations providing 50% inhibition of lipid peroxidation (LC_{50}) were calculated from the graph plotted between the inhibition of lipid peroxidation percentages and the sample concentrations.

Inhibition of Metal Ion Chelating Activity

The metal ion chelating activity of the extracts was assayed by the modified ferrous ion chelating method [11]. In Brief 100 µl of the samples or standard EDTA at concentration range of 0.0001, 0.001, 0.01, 0.1 1.0mg/ml, 50 µl of 2mM $FeCl_2$ in distilled water and 50 µl of 5mM ferrozine were added to a 96 well micro plate, Then, the mixture was incubated at room temperature for 15min. The absorbance was measured at 570nm by a micro plate reader. All experiments were conducted in quadruplet. The percentages of ferrozine–Fe^{2+} complex formation inhibition were calculated by the following equation: (%) = $\{(A_o-A_1)/A_o\}$ x 100. Where A_o is the absorbance of the control and A_1 is the absorbance of the sample. The concentrations providing 50 % metal chelating effect (MC_{50}) were calculated from the graph plotted between the percentages of metal chelating and the sample concentrations.

Determination of Total Phenolic Content (TPC)

Total phenolic content of the plant extracts was determined by Folin-Ciocalteau reagent according to the method of Antolovich *et al.* [2], with minor modifications as reported by [15]. In Brief, 20 µL of extracts were mixed with 100 µl of 1:10 Folin-Ciocalteu reagent followed by the

addition of Na_2CO_3 (80 µL, 7.5%). The assay was carried out in micro- plate. After incubation at room temperature for 2 hours in dark, the absorbance at 600 nm was recorded. Gallic acid was used as the standard reference. TPC (total phenolic content) was expressed as mg Gallic acid equivalents per gram of dried extract (mg GAE g-1) (Gallic acid equivalent)

RESULTS AND DISCUSSION

An antioxidant property of plants is an indispensable companion in both health and disease Conditions. This is so especially when the body's internal antioxidant system is overwhelmed so as to prevent oxidant related degenerative diseases. Many diseases such as cancer, cardiovascular diseases, Muscular degeneration atherosclerosis are attributed to generation of oxidant compounds in the body such as free radicals [22]. Presently there is surge in research for plant extracts with antioxidant capacity to improve knowledge about antioxidant activity [17]. In this present study the antioxidant activity and total Phenolic content of three Nigerian medicinal plants were analysed. Because antioxidant compound in plants have different polarities two solvents, water and ethanol were used to extract the plant materials, and it is often observed that the antioxidant activity and the yield of plant extracts depends on the solvent us.

Table 1: Plants names, Parts used and Percentage yield of Extracts

Plants	Local name	Part used	Abbreviation	% yield (w/w)
Leptadaenia hastata (Pers.) Decne. (Asclepiadaceae)	Njara (Kanuri) Yadiya (Hausa)	Root	LAqE, LEtE	6.2 11.6
Khaya senegalensis A. Juss (Meliaceae)	Kagum (Kanuri) Madaci (Hausa)	Stem bark	KAqE, KEtE	6.1 6.4
Parkia biglobosa Fabaceace – pea	Runo (Kanuri) Dorawa (Hausa)	Stem bark	PAqE, PEtE	10.5 3.5

LAqE; *Leptadaenia hastata aqueous extract,* LEtE; *Leptadaenia hastate ethanolic extract,* KAqE; *Khaya senegalensis aqueous extract,* KEtE; *Khaya senegalensis ethanolic extract,* PAqE; *Parkia biglobosa aqueous extract,* PEtE; *Parkia biglobosa ethanolic extract.*

Free Radical Scavenging Activity

Radical scavenging activities of the plants extracts were measured using DPPH method. All the extracts of *Khaya senegalensis* and *Parkia biglobosa* showed a percentage scavenging activity between 61% and 66% at the final concentration of 0.5mg /ml when compared with the standard vitamin C at the same final concentration which showed scavenging activity of 98%. This was further buttressed by their SC_{50} (the concentration at 50% scavenging activity of DPPH

is carried out) which showed no statistically significant difference with that of the reference standard vitamin C (Table 2). The lower the SC_{50}, the higher the scavenging activity of the component tested [14]. However, *Laptadenia hastata* extracts showed low percentage inhibition of 1.2% to 3.5% at 0.5mg/ml for LEtE and LAqE respectively as well as significantly high SC_{50} which portray it as having poor scavenging activity compare to the other two plants. In a similar work on ethanolic leave extract of *L. hastata* a strong but relatively poor radical scavenging activity when compared to reference standard vitamin C was revealed by Abubakar *et al.,* [29]

Inhibition of Lipid Peroxidation Activity

Inhibition of lipid peroxidation is one important activity of antioxidants. Lipid peroxidation is the oxidative deterioration of lipid containing a number of carbon-carbon double bonds [20] which are components of membranes. Membrane integrity is paramount for normal metabolic activity and survival of both cell and sub cellular organelles, presence of polyunsaturated fatty acid in the membrane bi-layer confer a unique characteristic of membrane fluidity required for normal function. Lipid peroxidation tends to disrupt this characteristic, causing highly detrimental consequence to both functionality and survival of cell [19]. KAqE, PAqE and PEtE showed a very unique capability of inhibiting lipid peroxidation in vitro with significantly lower LP_{50} when compared with the reference standard vitamin E (Table 2) a lipid soluble antioxidant which is a known inhibitor of oxidative damage due to lipid peroxidation [16] however there was no activity detected with the ethanolic extracts of *Laptednia hastata* and weak activity of it aqueous extract is also noted. In fact, relatively poor activity of all the ethanolic extracts is observed compared to aqueous extracts; this finding may be attributed to the solubility of active plant ingredients in water than in alcohol.

Table 2: Antioxidant activities of the three plants

S/N	Test samples	Radical scavenging activity		Inhibition of lipid peroxidation		Inhibition of metal ion chelating	
		SC_{50}(mg/ml)	Folds of Vit.C	LP_{50}(mg/ml)	Folds of Vit.E	MC_{50}(mg/ml)	Folds of EDTA
1.	LAqE,	1.959±0.032*	0.009	2.996±0.226*	0.480	NA	NA
2.	LEtE	0.524±0.031*	0.032	NA	NA	13.838±2.830*	0.033
3.	KAqE,	0.025±0.001	0.680	0.257±0.047**	5.603	6.688±1.681*	0.067
4.	KEtE	0.026±0.001	0.654	2.086±0.303*	0.690	12.353±2.746*	0.036
5.	PAqE	0.026±0.001	0.654	0.792±0.490	1.818	3.691±0.917*	0.122
6	PEtE	0.027±0.001	0.630	1.287±0.308	1.119	NA	NA
7.	Vitamin C	0.017±0.001	1.000	NT	NT	NT	NT
8.	Vitamin E	NT		1.440±0.300	1.000	NT	NT
9.	EDTA	NT		NT	NT	0.450±0.02	1.000

(NA= no activity; NT= not tested): Values are means ± Standard deviation of four replications
*P<0.05 less activity when compared with reference standard
**P<0.05 higher activity when compared with the reference standard.

Inhibition of Metal Ion Chelating Activity

Another very important activity of a good antioxidant compound is its ability to inhibit metal ion chelating in the system. Transition metal ions, especially iron can stimulate lipid per oxidation by Fenton reaction ($H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} OH^- + OH$) and can also accelerate lipid

peroxidation by decomposing lipid hydro peroxides into peroxyl and alkoxyl radicals that can perpetuate the chain reaction [21]. All the plant extracts showed significantly low inhibition of ferric ion chelating activity compared to the reference standard EDTA except aqueous extract of *Parkia bigbolosa* that exhibited apparent but not significant ability to chelating ion, an activity that is very essential in reducing the level of transition metals available that can catalyse lipid peroxidation [21]. The relatively mild iron (ii) chelating activity of the plant extract is of great significance, because it has been proposed that the transition metal ions contribute to the oxidative damage in neurodegenerative disorders, like Alzheimer's and Parkinson's diseases and one of the lines of treatments currently under investigation is selective low affinity binding of transition metals [5, 27].

Total Phenolic Content (TPC)

Phenolics are compounds possessing one or more aromatic rings with one or more hydroxyl groups [12]. Polyphenols are very important component of plants they are reported to have several biological effects such as antioxidant activity, decrease in oxidative stress related diseases such as cancer, and diabetes [9, 22], preventing neuron degenerative disease such as Parkinson and Alzheimer disease [7] as cited in Bello *et al.,* [4]. The total phenolic content of extracts is presented in **Figure 1.** *Khaya senegalensis* bark extracts showed the highest total phenolic content followed by *Parkia bigbolosa* and then *Leptadenia hastata* extracts, this could likely explain why the scavenging activity of *L. Hastata* is low. Even within the same plant the phenolic content differs depending on the solvent used in extracting the plant. This finding is corroborated by the findings of Bello *et al;* [4] that reported not too high concentration of polyphenol in acetone, methanol and aqueous leaf extracts of *Leptadenia hastata* with solvent - dependent variability in concentration.

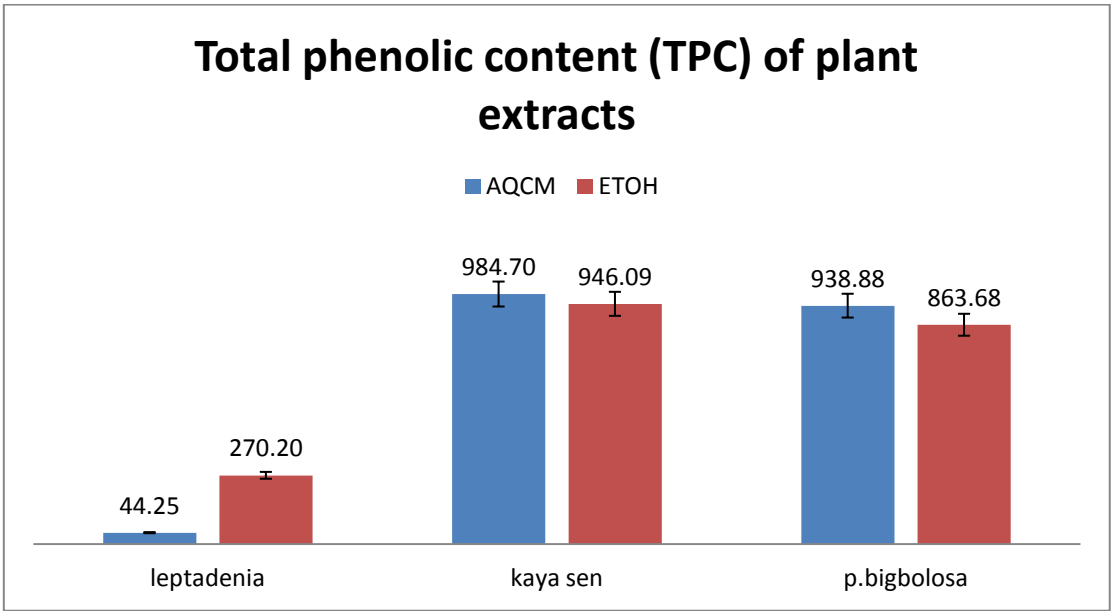

Figure 1: Total Phenolic Contents of the three plants extracts. Values are expressed as mg/g GAE. AQCM: aqueous cold maceration, ETOH: ethanolic extract.

Table 3: Pearson Correlation between total phenolic content (TPC) and the various IC_{50} of the antioxidant activities assayed

	TPC	SC50	LP50	MC50
TPC	1	-0.896**	-0.771**	-0.598*
SC50	-0.896	1	0.390	0.630
LP50	-0.771	0.390	1	-0.122
MC50	-0.598	0.630	-0.122	1

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Correlation between phenolic contents of the plant extracts and the IC_{50} values of the different antioxidant activity studied showed a strong negative relationship invariably indicating that there is strong antioxidant property due to the phenolic contents of the plant. The relationship is negative because the lower the IC_{50} the higher the antioxidant capacity of the plant [14].

CONCLUSION

The aqueous and the ethanolic extracts of the plant have exhibited varying degrees of antioxidant activity through different mechanisms ranging from radical scavenging activity to inhibition of lipid peroxidation and inhibition of metal ion chelating. These are all mechanisms through which free radicals can exert their oxidative stress on the body to cause morbidity and in most cases leading to mortality if not properly handled. This is further corroborated by the existence of high phenolic content in these plants, some of which (polyphenols) have proof to have immense medicinal benefit such as the flavonoids. As some of these ailments treated with these plants are directly or indirectly related to oxidative stress, these findings justify the use of these plants in the traditional medicine and could serve as a potential source modern drug.

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Conflict of Interest

The authors declare no conflict of interest.

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