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In vitro studies to assess the antioxidative, radical scavenging and arginase inhibitory potentials of extracts from *Artocarpus altilis*, *Ficus exasperate* and *Kigelia africana*

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PEER REVIEW

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Comments

This paper that describes the antioxidant, anti-lipid peroxidation and anti-arginase activities of selected Nigerian plants is indeed interesting. The antioxidant activity and the inhibitory potential of arginase activity exerted by *A. altilis* and Ficus exasperata extracts, although at relatively high doses, are promising.
Details on Page S498

ABSTRACT

Objective: To justify the use of *Artocarpus altilis* (*A. altilis*), *Ficus exasperata* (*F. exasperata*) and *Kigelia africana* (*K. africana*) in ethnomedicine for the treatment of several ailments and to evaluate the *in vitro* antioxidant, radical scavenging and arginase inhibitory potentials of these herbs and compared with catechin (Standard).

Methods: Antioxidant activities were determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH), nitric oxide, hydrogen peroxide (H_2O_2) and hydroxyl (OH) radicals scavenging methods. The flavonoids and phenolics content, inhibition of arginase activity, Fe^{2+}/ascorbate-induced lipid peroxidation (LPO) and reducing power were also determined.

Results: The *A. altilis*, *F. exasperata* and *K. africana* showed dose-dependent and significant scavenging of DPPH, H_2O_2 and OH radicals *in vitro* relative to catechin. The *A. altilis* and *F. exasperata* effectively scavenged DPPH radical with IC_{50} of 593 and 635 μg/mL and, OH radical with IC_{50} of 487 and 514 μg/mL, respectively. The DPPH and OH radicals scavenging activities followed the order *A. altilis*>*F. exasperata*>*K. africana*. In addition, *A. altilis* and *F. exasperata* significantly ($P<0.05$) inhibited LPO in a dose-dependent manner. The *A. altilis* extract had the most potent inhibitory activity against LPO with 79% relative to catechin (28%) at 750 μg/mL. The reducing power followed the order: *A. altilis*>Catechin>*F. exasperata*>*K. africana* at 1 000 μg/mL. The *A. altilis* at 500 and 750 μg/mL significantly ($P<0.05$) inhibited arginase activity by 63% and 67%, respectively. The flavonoids contents were found to be highest in *A. altilis*.

Conclusions: Extracts of *A. altilis* and *F. exasperata* are potent antioxidative agents with strong radical scavenging activity and inhibition of lipid peroxidation.

KEYWORDS
Antioxidant, lipid peroxidation, Arginase, Free radicals

1. Introduction

A free radical is a molecule with one or more unpaired electrons in the outer orbital[1]. Many of the free radicals, in the form of reactive oxygen species (ROS) and reactive nitrogen species are an integral part of normal physiology. These ROS are generated in the organelles such as mitochondria and microsomes under normal physiological conditions[2]. They can also be produced when a living system is exposed to radiation, toxic chemicals, cigarette smoking, alcohol intake and consumption of oxidized fats[3-5]. Overproduction of ROS can result in oxidative damage to various biomolecules including lipids, proteins, DNA and cell membranes[6,7]. Formations of reactive species have been linked to the development of diseases such as coronary heart diseases, cancer, diabetes, hypertension and neurodegenerative disorders[8-10]. It is known that antioxidants or endogenous compounds capable

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of scavenging free radicals possess great potential in ameliorating ROS-induced diseases[11,12]. The endogenous defense enzymes, *viz.*; catalase, superoxide dismutase, glutathione and associated enzymes may become depleted thereby limiting their functions during ROS overload[13]. Also, common synthetic antioxidants such as butylated hydoxyanisole, butylated hydoxytoluene, propylgallate used as supplements in foods are limited by shelves lifespan and adverse side effects[14]. Therefore, the search for natural antioxidants having little or no side effects to replace synthetic antioxidants for use in foods or as medicine is still an active area of research.

Breadfruit (*Arocarpus altilis*) (*A. altilis*) is a member of the Moraceae. The fruit is an excellent source of fiber, calcium, copper, iron, magnesium, potassium, thiamine, niacin, carbohydrates, vitamins and very low in fat[15]. Fruits can be eaten at all stages of growth as it can be baked, boiled, roasted, fried or steamed[16]. In Africa traditional herbal homes, the leaves of the plant are used for the treatment of liver disorders, hypertension and diabetes[17]. However, little information is available to give scientific support to the traditional use of breadfruit.

Ficus exasperata (*F. exasperata*), commonly known as "sand paper tree", is a deciduous shrub. Phytochemical and toxicological analyses of the leaf and stem extracts of the plant revealed the presence of flavonoids, tannis, saponins, alkaloids and cyanogenic glycosides[18]. In ethnomedicine, the leaf extract from the plant has been used to treat patients with hypertension[19], heamostative opthalmia, coughs and haemorrhoid[20]. However, there is dearth of information on the scientific basis of its use in traditional medicine.

Kigelia africana (*K. africana*) is a member of Bignoniaceae family. In traditional herbal homes, the bark is used for the treatment of rheumatism, dysentery and veneral diseases. In addition, the bark, leaves and roots of the plant are used for treatment against ringworm, tapeworm, haemorrhagic conditions, malaria, diabetes, hypertension and pneumonia[21]. Furthermore, studies showed that various parts of the plant elicited antimicrobial, anti-malarial, anti-inflammatory, anticancer and hepatoprotective effects[22-25]. From the aforementioned, this study was designed to evaluate and compare the antioxidant and free radical scavenging activities of these selected medicinal plants using panels of *in vitro* assays.

2. Materials and methods

2.1. Collections and extraction of plant materials

The plant parts; stem bark of *A. altilis*, leaves of *F. exasperata* and fruits of *K. africana* were collected in Ibadan (Oyo State) and Iwo (Osun State) of Nigeria. The authentication was done in the Botanical Garden, University of Ibadan. The stem bark and leaves were air-dried and crushed into fine powder. The powdered samples were de-fatted with *n*-hexane and, extracted with methanol using soxhlet extractor. The methanolic extracts were concentrated in vacuum at 40 °C with rotary evaporator and water bath to dryness. The fruits of *K. africana* were sliced and soaked in water for 48 h. The water extract was concentrated in vacuum at 40 °C with rotary evaporator and water bath to dryness.

2.2. Chemicals

Ethylenediamine tetra-acetic acid (EDTA), 2,2-diphenyl-1-picryhydrazyl (DPPH), 2- deoxyribose, Folin-Ciocalteu reagent, catechin, 2-thiobarbituric acid (TBA), Trichloroacetic acid (TCA) and ascorbic acid were purchased from Sigma Chemical Co., Saint Louis, MO, USA. Ferrous ammonium sulphate, hydrochloric acid, naphthylenediamine dihydrochloride, phosphoric acid and sodium hydroxide were procured from British Drug House (BDH) Chemical Ltd., Poole, UK. Other chemicals were of analytical grade and purest quality available.

2.3.1. DPPH- radical scavenging activity

The radical scavenging activity of plant extracts were measured as described by Mensor *et al*[26]. The stable 2,2-diphenyl-1-picryldydrazyl (DPPH) radical was used for the determination of free radical scavenging activities of the extracts. A portion (1 mL) each of the different concentrations (10-1 000 ug/mL) of the extracts or standard (Catechin) was added to 1 mL of 1 mmol/L DPPH in methanol. The mixtures were vortexed and incubated in a dark chamber for 30 min after which the absorbance was measured at 517 nm against a DPPH control containing only 1 mL of methanol in place of the extract. All calculations were carried out in triplicates. The inhibition of DPPH was calculated as a percentage using the expression:

$$\% I = \frac{A_{control} - A_{sample} \times 100}{A_{control}}$$

Where % I is the percentage inhibition of the DPPH radical; $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of the test compound.

2.3.2. Reducing power

This was determined according the method of Oyaizu[27]. The extract or standard (100 μg/mL) was mixed with phosphate buffer (pH 6.6) and potassium ferricyanide. The mixture was incubated at 50 °C for 20 min and trichloroacetic acid (10%, 2.5 mL) was added to the mixture. A portion of the resulting mixture was mixed with $FeCl_3$ (0.1%, 0.5 mL) and the absorbance was measured at 700 nm in a spectrophotometer. Higher absorbance of the reaction mixture indicated reductive potential of the extract.

2.3.3. Determination of total phenolic content

The total phenolic content of the extract was determined using the method of Singleton *et al.*[28] with slight modifications. Folin-C reagent (1 mL) was added to 1mL of extract or standard. After 3 min, 1 mL of 15% Na_2CO_3 was added and the solution was made up to 5 mL with distilled water. The reaction mixture was kept in the dark for 90 min with intermittent shaking or placed in a water bath

at 40 °C for 20 min. The absorbance was measured by a spectrophotometer at 760 nm. All experiments were done in triplicate. Catechin was used as standard.

2.3.4. Determination of total flavonoids

The total flavonoids content was determined by the method described by Meda *et al*[29] with slight modification. The extract (10-1 000 μg) in 1 mL of distilled water was added to 75 μL of 5% $NaNO_2$. After 5 min, 150 μL of 10% $AlCl_3.6H_2O$ was added, followed by 500 μL of 1 mol/L NaOH and 275 μL of distilled water. The solution was properly mixed and the colour intensity of the mixture read at 510 nm after 15 min. Catechin was used as the standard. All experiments were done in triplicate.

2.3.5. Scavenging of hydrogen peroxide

The ability of the extracts to scavenge hydrogen peroxide was determined according to methods described by Nabavi *et al*[30,31]. A solution of hydrogen peroxide (40 mmol/L) was prepared in phosphate buffer (pH 7.4). The concentration of hydrogen peroxide was determined at 230 nm using a spectrophotometer. Extracts (10-1 000 μg/mL) in distilled water were added to hydrogen peroxide solution (0.6 mL, 40 mmol/L). The absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging by the extracts and catechin (standard) was calculated as follows:

$$\%\ \text{Scavenged}\ [H_2O_2] = \frac{Ao - A1}{Ao} \times 100$$

where Ao is the absorbance of the control and A1 is the absorbance in the presence of the extracts or standard.

2.3.6. Nitric oxide radical scavenging activity

The scavenging effect of extract on nitric oxide radical was measured according to the method of Ebrahimzadeh *et al*[32]. Sodium nitroprusside (5 mmol/L, 1 mL) in phosphate buffered saline was mixed with different concentration of extracts (10-1 000 μg/mL) and distilled water. This was incubated at room temperature for 150 min after which 0.5 mL of Griess reagent was added. The absorbance of the pink chromophore formed was read at 546 nm. Catechin was used as standard. All experiments were done in triplicate. The percentage inhibition was calculated as:

$$\%\ \text{Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

2.3.7. Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of extracts was carried out as described by Halliwell *et al*[33]. The assay was performed by adding 0.1 mL of EDTA, 0.01mL of $FeCl_3$, 0.1 mL of H_2O_2, 0.36 mL of deoxyribose, 1.0 mL of plant extract (10-1 000 ug/mL), 0.33 mL of phosphate buffer (50 mmol/L, pH 7.4) and 0.1 mL of ascorbic acid in sequence. The mixture was then incubated at 37 °C for 1 h. About 1.0 mL of 10% TCA and 1.0 mL of 0.5% TBA were added to develop the pink chromogen and, was measured at 532 nm. The assay was conducted in triplicates and catechin served as standard.

$$\text{Hydroxyl radical scavenging activity}\ (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where $A_{control}$ is absorbance of control and A_{sample} is absorbance of extract or catechin.

2.3.8. Determination of Fe^{2+}/ascorbate-induced lipid peroxidation

Lipid peroxidation was carried out by the method of Ohkawa *et al*[34]. The reaction mixtures contained 0.2 mL of rat liver homogenate in varying concentration of 30 mmol/L tris buffer, 0.38 mL of 0.16 mmol/L ferrous ammonium sulphate, 0.06 mL ascorbic acid and different concentration of the extracts (10-1 000 μg) and, were incubated for 1 h at 37 °C. The resulting thiobarbituric acid reacting substance formed was measured as followed; briefly, an aliquot (0.4 mL) of the reaction mixture was mixed with 1.6 mL of 0.15 mol/L Tris-KCl buffer and 0.5 mL of 30% TCA (to stop the reaction), and placed in a water bath for 45 min at 80 °C. After which it was cooled in ice and centrifuged at room temperature for 15 min at 3 000 r/min to remove precipitates. The absorbance of the clear pink coloured supernatant was measured against blank at 532 nm. Catechin was used as standard and experiment done in triplicate.

2.3.9. Determination of cardiac arginase activity

Arginase (l-Arginine amido hydrolase, EC. 3.5.3.1) assay was based on the method of Campbell[35]. Assay reaction mixture contained in 1 mL: 50 μmol $NaHCO_3$ buffer (pH 9.5), 20 μmol arginine, 0.5 μmol $MnCl_2$, 0.2 mL crude extracts (10-1 000 ug) and 0.79 mL of 10% rat heart homogenate. The reaction mixture was incubated at 37 °C for 1 h. The reaction was stopped with 1 mL of 0.5 mol/L $HClO_4$ and centrifuged to obtain clear supernatant and the urea formed was determined using urea kits.

2.4. Statistical analysis

Experimental results were expressed as mean±standard deviation (SD). All measurements were replicated three times. The results were analyzed using One-way analysis of variance (ANOVA). The level of significance used was $P<0.05$.

3. Results

3.1. Total phenolic and flavonoids content of the extracts

The methanolic extract of *A. altilis* and *F. exasperata* showed considerable higher phenol contents than catechin (standard) while the phenol contents of aqueous extract of *K. africana* is lesser than catechin at the concentration of 10 μg/mL, the absorbance of extracts of *A. altilis*, *F. exasperata*, *K. africana* and catechin were 0.041, 0.059, 0.048 and 0.034

respectively; while at 1 000 μg/mL, the absorbance of the extracts of *A. altilis*, *F. exasperata*, *K. africana* and catechin were 0.797, 0.847, 0.523 and 0.709. The total phenol contents of the extracts and catechin showed dose-dependent increase (Figure 1). The flavonoid contents of the extracts showed a dose dependent (Figure 2). At 1 000 μg/mL, the flavonoid contents followed the order *A. altilis*> *F. exasperata*> Catechin> *K. africana*.

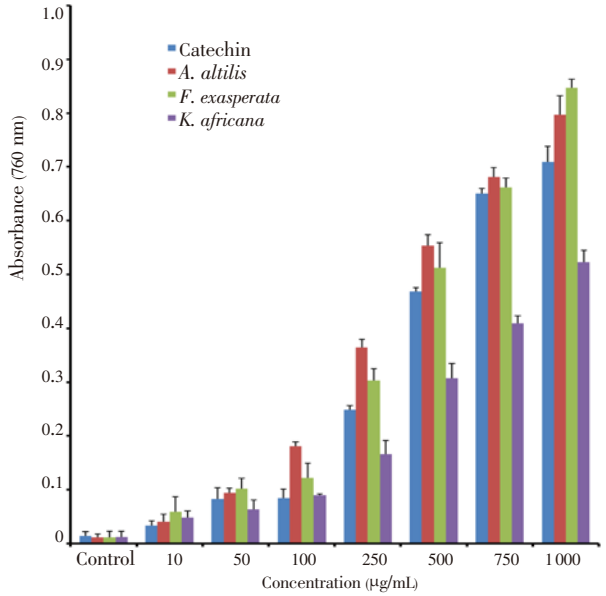

Figure 1. The total phenolic contents in the methanolic extracts of *A. altilis*) and *F. exasperate* as well as aqueous extract of *K. africana* and the standard (Catechin).

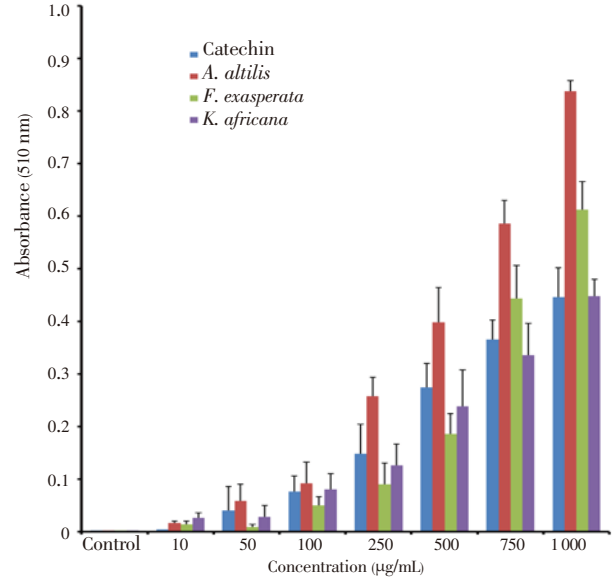

Figure 2. The flavonoid contents in the methanolic extracts of *A. altilis* and *F. exasperate* as well as aqueous extract of *K. africana* and the standard (Catechin).

3.2. Reducing power of extracts

The extracts of *A. altilis*, *F. exasperata* and *K. africana* showed a dose-dependent increase in the ferric ion reducing potential (Figure 3). At 10 μg/mL, the absorbance of *A. altilis*, *F. exasperata*, *K. africana* and catechin were 0.039, 0.049, 0.066 and 0.023, respectively while at 1 000 μg/mL the absorbance were 0.889, 0.236, 0.145 and 0.610, respectively.

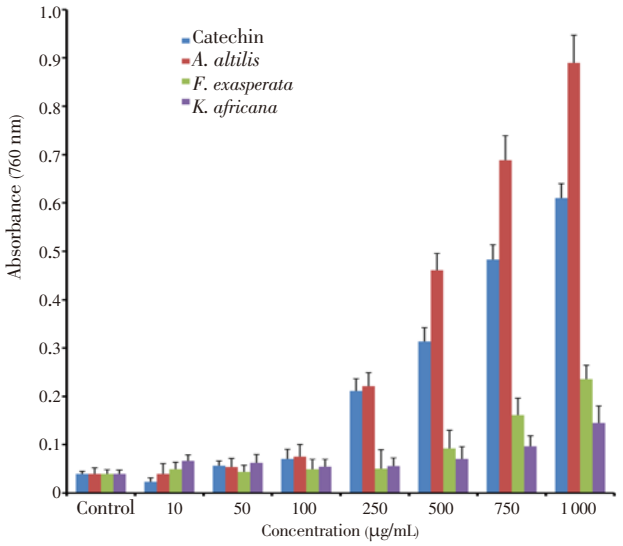

Figure 3. The reducing property of methanolic extracts of *A. altilis* and *F. exasperate* as well as aqueous extract of *K. africana* and the standard (Catechin).

3.3. DPPH radical and nitric oxide scavenging activity of extracts

There were significant ($P<0.05$) and dose-dependent increases in scavenging activity of extracts on DPPH radicals (Table 1). At 100 μg/mL the percentage DPPH radical scavenging activity of the extracts were 2.3%, 10.5%, 22.7% and 42.2% while at 750 μg/mL the scavenging activity were 28.1%, 58.7%, 62.5% and 67.8% for *K. africana*, *F. exasperata*, *A. altilis* and catechin, respectively respectively. The aqueous extract of *K. africana* was found to have the highest scavenging activity on NO radical (Table 2). At 500 μg/mL, the scavenging activity of *A. altilis*, *F. exasperata*, *K. africana* and catechin on NO radical were 22.2%, 16.3%, 43.3% and 20.9% respectively.

Table 1

The scavenging activity of *A. altilis*, *F. exasperata* and *K. africana* extracts on 2, 2- diphenyl-1-picrylhydrazyl radical (DPPH) *in vitro*.

Conccentration μg/mL	% Scavenging activity			
	Catechin	*A. altilis*	*F. exasperata*	*K. africana*
Control	0.000	0.000	0.000	0.000
100	42.2±4.40*	22.7±5.88*	10.5±1.09*	2.3±4.16*
300	47.6±1.55*	50.4±3.13*	16.2±3.17*	11.1±1.61*
500	63.1±5.13*	51.1±2.27*	50.9±4.63*	23.8±5.18*
750	67.8±3.92*	62.5±8.53*	58.7±3.78*	28.1±5.43*

Data are expressed as mean±SD (n=3); * Dose-dependent increase from 100-750 μg/mL.

Table 2

The scavenging activity of *A. altilis*, *F. exasperata* and *K. africana* extracts on nitric oxide radical *in vitro*.

Conccentration μg/mL	% Inhibition			
	Catechin	*A. altilis*	*F. exasperata*	*K. africana*
Control	0.000	0.000	0.000	0.000
100	22.7±3.69	32.9±2.59	27.7±5.41	37.5±3.37
300	22.4±2.56	25.1±2.98	24.8±5.86	48.2±1.60
500	20.9±4.24	22.2±7.49	16.3±4.62	43.3±1.04
750	19.9±3.13	22.2±6.39	22.0±5.74	36.5±2.70

Data are expressed as mean±SD (n=3).

3.4. Inhibition of Fe^{2+}/ascorbate- induced lipid peroxidation by the extracts

The lipid peroxidation (LPO) inhibition potential of extracts from *A. altilis*, *F. exasperata* and *K. africana* was compared with catechin. Extracts of *A. altilis*, *F. exasperata* and catechin exhibited dose-dependent and significant ($P<0.05$) inhibition of Fe^{2+}/ascorbate-induced lipid peroxidation *in vitro*. The order of inhibition of LPO was *A. altilis*> *F. exasperata*> catechin> *K. africana* at 750 μg/mL (Table 3).

Table 3
Inhibition of Fe^{2+}/ascorbate-induced lipid peroxidation by extracts of *A. altilis*, *F. exasperata* and *K. africana in vitro*.

Conccentration μg/mL	% Inhibition			
	Catechin	*A. altilis*	*F. exasperata*	*K. africana*
Control	0.000	0.000	0.000	0.000
100	21.6±8.17*	60.6±1.15*	60.5±0.31*	−75.4±7.09
300	22.5±4.03*	74.5±5.67*	62.8±8.03*	−70.8±5.79
500	26.8±5.65*	78.2±2.06*	63.7±5.49*	−01.8±7.17
750	28.4±4.42*	79.0±1.11*	66.8±3.70*	−65.1±3.43

Data are expressed as mean±SD (n=3); *Dose-dependent increase from 100-750 μg/mL.

3.5. Scavenging of hydrogen peroxide and hydroxyl radical by the extracts

The extracts (*F. exasperata* and *K. africana*) showed low scavenging activity of hydrogen peroxide when compared with catechin. The highest activity was found in *A. altilis* (32%) when compared with catechin (29%) at 1 000 μg/mL. At 750 μg/mL, the scavenging activity of *A. altilis*, *F. exasperata*, *K. africana* and catechin were 31%, 19%, 10% and 29%, respectively (Table 4). The hydroxyl radical scavenging activities of the three extracts were compared with catechin in Table 5. The hydroxyl radical scavenging activities of *K. africana* and *F. exasperata* showed dose-dependent and significant increase from 100-750 μg/mL. At 100 μg/mL, both catechin and *A. altilis* showed highest hydroxyl radical scavenging activities which were 90% and 81%, respectively.

Table 4
The hydrogen peroxide scavenging activity of extracts from *A. altilis*, *F. exasperata* and *K. africana in vitro*.

Conccentration μg/mL	Scavenging activity (%)			
	Catechin	*A. altilis*	*F. exasperata*	*K. africana*
Control	0.000	0.000	0.000	0.000
100	19.5±1.56*	9.6±1.05*	4.8±1.23*	22.4±1.50
300	26.8±0.98*	28.6±1.04*	5.4±1.19*	23.1±6.23
500	28.2±1.15*	30.8±1.12*	8.3±0.52*	17.2±2.82
750	28.8±1.21*	31.4±1.21*	18.9±1.06*	10.1±0.84
1000	29.0±1.12*	31.6±1.15*	25.1±2.15*	6.4±1.34

Data are expressed as mean±SD (n=3); *Dose-dependent increase from 100-1 000 μg/mL.

Table 5
The hydroxyl radical scavenging activity of *A. altilis*, *F. exasperata* and *K. africana* extracts *in vitro*.

Conccentration μg/mL	% Scavenging activity			
	Catechin	*A. altilis*	*F. exasperata*	*K. africana*
Control	0.000	0.000	0.000	0.000
100	89.9±3.62	80.9±1.99	64.6±4.25*	39.5±7.04*
300	73.9±8.73	80.2±3.42	68.4±3.92*	40.6±8.19*
500	82.5±8.38	73.8±5.38	70.9±2.12*	56.7±6.77*
750	69.9±8.19	76.9±4.91	72.5±7.06*	58.9±7.14*

Data are expressed as mean±SD (n=3); * Dose-dependent increase from 100-750 μg/mL.

3.6. Inhibition of cardiac arginase activity in vitro by the extracts

The ability of the extracts to inhibit cardiac arginase activity expressed as the amount of urea liberated per minute per milligram protein is given in Table 6. The extracts; *F. exasperata* and *K. africana* did not produced significant ($P>0.05$) effect on the activity of cardiac arginase *in vitro* from 50-750 μg/mL. However at 500 and 750 μg/mL, *A. altilis* and catechin significantly ($P<0.05$) inhibited cardiac arginase activities by 63%, 67% and 42%, 52%, respectively when compared with the control (Table 6).

Table 6
The inhibitory effects of *A. altilis*, *F. exasperata* and *K. africana* on cardiac arginase activity *in vitro*.

Conccentration μg/mL	Activities of arginase (mg Urea/ min/ mg protein)			
	Catechin	*A. altilis*	*F. exasperata*	*K. africana*
Control	1.13±0.05	1.29±0.15	1.19±0.09	1.19±0.15
50	1.01±0.17	1.21±0.12	1.04±0.13	1.12±0.13
100	1.03±0.11	1.26±0.21	1.07±0.08	1.08±0.07
250	0.97±0.06	1.01±0.06	1.01±0.10	1.08±0.10
500	0.66±0.08*	0.48±0.07*	1.07±0.11	1.13±0.10
750	0.54±0.05*	0.42±0.06*	1.03±0.14	1.06±0.12

Data are expressed as mean±SD (n=3); *Significantly different from control ($P<0.05$) at 500 and 750 μg/mL.

4. Discussion

Several techniques have been used to determine the antioxidant activity *in vitro* in order to allow rapid screening of substances. Free radicals are known to play a definite role in a wide variety of pathological manifestations. Antioxidants combat free radicals and protect from various degenerative diseases. They exert their action either by scavenging the ROS or protecting the antioxidant defense mechanisms[36]. The electron donation ability of natural products can be measured by DPPH purple-coloured solution bleaching[37]. The method is based on scavenging of DPPH through the addition of a radical species or antioxidant that decolourizes the DPPH solution. The degree of colour change is proportional to the concentration and potency of the antioxidants. A large decrease in the absorbance of the reaction mixture indicates significant free radical scavenging activity of the compound under test[38]. In the present study, the three extracts (*A. altilis*, *F. exasperata* and *K. africana*) showed dose-dependent scavenging of DPPH radical. However, *A. altilis*

and *F. exasperata* at 500 μg/mL significantly scavenged DPPH radical by over 50% which is directly related to the high phenolic contents of *A. altilis* and *F. exasperata*. The results indicate *A. altilis* and *F. exasperata* contain phytochemical constituents that are capable of donating hydrogen to a free radical to scavenge the potential damage.

One of the mechanisms of action of antioxidants is to chelate and deactivate transition metals thereby preventing such metals from participating in the initiation of LPO and oxidative stress through metal catalysed reaction[39]. Overproduction of ROS may have a direct attack on the polyunsatureated fatty acids of the cell membrane to induce peroxidation reactions[39]. The deleterious effect caused by iron is done by reacting with hydrogen peroxide to produce hydroxyl radical (OH.) through Fenton reaction. Superoxide can also react with Fe^{3+} to regenerate Fe^{2+} which can participate in the Fenton reaction[40]. In this study, LPO of rat liver homogenates was induced by ferric ion plus ascorbic acid. The LPO inhibitory activity of *A. altilis* and *F. exasperata* was found to be very high, significant and dose-dependent. The inhibition of LPO by *A. altilis* was the highest relative to others (*F. exasperata* and *K. africana*) at concentration of 750 μg/mL. These results indicated that *A. altilis* and *F. exasperata* have potential to be studied for use in treating liver disease. Hydroxyl radical is one of the potent ROS in the biological system. It reacts with polyunsatureated fatty acids moieties of cell membrane phospholipids and causes damage to cell[41]. The hydroxyl radical is regarded as a detrimental species in pathophysiological processes and capable of damaging almost every molecule of biological system and contributes to carcinogenesis, mutagenesis and cytotoxicity[42]. It is known that the mutagenic capacity of free radicals is due to the direct interactions of hydroxyl radicals with DNA and therefore playing an important role in cancer formation[43]. The results demonstrated that the methanolic extract of *A. altilis* and *F. exasperata* had appreciable OH radical scavenging effects when compared to catechin. The results indicate that both *A. altilis* and Fe may serve as anticancer agents by inhibiting the interaction of hydroxyl radical with DNA. The ability of these extracts to quench hydroxyl radicals might directly relate to the prevention of lipid peroxidation.

Hydrogen peroxide occurs naturally at low levels in the air, water, human body, plants, microorganisms and food[44]. H_2O_2 is rapidly decomposed into oxygen and water, and may produce hydroxyl radicals that can initiate LPO and cause DNA damage[45]. The extracts; *A. altilis*, *F. exasperata* and *K. africana* exhibited low scavenging effect on hydrogen peroxide *in vitro* at 100-300 μg/mL. However, at higher concentrations (500-1 000 μg/mL), the hydrogen peroxide scavenging effect of *A. altilis* is statistically similar to catechin (about 30%). Hence, *A. altilis* showed appreciable scavenging of hydrogen peroxide and may be attributed to the presence of phenolic groups that could donate electrons to hydrogen peroxide, thereby neutralizing it into water. In reducing power assay, the yellow colour of the test solution changes to green depending on the reducing power of the extracts. The presence of the reductants in the solution causes the reduction of the Fe^{3+}/ ferricyanide complex to the ferrous form. Therefore, Fe^{2+} can be monitored by absorbance measurement at 700 nm. It is known that the reducing properties of an extract could serve as a measure of its antioxidant action by donating hydrogen atom to break the free radical chain[46]. Increasing absorbance at 700 nm indicates an increase in reducing ability. The antioxidants present in the fractions of *A. altilis*, *F. exasperata* and *K. africana* caused the reduction of Fe^{3+}/ ferricyanide complex to the ferrous form, and thus proved their reducing power. The reducing power of the plant extracts followed the order; *A. altilis*>*F. exasperata*>*K. africana* at 1000 μg/mL. The release of cyanide and/or nitric oxide in sodium nitroprusside can cause cytotoxicity[47]. NO is a free radical with a short half-life (<30 s) and its independent action may cause neuronal damage, especially in conjunction other ROS such as superoxide radical to form peroxynitrite radical[47]. However, the result revealed that these extracts had higher NO scavenging potentials than standard at 750 μg/mL. Hence, these extracts may elicit inhibitory action against NO-induced cellular damage.

Arginine metabolism is important to vascular function in health and disease. The key enzymes required in arginine metabolism in the vascular systems are the nitric oxide synthases and the arginases, both of which use arginine as a substrate[48], and dysregulated activity of these enzymes has been linked to multiple types of endothelial dysfunction and cardiovascular disease[49]. Cardiac arginase is hemodynamically sensitive to blood pressure fluctuations and arginase inhibitors such as hydrazalazine and nor-hydroxyl arginine have been demonstrated to hold promise as future antihypertensive agents courtesy of their abilities to cause reduction in arginase activity by up to 30% and arterial blood pressure by 30-35 mmHg, modulate arterial resistance and promote blood flow[50]. It is important to note that cardiovascular diseases such as hypertension, arrythmias, angina pectoris, myocardial infarction, stroke, and left ventricular hypertrophy have become a major cause of morbidity and mortality in the world with increasing prevalence in developing countries[51]. In the present study, the plant extract (*A. altilis*) caused significant reduction in the activity of cardiac arginase *in vitro* at concentrations of 500 and 750 μg/mL. Therefore, the observed reduction in arginase activity is a pointer to the possible antihypertensive action of *A. altilis*.

In conclusion, the replacement of synthetic with natural antioxidants because of implications for human health may be advantageous. In the present study, methanolic extracts of *A. altilis* and *F. exasperata* had stronger hydroxyl and DPPH radical scavenging activities and inhibitory activity against lipid peroxidation than *K. africana*. The reducing capacity of *A. altilis* and *F. exasperata* on ferrous ion was higher than that of other extract (*K. africana*). In addition, the potent antioxidative activities of *A. altilis* and *F. exasperata* might result from their high contents of phenolic compounds. Hence, the methanolic extracts from *A. altilis* and *F. exasperata* could be used as a health-care food supplement or in the pharmaceutical industry as natural antioxidants. Further studies may be necessary to identify, isolate and

characterize the active components in *A. altilis* and *F. exasperata* that is responsible for the observed effects.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Free radicals are essential in human physiology, but may be responsible of various diseases when overproduced. The search for active molecules from natural sources has become attractive since synthetic medicines are costly and present some severe side effects.

Research frontiers

This study is carried out to investigate the free radical scavenging (DPPH, NO, H_2O_2, OH) activity by extracts from Nigerian plants that are extensively used in traditional medicine). Total phenols and flavonoid contents, inhibition of arginase activity and Fe^{2+}-ascorbate-induced lipid peroxidation were also assessed.

Related reports

The results indicate that *Artocarpus altilis* and *F. exasperata* extracts showed a high and dose-dependent DPPH scavenger activity. The above extracts also inhibited lipid peroxidation efficiently. Artocarpus artilis extracts inhibited arginase activity by 63% and 67% at concentrations of 500 and 750 μg/mL, respectively.

Innovations and breakthroughs

Authors have demonstrated new biological activities that have never been reported for the plants involved in this study. This opens new perspectives of research using these plants.

Applications

The preliminary results reported in this study can be considered as the premise for supporting the use of this plant in traditional medicine.

Peer review

This paper that describes the antioxidant, anti-lipid peroxidation and anti-arginase activities of selected Nigerian plants is indeed interesting. The antioxidant activity and the inhibitory potential of arginase activity exerted by *A. altilis* and *F. exasperata* extracts, although at relatively high doses, are promising.

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