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Original Research Article

Evaluation of the Antioxidant Activity of the Stem Bark Extracts of *Anacardium occidentale* (Linn) Anacardiaceae

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ABSRTACT

Anacardium occidentale is a known Nigerian indigenous plant used in folklore medicine in the management of various ailments. The antioxidant properties of *A.occidentale* was investigated via the evaluation of the radical scavenging activity of the various fractions of the extracts using 2, 2-diphenyl-1-picrylhydrazyl and 2,4,6-tri[2-pyridyl]-5-triazine assays. Phytochemical screening was conducted on the extract to ascertain the presence of vital phytoconstituents, and the phenol and flavonoid contents of the various fractions of the extracts were quantified spectrophotometrically. The results obtained revealed that *A.occidentale* contains vital phytoconstituents with the highest total phenol contents of 130.63±0.89 GAE/mg of extract in the crude sample, while the lowest value of 11.19±0.21 GAE/mg of extract was obtained in 50:50 n-hexane/ethylacetate fraction respectively. The total flavonoid content was highest in the crude sample (62.29 ± 3.81 mg QE/g extract dry weight) and least in the methanol fraction 0.31 ± 0.07 mg QE/g of dry extract. The Ferric Reducing Antioxidant Power (FRAP) assay shows that the extract and fractions showed a considerable antioxidant effect from the absorbance of 0.208nm to 1.276 nm with 100% methanol having the highest value. Similarly, the DPPH assay result of the extracts and fractions was concentration-dependent. DPPH radical scavenging activity revealed that the highest radical scavenging was observed in 90% Methanol-10% Water fraction with an IC_{50} value of 0.43µg/mL. These findings validate *A.occidentale* as a rich source of antioxidant and could be recommended for therapeutic use in the management of oxidative related ailments.

Keywords: *A. occidentale*, Antioxidant, Phytochemical, Radical Scavenging

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Introduction

Anacardium occidentale, is a botanical species native of Eastern Brazil.[1] The significant of the process of oxidation in the production of energy for fueling biological activities cannot be overstated. However, oxygen-centred free radicals and other reactive oxygen species that are continuously produced *in-vivo*, result in cell death and tissue damage. Oxidative damage caused by free radicals may be related to aging and diseases, such as diabetes, cancer and cirrhosis.[22,23.] The degenerative diseases associated with aging include cancer, cardiovascular disease, immune-system decline, brain dysfunction and cataracts.

Free radicals are neutral, short lived, unstable and highly reactive chemical species associated with an odd or unpaired electron. They have the ability to alter the proper functioning of healthy cells of the body, causing them to lose their structure and function.

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Cell damage caused by free radicals appears to be a major contributor to aging and degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, immune system decline, liver diseases, diabetes mellitus, inflammation, renal failure, brain dysfunction and stress among others.

In traditional medicine, *A. occidentale* has been reported to be efficacious in the treatment of different ailment. The traditional Nigerian pharmacopoeia reported that the stem-bark of *A. occidentale* is known for treatment of malaria fever, asthma, dysentery, toothache and sore gum. The stem barks and leaves are also used to cure dermatitis, other skin diseases and pile.

Akinpelu (2001)[5] reported the use of the leaves of *A. occidentale* for treating dysentery, diarrhea, piles, and an infusion of bark and leaves are applied to relief toothache and sore gums.[5] Research done by Andarwulan *et al.,* evaluated the leaves extract of the plant and discovered that it could be used for the management of rheumatism and hypertension.[6'7] and that it also possesses antidiabetic, anti-inflammatory, antimicrobial and analgesic properties.[8]

We therefore, wish to scientifically verify and validate the promising antioxidative properties of *A. occidentale* that has been reported folklorically.

Materials and Methods

Collection and preparation of stem bark of A. occidentale

The Stem bark of *A. occidentale* was collected from the Faculty of Agriculture, University of Benin, in August, 2017. The plant sample was identified by Dr. Henry Akinibosun, from the Department of Plant Biology and Biotechnology, University of Benin, with a voucher

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specimen number UBH-A389 deposited. The Stem bark was rinsed with water and dried at ambient temperature (28°C - 30°C) for fourteen (14) days. The well dried stem bark was ground to powder by means of a mechanical grinder. The powdered stem bark sample was stored in clean and air-tight glass container and stored for further analysis of the stem bark.

Extraction of crude powdered sample
The powdered stem bark material (2.1k g) was extracted with 10.5 L of methanol by maceration at room temperature for four days and thereafter filtered. The filtrate was concentrated using a rotary evaporator *in vacuo* at 45°C. The concentrated extract was kept in tightly stoppered bottles in a refrigerator at 4°C for further analysis.

Phytochemical screening of A. occidentale
Preliminary phytochemical screening was done with the method of Sofowara with some modifications].[24]
Proximate analysis on stem bark of A. occidentale
Proximate analysis of the stem bark of *A. occidentale* crude powder was carried out using methods described by Khandelwal with slight modifications.[9]

Determination of total phenolic in the stem bark of A. occidentale
The total phenolic content in the stem bark of *A. occidentale* was determined in terms of gallic acid equivalents (GAE) using Folin Ciocalteu method.[10]

Determination of total flavonoids in the stem bark of A. occidentale
Total flavonoid contents were estimated using the standard method described by Ebrahimzadeh *et al*.,.[11]

Antioxidant activity
DPPH radical scavenging assay was used in the determination of free radical scavenging activitiy of crude methanol extract and fractions of *A. occidentale* using 2, 2-diphenyl-1-picrylhydrazyl. The scavenging effect of crude methanol extract and fractions of *A. occidentale* stem bark on DPPH radical was carried out by the method described by Jain *et al.,* A solution of 0.2 mM DPPH (394.32g/mol) in methanol was prepared, and 1.0 mL of this solution was mixed with 3.0 mL of sample in methanol containing 1-200 µg/mL of the sample. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 minutes. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid was used as a reference standard. The ability to scavenge DPPH radical was calculated by the following equations:

DPPH radical scavenging activity (%) = $[(A_0-A_1)/(A_0)] \times 100$.
Where; A_0 was the absorbance of DPPH radical + methanol
A_1 was the absorbance of DPPH radical + sample extract.
The 50% inhibitory concentration value (IC_{50}) is indicated in Table 5 as the effective concentration of the sample that is required to scavenge 50% of the DPPH free radical.[15]

FRAP assay:
The FRAP assay was carried out according to the method described by (Benzie and Strain) with slight modification, in the estimation of the antioxidant activity of the stem bark of *A. occidentale*.
The fresh FRAP solution was prepared by mixing 25 mL of 300mM of acetate buffer (pH 3.6), 2.5 mL of 10mM TPTZ (2,4,6-tri[2-pyridyl]-5-triazine) solution in 40mM HCl, and 2.5 mL 0f 20mM $FeCl_3.6H_2O$ (Ferric chloride hexahydrate) solution and then warmed at 37ºc before use. The extract (1.5 mL of 1mg/mL) was mixed with 2.8 mL of the FRAP solution, and incubated at room temperature for 30min in the dark. Readings of the colored products (ferrous tripyridyltriazine complex) was then taken at 593nm.
The calibration curve was prepared using $FeSO_4.7H_2O$ (ferric sulphate heptahydrate) at concentration of (1, 2, 5, 10, 25, 100, 200mM) and the absorbance values were measured. Results are expressed in %mM Fe(II)/g dry plant material.[13]

Statistical analysis
The results obtained were expressed as mean ± standard error of the mean (SEM) of six replicates. The data were subjected to one-way analysis of variance (ANOVA) and the difference between means were determined by Duncan's multiple range tests using the Statistical Analysis System (SPSS Statistics 17.0) where applicable. P values <0.05 were considered as significant.

Results and Discussion

The phytochemical are known to be biologically active compounds which act as free radical scavengers to help eliminate highly Reactive Oxygen Species (ROS) that are by-products of metabolized oxygen and are rich in offering numerous health benefits.
The phytochemical screening of the pulverized aqueous fraction of *A. occidentale*, revealed the presence of alkaloids, carbohydrate, reducing sugar, deoxysugar, saponins, phenolics, tannins, terpenoids, flavonoids and proteins (Table 1).
The obtained results are in agreement with the reports of Mustapha *et al*., that the phytochemical analysis of propanolic extract of the leaves of *A. occidentale* revealed the presence of phenols, tannins, flavonoids, glycosides and terpenoids.[14] Meanwhile, the extract from the stem as reported by Dietewa 2004, showed the presence of tannins, flavonoids, glycosides, terpenoids, reducing sugars and saponins.
In folkloric medicine, alkaloids are widely known for their medicinal roles. One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms. These activities have been widely studied for their potential use in the elimination and reduction of human cancer cell lines.[15] Flavonoids also exhibit a wide range of biological activities such as antimicrobial, antiinflammatory, analgesic, and cytostatic, hypoglycemic and antioxidant properties.[16]
The proximate analysis result for the stem bark of *A. occidentale* as presented in Table 2 revealed moisture content (5.84 ± 0.31), total ash (9.69 ± 0.05), acid-insoluble ash (12.89 ± 0.25), water-souble ash (0.29 ± 0.16), alcohol extractive value (2.11 ± 0.21), and water extractive value (1.49 ± 0.25). The moisture content (5.84 ± 0.31) of stem bark of *A. occidentale* shows that the stem bark has promising minimal vulnerability to microbial and hydrolytic degradation during storage.[23]
The ash content (9.69 ± 0.05), was significantly higher than the results reported by Aremu *et al*., and Omusuli *et al*., for cashew nut flour (4.4%) and roasted/defatted cashew nut flour (4.41%), respectively.[18,19]
The ash is the residue left after incineration of the plant sample which gives information regarding the mineral content of the sample, it varies within definite limits according to the environment (air/water) and on soils in which the plants grow. The high value of ash content observed shows the possible richness of the stem bark of *A. occidentale* in mineral content.

Table 1: Phytochemical composition of pulverized aqueous *Anacardium occidentale*

S/N	Phytochemicals	Inferences
1.	Alkaloids	+
2.	Carbohydrates	+
3.	Reducing Sugar	+
4.	Deoxysugar	+
5.	Saponins	+
6.	Phenolics	+
7.	Flavonoids	+
8.	Tannins	+
9.	Terpenoids	+
10.	Proteins	+

+ indicates the presence of the phytochemical

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The water extractive value and alcohol extractive values were (1.49 ± 0.25) and (2.11 ± 0.21) respectively. The extractive values (water and alcohol) are pointers on which solvent is a better solvent extractor. The result obtained shows that alcohol extracts better phyto-constituent than water thus a recommended solvent extractor for the extraction of the stem bark of *A. occidentale*.

Antioxidant assay
The various concentrations in percentage mmole of the crude and different fractions of the stem bark presented in Table 3 in the FRAP assay, were from the plot of Y=0.0084X - 0.041. The presence of an antioxidant at low concentrations in relation to the oxidizable substrate could significantly delay or inhibit oxidative processes.[20] The crude extract and fractions showed a considerable antioxidant effect from the absorbance of 0.208nm to 1.276nm with 100% methanol having the highest value (Table 3). The coefficient of determination; R^2=0.9961 as shown in Figure 1, shows that 99.61 percent of the variation in absorbance is due to difference in the concentration of the standard and thus, the more intense the colour of the analyte, the higher the absorbance, which shows a higher ferric reducing antioxidant capacity of the stem bark of *A. occidentale* (with highest value in methanol fraction).
The below DPPH Table 4 shows that the crude extract and fractions showed concentration-dependent DPPH radical scavenging. It is observed that as the concentration increases, the % inhibition also increases while absorbance decreases. In this assay, the purple chromogen radical 2,2-diphenyl1-picrylhydrazyl (DPPH•) becomes a stable molecule on accepting an electron or hydrogen atom(pale yellow hydrazine is formed). The activity is expressed as inhibitory concentration IC_{50}, that is the amount of antioxidant necessary to decrease by 50% the initial DPPH• concentration.[21] The lower IC_{50}, the higher is the "antiradical efficiency". In this vain, the results obtained shows that the fraction (90% Methanol-10% Water) has the highest antioxidant activity with IC_{50} value of 0.43µg/ml while on the other hand, n-Hexane fraction shows no activity.
This variation in activity could be attributed to the nature of solvent employed, as polyphenols tend to be more soluble in polar protic solvents.
However, the results shows that *A. occidentale* has a very strong antioxidant activity. The antioxidative activities observed can be attributed to either the different mechanisms exhibited by different polyphenolic compounds that is, tocopherols, flavonoids and other organic acids and to the synergistic effects of different compounds.
The total phenolic content was determined using Folin-Ciocalteau method. In this study, the absorbance of a series of concentrations of gallic acid were plotted to their concentration to yield a linear calibration curve of gallic acid (y = 0.0049x + 0.0436) with coefficient of correlation (r) value of 0.9862. The total phenolic content of the various fractions obtained are as shown in figure 2.0. The chart indicate the highest total phenol content at 130.63±0.89 GAE/mg of extract for the crude sample, while the lowest total phenol content at 11.19±0.21 GAE/mg of extract for the 50:50 n-hexane/ethylacetate fraction. 130.63±0.89 GAE/mg which was obtained for the crude sample, reveals that each 100 g dry weight of the stem bark of *A. occidentale* contained total phenolics that was equivalent to gallic acid of 130.63±0.89g. The descending order of total phenolic content of the various fractions in stem bark is as follows: Crude > Methanol/H_2O > Ethyl acetate/Methanol > Ethyl acetate > Methanol > n-Hexane > n-Hexane/ Ethyl acetate.
The total flavonoid content was determined using quarcetin method. In this study, the absorbance of a series of concentrations were plotted to their concentration to yield a linear calibration curve of quarcetin acid (y = 0.0179x + 0.0036) with coefficient of correlation (r) value of 0.9949. In the study, total flavonoid content of the various fractions obtained are as shown in Figure 3, the highest flavonoid content was measured in the crude fraction with 62.29 ± 3.81 mg QE/g extract dry weight and the lowest content in Methanol at 0.31 ± 0.07 mg QE/g of dry extract. As shown in the chart, the total flavonoid contents in the stem bark of *A. occidentale* in the various fractions are ranked in the following order: Crude > n-Hexane/ Ethylacetate > Ethylacetate > n-Hexane > Ethyacetate/Methanol > Methanol. The trend observed shows that the total flavonoid contents were higher in the non-polar organic solvent.

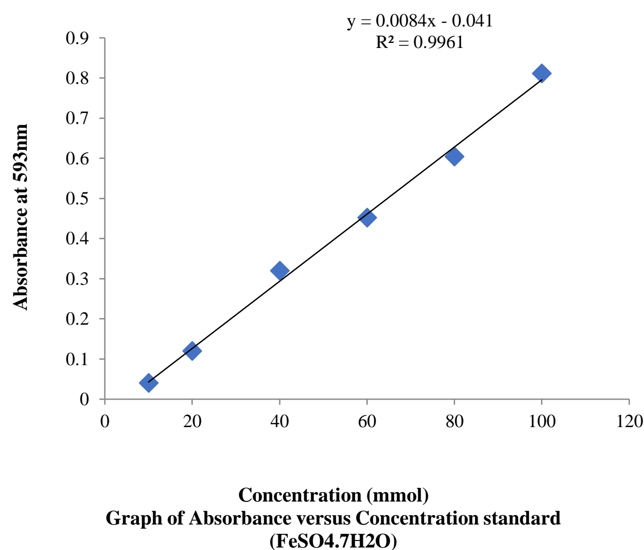

Figure 1: Plot of absorbance against concentration of standard solution

Table 2: Proximate composition of the stem bark of *A. occidentale*

	Quantitative Parameter	Mean
1	Moisture content	5.84 ± 0.31
2	Total Ash	9.69 ± 0.05
3	Acid-insoluble ash	12.89 ± 0.25
4	Water-soluble ash	0.29 ± 0.16
5	Alcohol extractive value	2.11 ± 0.21
6	Water extractive value	1.49 ± 0.25

Values are mean ± standard deviation

Table 3: Absorbance and %mmol of the crude extract and different fractions of the stem bark of *A. occidentale*

Fractions	Absorbance (mean)	Mean Conc. (mmol) ± SD
n-Hex	0.366	$48.45^b \pm 9.96$
n-Hex/EtoAC	0.208	$29.64^a \pm 3.94$
EtoAC	0.531	$68.09^c \pm 3.04$
EtoAC/MeOH	1.177	$145.00^d \pm 2.70$
MeOH	1.276	$156.78^f \pm 2.31$
MeOH/H_2O	0.773	$135.59^g \pm 0.79$
CR	0.909	$113.09^h \pm 3.40$

Values with different superscripts are significantly different ($p < 0.05$).

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Table 4a: DPPH scavenging activity (% inhibition) of *A. occidentale* stems bark

Conc. µg/mL	Ascorbic acid	Hex	n-Hex/EA	EA	EA/MeOH	MeOH	MeOH/H_2O
1	41.73 ± 0.22	18.51 ± 0.53	29.99 ± 0.06	39.89 ± 0.15	44.47 ± 0.35	41.74 ± 0.37	42.84 ± 0.37
2	77.75 ± 0.50	21.79 ± 0.15	30.94 ± 0.26	54.64 ± 0.49	58.13 ± 2.25	45.63 ± 1.48	44.72 ± 0.22
5	92.89 ± 0.37	22.47 ± 0.39	34.77 ± 0.22	82.17 ± 0.26	84.22 ± 0.26	84.08 ± 0.24	84.06 ± 0.39
10	95.69 ± 0.29	24.45 ± 1.99	47.68 ± 1.67	84.29 ± 0.34	88.32 ± 1.02	87.43 ± 0.53	86.91 ± 0.31
25	95.69 ± 0.29	25.89 ± 1.06	48.22 ± 0.20	91.59 ± 0.17	90.64 ± 0.87	91.53 ± 0.57	91.05 ± 0.39
50	96.24 ± 0.15	32.11 ± 0.53	68.03 ± 0.44	92.69 ± 0.20	91.94 ± 0.24	92.36 ± 0.05	92.62 ± 0.42
100	96.72 ± 0.17	35.11 ± 1.44	77.12 ± 1.54	93.72 ± 0.11	92.08 ± 0.11	92.89 ± 0.06	92.83 ± 0.59
200	97.88 ± 1.11	35.52 ± 0.39	92.28 ± 0.69	94.19 ± 0.15	92.76 ± 0.31	93.59 ± 0.58	95.35 ± 0.46

Data represent mean± Standard Deviation of triplicate analysis

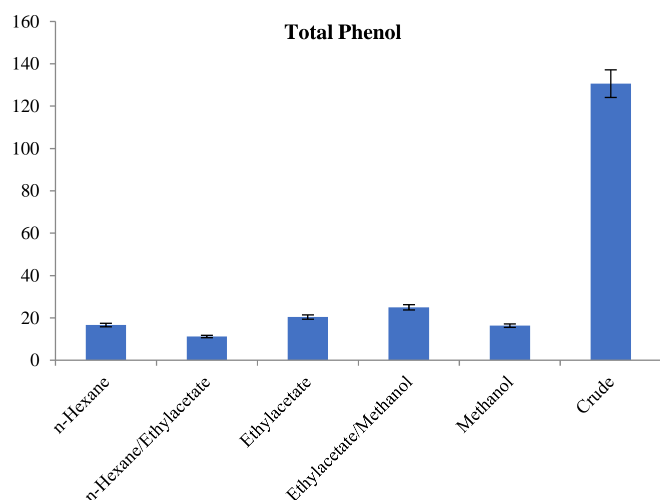

Figure 2: showing the mean total phenolic content of the fractions

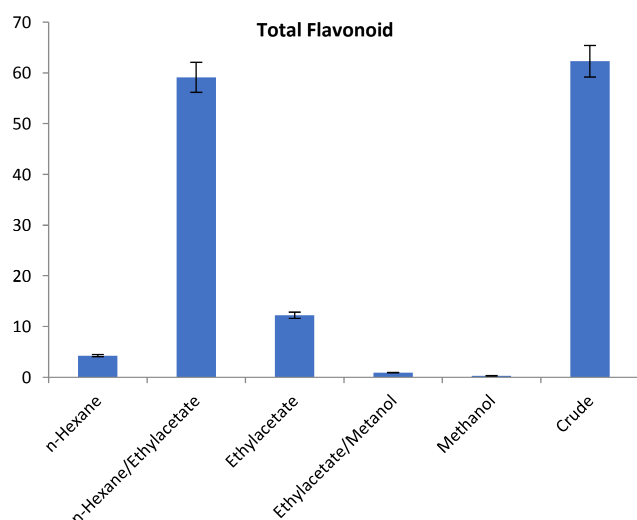

Figure 3: Showing the mean total Flavonoid Content in the stem bark of *A. occidentale*

Table 4b: DPPH scavenging activity (% inhibition) of *A. occidentale* stems bark

Concentration (µg/mL)	CR
1	51.36 ± 0.29
2	55.74 ± 0.44
5	65.78 ± 0.09
10	67.42 ± 0.19
25	72.27 ± 0.24
50	74.59 ± 1.19
100	77.53 ± 0.28
200	77.53 ± 0.06

Table 5: 50% Inhibitory concentration of DPPH *A. occidentale* stem bark

Fractions	IC_{50} (µg/mL)
Ascorbic acid	0.13^a
Crude	0.44^b
n-Hexane	No Activity
50% Ethylacetate.50% n-Hexane	10.99^c
100% Ethylacetate	1.38^d
50% Methanol.50% Ethylacetate	1.26^e
100% Methanol	1.53^f
90% Methanol.10% Water	0.85^g

Values with different superscripts are significantly different ($p < 0.05$)

Conclusion

This study has revealed that the stem bark of *A. occidentale* possesses considerable antioxidant properties and could be a potential source for therapeutic applications in the management of oxidative related ailment and as an agent that could be used against free radical species. Finally, it can also act as natural antioxidants in diet.

Conflict of Interest

The authors declare no conflict of interest.

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Authors' Declaration

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