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Antioxidant Activity of *Coula edulis* Baill. Seed Extracts

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Authors' contributions

This work was carried out in collaboration between both authors. Author BNI designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author GIN managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: In this work, the antioxidant activity of organic and aqueous extracts of seed of *Coula edulis* Baill. was evaluated.
Study Design: Seeds of *Coula edulis* were collected, authenticated and extracted with acetone, ethanol and water respectively. The resulting extracts were used for antioxidant assay.
Place and Duration of Study: This work was done at the Department of Chemistry, University of Uyo between November 2015 and July 2016.
Methodology: Air-dried seeds of *Coula edulis* (800 g) were pulverized and macerated with 2.5 L of acetone, ethanol and water separately for 24 hrs at room temperature and then filtered. The acetone and ethanol filtrates were concentrated *in vacuo*, while the aqueous extract was freeze-dried to obtain the crude acetone (AcE), ethanol (EtE) and aqueous (AqE) extracts respectively. Content of total phenolics, flavonoids and vitamin C in the extracts were determined. Also, the DPPH and ABTS scavenging activity as well as the metal chelating and reducing power of the extracts were also evaluated.

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Results: Our results indicated that *Coula edulis* seed is a rich source of total phenolics, flavonoids and vitamin C. Amongst the tested extracts, the acetone extract contained the highest amount of total phenolics (28.3 mgGAE/g), could chelate Fe^{2+} ions more effectively (IC_{50} = 46.2 µg/mL) and showed the best DPPH radical scavenging ability (IC_{50} = 19.2 µg/mL). The aqueous extract was the richest in flavonoids and vitamin C. Also, it showed the best reducing power and ABTS radical scavenging abilities with an IC_{50} of 25.2 µg/mL.
Conclusion: *Coula edulis seed is* a good source of natural antioxidants that can be exploited in food and therapeutic applications.

Keywords: Coula edulis; antioxidant activity; metal chelating activity; ferric reducing activity.

1. INTRODUCTION

The family Olacaceae encompasses about 250 species, of which *Coula edulis* Baill. is a member. This plant, also known as the African walnut, is abundant in Nigeria. The seed is edible, has an agreeable taste and is rich in oleic acid, potassium and phosphorus. Traditionally, its organs are used to dress sores, stimulate appetite; and treat stomach ache, skin diseases and dysentery [1-2]. The biological value, chemical composition, antimicrobial, antiplasmodial and antidermatophytic potential of extracts from organs of this plant have been reported [3-6]. In addition, the plant is reported to contain acetylenes with anticancer property [7].

In recent times, the search for natural antioxidants in foods has been on the increase. These compounds possess the ability to neutralize reactive oxygen / nitrogen species which may induce oxidative stress in living cells. When in excess, these reactive species can damage lipids, proteins, or DNA resulting in impaired functioning despite the presence of endogenous antioxidant defences in the body [8]. Diseases such as cancer, diabetes, neurodegenerative, digestive and gastrointestinal disorders have been attributed to oxidative stress. Therefore, consumption of foods rich in natural antioxidants may provide protection against diseases related to oxidative damage [9].

Despite its medicinal values and consumption, no scientific data exist on the antioxidant potential of this edible seed. This work reports the antioxidant potentials of organic and aqueous extracts of *Coula edulis* seeds.

2. MATERIALS AND METHODS

2.1 Sample Collection and Extraction

Fresh fruits of *Coula edulis* were harvested from a farm in Akwa Ibom State, Nigeria and the kernels manually removed from seeds. The seeds were dried, and pulverized with a blender. 800 g of this powder was macerated in 2.5 L acetone, ethanol and water separately for 24 hrs at room temperature, and then filtered. The acetone and ethanol filtrates were concentrated *in vacuo*, while the aqueous extract was freeze-dried to obtain the crude acetone (AcE), ethanol (EtE) and aqueous (AqE) extracts with yields of 1.79%, 2.14% and 2.87% respectively.

2.2 Chemicals

1,1- diphenyl-2-picryl hydrazyl (DPPH), 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), disodium salt of 3-(2-pyridil)-5,6-bis(4- phenylsulfonic acid)-1,2,4-triazine (ferrozine), Folin Ciocalteu reagent, gallic acid, quercetin, 2,4 dinitrophenyl hydrazine (DNPH), butylated hydroxyanisole (BHA) and trichloroacetic acid (TCA) were purchased from Sigma – Aldrich. Other reagents used were of analytical grade or better.

2.3 Determination of Total Phenolic Content

The Folin- Ciocalteu reagent was used to determine the total phenolic content of the seed extracts. Briefly, 10 µL of each extract was taken and the volume made to 2 mL with distilled water. 0.5 mL of Folin-Ciocalteu reagent was then added and the sample allowed to stand for 3 min. Then, 2 mL of Na_2CO_3 (20%w/v) was added, placed in boiling water for 1 min and allowed to cool to room temperature. The absorbance of this mixture was then read at 765 nm, and the absorbance of the control subtracted. Total phenolic content was expressed in mgGAE/g extract based on a standard calibration curve of gallic acid (Fig. 5) [10].

2.4 Determination of Flavonoid Content

Content of flavonoid in the seed extracts was determined according to the modified method of Kumar et al. [11]. Briefly, 10μL of each plant

extract was diluted with distilled water and the volume made up to 2 mL. This mixture was kept at room temperature for 3 min. Thereafter, 3 mL of 5% $NaNO_2$ and 0.3 mL of 10% $AlCl_3$ were added allowed to stand for a further 6 min. Then, 2 mL of 1M NaOH was added and the final volume adjusted to 10 mL with distilled water. The absorbance of this mixture was read at 510 nm. Flavonoid content was calculated using a standard calibration curve prepared from quercetin and expressed as mgQE/g (Fig. 5).

2.5 Determination of Ascorbic Acid Content

To 10 µL of each seed extract, distilled water was added and the volume made up to 2 mL. Thereafter, 2 mL of 2, 4-dinitrophenylhydrazine (DNPH) and 1 drop of 10% thiourea were added to this mixture, heated in a water bath for 15 min and allowed to cool. This was followed by the addition of 5 µL of H_2SO_4 (80%v/v) to the mixture at 0℃ in an ice bath. The absorbance of this mixture taken at 521 nm, with ascorbic acid used as the standard (Fig. 5) [11].

2.6 Evaluation of DPPH Activity

To evaluate the DPPH activity, 1 mL of each seed extract at varying concentrations was mixed with 1 mL of DPPH solution in methanol (0.004%). The mixture was shaken vigorously and allowed to stand for 30 min at room temperature in the dark. The reduction of the DPPH radical was determined by measuring the absorption at 517 nm. The procedure was repeated for the blank and control. The radical scavenging activity was calculated using the equation:

$$\text{DPPH scavenging effect (\%)} = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

Sample concentration providing fifty percent inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration. BHA and vitamin E were used as standards [12].

2.7 Evaluation of ABTS Activity

$ABTS^{\cdot+}$ was produced by reacting 7 mM ABTS solution (absorbance = 0.7± 0.02 at 734 nm) with 2.45 mM potassium persulfate. This mixture was allowed to stand for 12 hrs in the dark at room temperature. Then, 2.94 mL of this $ABTS^{\cdot+}$ solution was mixed with 60 µL of each extract and incubated at 37℃ for 20 min in the dark. After incubation, the absorption was read at 734 nm. The percentage inhibition was calculated using the equation:

$$\text{\% inhibition} = [A_{blank} - A_{sample})/A_{blank}] \times 100$$

Sample concentration providing fifty percent inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration. BHA and vitamin C were used as standards [3].

2.8 Evaluation of Reducing Power (RP)

Reducing power of the seed extracts was determined according to the method of Oyiazu [13]. Each extract (10 -300 µg/mL) dissolved in ethanol (2.5 mL) was mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide and the mixture allowed to stand at 50℃ for 20 minutes. Thereafter, 2.5 mL of 10% trichloroacetic acid (w/v) was added and the mixture centrifuged at 200 g for 19 minutes. The upper layer (5 mL) was mixed with 5 mL of deionised water and 1 mL of 0.1% ferric chloride and the absorbance measured at 700 nm against a blank. A higher absorbance indicated a higher reducing power. Effective concentration at which the absorbance was 0.5 for reducing power (IC_{50} in µg/mL) obtained by interpolation. BHA and ascorbic acid were used as positive controls.

2.9 Evaluation of Metal Chelating (MC) Activity

The modified method of Decker and Welch [14] was used to evaluate the metal chelating activity of the seed extracts. Briefly, 0.5 mL of each seed extract at varying concentrations was mixed with 0.05 mL of 2 mM $FeCl_2$ and 0.1 mL of 5 mM ferrozine and the total volume made to 2 mL with methanol. This mixture was shaken vigorously and left standing at room temperature for 10 min. After the mixture had reached equilibrium, the absorbance of the solution was measured spectrophotometrically at 562 nm. The percentage inhibition rate of ferrozine – Fe^{2+} complex formation was calculated using the formula:

$$\text{Scavenging activity (\%)} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

where $A_{control}$ = absorbance of ferrozine – Fe^{2+} complex, and A_{sample} = absorbance of sample. EDTA was used as positive control.

2.10 Statistical Analysis

Microsoft Excel was used for all statistical analysis, and all experiments were performed in triplicates.

3. RESULTS AND DISCUSSION

Phenolic compounds from plants have received considerable attention in recent times due to their promising biological activities [5,15]. Our study indicates that *Coula edulis* seed is rich in phenolics, flavonoids and ascorbic acid, but their concentrations were dependent on the polarity of the solvent (Table 1). Total phenolics in the extracts were in the range 18.6 -28.3 mgGAE/g, with the acetone extract being the richest. Flavonoids were less than phenolics, with the aqueous extract having the highest content (13.9 mgQE/g), and the acetone extract the lowest (9.2 mgQE/g). Ascorbic acid increased with increasing polarity of the solvent (14.6 - 49.3 mgAAE/g). However, results obtained are higher than that reported by Oktay [16] for ethanol and water extracts from seeds of *Foeniculum vulgare*.

The antioxidant activity of the seed extracts were assessed by the DPPH and ABTS assays. In the DPPH assay, extracts from seeds of *Coula edulis* exhibited effective radical scavenging activity in a dose dependent manner (Fig. 1). At 300 µg/ml, the acetone, ethanol and aqueous extracts were able to scavenge 71.1%, 79.1% and 75.3% of the DPPH radical respectively. Acetone extract showed the highest DPPH scavenging activity the aqueous extract showed the lowest activity. Based on IC_{50} values, DPPH radical scavenging activity decreased in the order: acetone extract (IC_{50} = 19.2 µg/mL) > ethanol extract (IC_{50} =30.2 µg/mL) > water extract (IC_{50} =75.1 µg/mL). Our reports are higher than values obtained from hydroalcoholic seed extracts of *Annona coriacea* and *Annona sylvatica* which had IC_{50} values of 330.55 + 2.34 and 724.14 + 17.79 µg/mL respectively [17]. Also, DPPH activity (IC_{50}) correlated positively with flavonoids (R^2 = 0.9997), suggesting a greater contribution from flavonoids; this is in consonance with reports in literature [18]. In the ABTS assay, the aqueous extract exhibited the highest activity (IC_{50}= 25.2 µg/mL) while the ethanol extract showed the lowest activity (IC_{50}= 53.2 µg/mL); the activity of the acetone extract was close to that of the aqueous extract (IC_{50}= 26.5 µg/mL). Lower ABTS values were reported for hexane seed extracts of *Nephelium lappaceum* [19]. These results reveal that the aqueous and acetone extracts of *Coula edulis* seeds are better scavengers of the proton radicals than the ethanol extract based on the ABTS model. However, in consonance with our findings, the antioxidant potential of plants is attributed to their high DPPH scavenging activity [11].

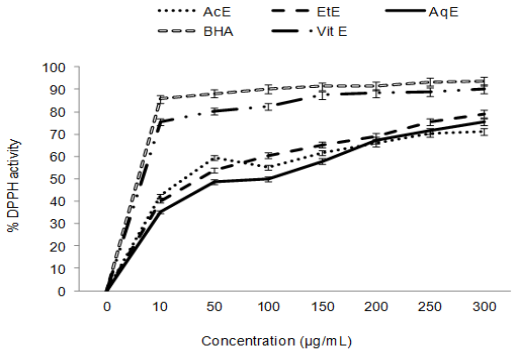

Fig. 1. DPPH radical scavenging activity of *Coula edulis* seed extracts

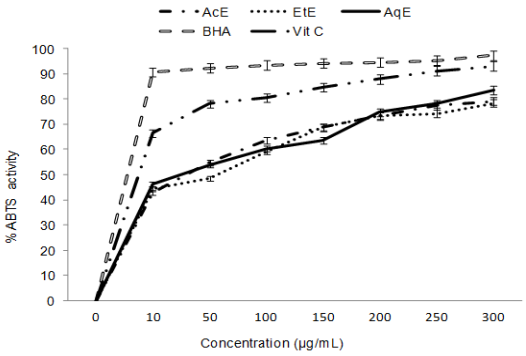

Fig. 2. ABTS radical scavenging activity of *Coula edulis* seed extracts

In addition to the DPPH and ABTS activity of the seed extracts, the reducing power of the extracts was also evaluated. This gives a measure of the electron donating ability of the extracts, and is an important mechanism of antioxidant activity. Like the antioxidant activity, the reducing power of the extracts increased with concentration. The aqueous extract exhibited the highest reducing power (IC_{50} = 18.3 µg/mL), followed by the ethanol extract (IC_{50} = 43.3 µg/mL) and the lowest reducing power was shown by the acetone extract (IC_{50} = 87.9 µg/mL). Reducing power is associated with the presence of reductones [20]. Our result indicate promising reducing power for extracts of *Coula edulis* seeds, capable of breaking free radical chains by hydrogen donation or prevention of peroxide formation. In comparison with other studies, our values are higher than reports for seeds of *Vitis vinifera* [21].

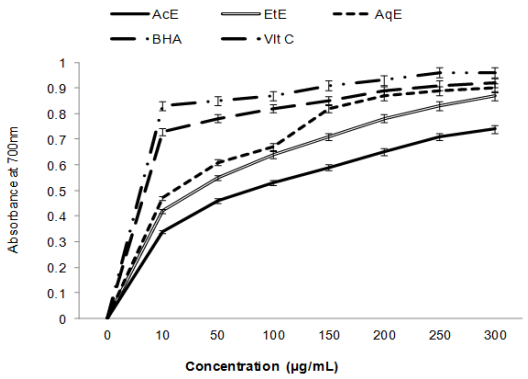

Fig. 3. Reducing power of *Coula edulis* seed extracts

Fe^{2+} chelating ability of the seed extracts was also evaluated. Fe^{2+} can undergo Fenton type reactions, forming reactive hydroxyl radicals which may contribute to oxidative stress [22] Thus, the ability of substances or plant extracts to chelate Fe^{2+} indicates an important mechanism of antioxidant action as this prevents transition metal ion from catalysing hydrogen peroxide decomposition and Fenton type reactions. It is evident from our results that extracts of *Coula edulis* seeds demonstrated potent Fe^{2+} chelating abilities and this increased with increase in extract concentration (Fig. 4). Based on IC_{50} values, the acetone extract demonstrated the highest Fe^{2+} chelating ability. Observed trend was: acetone extract (IC_{50}= 46.2 µg/mL) > ethanol extract (IC_{50} = 74.2 µg/mL) > aqueous extract (IC_{50} = 100.2 µg/mL). However, these values were inferior to the metal chelating ability of the standard, EDTA.

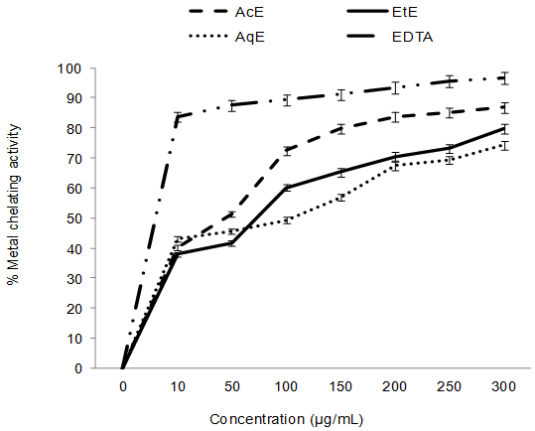

Fig. 4. Metal chelating activity of *Coula edulis* seed extracts

Table 1. Total phenolics, flavonoids, ascorbic acid and antioxidant activity of *Coula edulis* seed extract

	Extract / Control					
	AcE	**EtE**	**AqE**	**BHA**	**Vit C**	**EDTA**
Total Phenolics[a]	28.3 ± 0.9	21.4 ± 0.8	18.6 ± 0.6	-	-	-
Flavonoids[b]	9.2 ± 0.5	10.2 ± 0.6	13.9 ± 0.8	-	-	-
Ascorbic acid[c]	14.6 ± 0.3	39.4 ± 0.5	49.3 ± 0.7	-	-	-
DPPH activity*	19.2 ± 0.5	30.2 ± 0.4	75.1 ± 0.3	6.1 ± 0.3	7.2 ± 0.4	-
ABTS activity*	26.5 ± 0.4	53.2 ± 0.7	25.2 ± 0.8	7.5 ± 0.2	5.8 ± 0.3	-
MC activity*	46.2 ± 0.9	74.2 ± 1.1	100.2 ± 1.7			6.8 ± 0.3
Reducing power*	87.9 ± 2.1	43.3 ± 1.7	18.3 ± 0.7	5.3 ± 0.2	6.9 ± 0.3	

**IC_{50} value (µg/mL) is the effective concentration at which DPPH and ABTS radicals were scavenged by 50%, ferrous ions were chelated by 50%, and theabsorbance was 0.5 for reducing power. IC_{50} value was obtained by interpolation; a = mgGAE/g; b = mgQE/g; c = mgAAE/g*

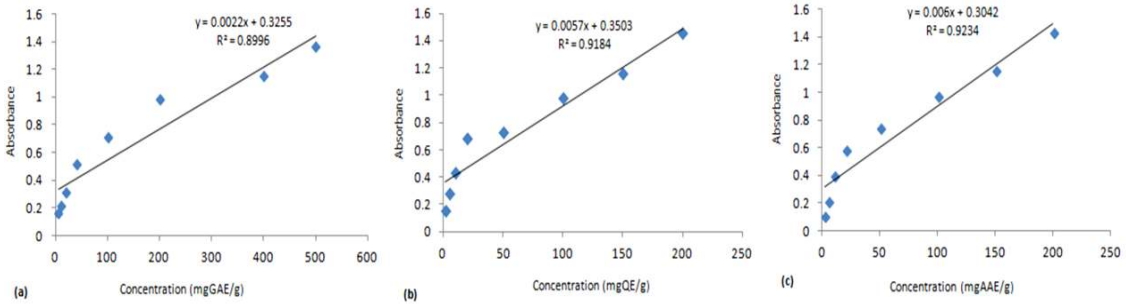

Fig. 5. Standard calibration curve for (a) Total phenolics (b) Flavonoids (c) Ascorbic acid

4. CONCLUSION

The present study has shown that *Coula edulis* seed is rich in total phenolics, flanonoids and vitamin C, with solvent dependent variations in their amounts. DPPH and ABTS assays revealed that the extracts exhibited promising antioxidant activities, with good metal chelating and reducing properties. Overall, the acetone extract was richer in total phenolics, showed higher antioxidant activities in the DPPH assay and could chelate metals most; while the aqueous extract contained higher amounts of flavonoids, vitamin C and exhibited good antioxidant potentials in the ABTS assay.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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