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Research Paper

Comparative evaluation of *in vitro* antioxidant properties of *Cajanus cajan* seed and *Moringa oleifera* leave extracts

ABSTRACT

Aims: This study was carried out to compare the antioxidant potentials of hydro-methanolic extracts of seeds of *Cajanus cajan* and the leaves of *Moringa oleifera*. These plants have been implicated in alleviating the oxidative stress related conditions.
Place and Duration of Study: Department of Biochemistry, Federal University of Agriculture, Abeokuta and Covenant University Sango, Nigeria, between August 2012 and September, 2012.
Methodology: : Different assays for determining antioxidant activities were used to compare the antioxidant potentials of the plants: hydroxyl radical scavenging assay, ascorbic acid value, total polyphenols, total flavonoids content, ferric reducing antioxidant power, 2,2-azino-bis (3-ethylbenzthiazoline-6- sulphonic acid (ABTS) scavenging assay, 2,2-diphenyl-2-picrylhydrazyl (DPPH) and inhibition of lipid peroxidation (lipoprotein).
Results: The total phenolic contents of *Cajanus cajan* and *Moringa oleifera* were 83.0 ±0.019 and 541.0 ± 0.021 mg gallic acid equivalents per gram dry weight (mg/GAE/g DW) respectively. Also, the results obtained for total flavonoids in *Cajanus cajan* and *Moringa oleifera* were 46.0±.009 and 645.0±0.101 mg pyrocatechol equivalent per gram dry weight respectively. It was observed that *Moringa oleifera leaves* extract has significantly higher antioxidant potentials than the *Cajanus cajan* seeds extract ($P < 0.05$).
Conclusion: Further research in determining the benefits of *Moringa Oleifera* leaves and *Cajanus cajan seeds* extracts due to their antioxidant potential is therefore recommended.

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Keywords: antioxidant, Moringa oleifera, Pigeon pea, flavonoids.

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1. INTRODUCTION

Moringa oleifera is the most widely cultivated species of a monogeneric family of Moringaceae. It has been given several names including horseradish tree, drumstick tree, benzolive tree, kelor, marango, mlonge, moonga, mulangay, nébéday, saijhan, sajna and ben oil tree. [1, 2] It is a rapidly-growing tree utilized worldwide. *Moringa oleifera* is considered one of the world's most useful trees, as almost every part of the tree has some nutritional, medicinal and other beneficial properties. [3] The medicinal properties have been attributed to its various parts; the roots, bark, leaves, flowers, fruits, and seeds.[4, 5] Though, the dry leaves of *Moringa oleifera* are widely utilized in the developing countries as a good source of protein, calcium and anti-oxidizing agents. The leaves, especially young shoots, are eaten as greens, in salads, in vegetable curries, and as pickles. The leaves can be eaten fresh, cooked, or stored as dried powder for many months without refrigeration, and

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reportedly without loss of nutritional values.[3] However, antioxidant properties are not limited to *Moringa oleifera* alone; other plants like *Cajanus cajan* have also been identified.

Cajanus cajan millsp.(Pigeon pea), also known as red gram, Congo pea, gungo pea, no eye pea, dhal, gandul, gandure, frijol de árbol, and pois cajan, a herbaceous plant of the botanical family Fabaceae occurs in several varieties. It is known as pigeon pea (English), Otili (Yoruba), and Waken turawa (Hausa). In developing tropical countries Pigeon peas are widely consumed. An excellent source of protein, the seeds and the pods are eaten as a vegetable, as a flour additive to other foods, in soups, and with rice. Preparations of the leaves are used to treat jaundice, the extracts or components of pigeon pea are commonly used all over the world for the treatment of diabetes, dysentery, hepatitis and measles, as a febrifuge to stabilize the menstrual period.[6] Nowadays, pigeon pea leaves are used for the treatment of wounds, bedsores and malaria [7]. Protective effects of extracts from pigeon pea leaf against hypoxic-ischemic brain damage and alcohol-induced liver damage have also been reported.[8] Chemical constituent investigations have indicated that pigeon pea leaves are rich in flavonoids and stilbenes, which are considered responsible for the beneficial efficacies of pigeon pea leaves on human health inflammation, and sores of the mouth [9, 10].

Moringa oleifera leave extracts and *Cajanus cajan* seed extracts are widely used as a result of their antioxidant properties. Antioxidants are compounds that inhibit or terminate the oxidation process which results in generation of free radicals and reactive oxygen species. They help to scavenge free radicals within the human body. Free radicals impair the proper functioning of the immune system and they have been implicated in various disease conditions such as diabetes mellitus, cancer, atherosclerosis, arthritis and neurodegenerative diseases as well as ageing process.[11, 12] The antioxidant ability of *Moringa oleifera* leave extracts and *Cajanus cajan* seed extracts have been documented. It was suggested that pigeon pea leaf extracts may be valuable natural antioxidant sources and are potentially applicable in both medicine and the healthy food industry.[13] The extent of the antioxidant properties of both *Moringa oleifera* leave extracts and *Cajanus cajan* is yet to be fully elucidated and compared with a substance with standard antioxidant properties. The aim of this work is therefore to investigate and compare the antioxidant properties of the Cajanus cajan seed extracts and Moringa oleifera leaves extracts with antioxidant properties of Vitamin C.

2. MATERIAL AND METHODS

2.1 Plant materials

The leaves of *Moringa oleifera* were collected from a biological garden in Ibadan Oyo state Nigeria and the herbarium specimens were deposited in Forestry Research Institute of Nigeria, Ibadan, Nigeria and the *Cajanus cajan* seeds was purchased from Bodija Market Ibadan. The plant materials were identified and authenticated by taxonomists at Forestry Research Institute of Nigeria, Ibadan, Nigeria. (Ugbogu O. A. & Shosanya O. S. Voucher Specimen no.108486)

Chemicals

2-Thiobarbituric acid (TBA), trichloroacetic acid, and 2, 2-diphenyl-2- picryl hydrazyl (DPPH) were purchased from Sigma (Deisenhofen, Germany). The other chemicals were of analytical grades.

2.1.1 Preparation of plant extracts

Fresh leaves of *Moringa oleifera* collected, were air- dried in a shed and milled into powder. The powder (50g) was extracted in a round bottom flask with 80% methanol at room temperature for 48 hours with 1:10 sample to solvent ratio. After 48 hours, the extract was filtered through filter paper and dried using rotary evaporator.

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The seeds of *Cajanus cajan* were also milled to powder and then defatted using n-hexane in a soxhlet apparatus. The residue was later subjected to 80% methanolic extraction. The stock solution was prepared by dissolving 0.1g of extract in 10ml of distilled water.

2.2 ANTIOXIDANT ASSAYS

2.2.1 Quantification of total phenolic content

The Folin–Ciocalteu method was used for total phenolic content determination .[14] The Folin–Ciocalteu reagent (2.5 ml) was diluted with water (1:10, v/v), and mixed with 2 ml of 75 g/L aqueous solution of sodium carbonate. The resultant solution was added to 0.5 ml of the extract. The mixture was kept for 5 min at 50°C before measuring the absorbance at 760 nm. The total phenolic content was expressed as mg gallic acid equivalent (GAE)/ g of extract.

2.2.2 Estimation of Flavonoid Content

Total flavonoids contents were estimated both in methanolic extracts of *Moringa oleifera* leaves and *Cajanus cajan* seed using pyrocatechol as standard.[15] Briefly, 0.5mL aliquot extract was added to 75µL of 5% $NaNO_2$ solution. After 6 minutes, 150µL of a 10% $AlCl_3$ $6H_2O$ solution was added and the mixture was allowed to stand for another 5 minutes. Then, 0.5mL of 1 mol NaOH and 2.5mL of distilled water was added. The solutions were mixed and absorbance was measured at 510 nm using Labo Med Inc. Spectrophotometer (USA). All experiments were carried out in triplicates. Total flavonoids content was calculated as mg/pyrocatechol equivalent/gram of dry samples.

2.2.3 Total antioxidant capacity (TAC)

TAC was measured according to the method described by Oboh with slight modification [16]. The reacting mixture contained 75 µl of (2g of 2, 4 Dinitro phenyl hydrazine, and 230 mg of thiourea and 270 mg of $CuSO_4.5H_2O$ in 100ml of 5 M H_2SO_4), 50 µl of extract, 350 µl of distilled water and 100 µl of 13.3% (w/v) TCA. The mixture was incubated for 3hrs at 37^0c after which 500 µl of 65% (v/v) H_2SO_4 was added. Absorbance was read at 520nm and vitamin C content was calculated from the formula:

(Absorbance of sample/Absorbance of standard) x concentration of standard (1)

2.2.4 Ferric reducing antioxidant potential (FRAP) assay

The FRAP assay was conducted according to the method of Oboh with slight modification [16]. 50µl aliquot extract was mixed with 450µl of 200mM sodium phosphate buffer (PH 6.6) and 250µl 1%pottassium ferricyanide was added. The mixture was incubated at 50^0C for 20 minutes. 250 µl of 10% Trichloroacetic acid was added. The mixture was centrifuged at 2000 rpm for 10mins. 10µl of the supernatant was mixed with 500µl 0.1% $FeCl_3$. Absorbance of the reaction mixture was measured at 700nm using spectrophotometer. All tests were run in triplicates. Increase in the absorbance of the reaction mixture indicated increased reducing power.

2.2.5 DPPH Radicals Scavenging Activity

The scavenging effect of extract on DPPH free radical was measured accordingly [17]. 2ml of each sample solution was added to 2 ml of 0.1mM DPPH dissolved in 95% ethanol. The mixture was shaken and left for 30 min at room temperature, and the absorbance of resulting solution was read at 517nm. Ascorbic acid was used as positive reference. The experiment was carried out in triplicates. The scavenging effect was expressed as shown in the following equation:

$$\% \text{ inhibition} = [(A_{blank} - A_{sample})/A_{blank}] \times 100 \quad (2)$$

Where A_{blank} = absorbance of blank and A_{sample} = absorbance of extract samples. A lower absorbance represents a higher DPPH scavenging activity

2.2.6 Hydroxyl Radical Scavenging Activity

Hydroxyl radical scavenging activity is commonly used to evaluate the free radical scavenging effectiveness of various antioxidant substances [16]. It was measured by studying the competition between deoxyribose and the extracts for hydroxyl radicals generated from the Fe^{2+}/EDTA/H_2O_2 system (Fenton reaction). The hydroxyl radical attacks

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deoxyribose, which eventually results in the formation of thiobarbituric acid reacting substances (TBARS). Reaction mixture contained 60µL of 1.0mM $FeCl_3$, 90µL of 1.0mM 1,10-phenanthroline, 2.4mL of 0.2M phosphate buffer (pH 7.8), 150µL of 0.17 M H_2O_2, and 1.5mL of extract. After incubation at room temperature for 5min, the absorbance of reaction mixture was noted at 560nm. The hydroxyl radicals scavenging activity was calculated according to the following equation and compared with ascorbic acid as standard:

% Inhibition = [(AB – AE)/AB] x 100 (3)

Where AB was the absorbance of blank (without extract) and AE was the absorbance of tested samples.

2.2.7 ABTS radicals scavenging activity assay

ABTS radical scavenging activity of extracts was determined according to a standard method [18]. A stock solution of ABTS radicals was prepared by mixing 5.0 ml of 7 mM ABTS solution with 88 µl of 140 mM potassium persulfate, and kept in the dark at room temperature for 16 hrs. An aliquot of stock solution was diluted with phosphate buffer (5 mM, pH 7.4) containing 0.15 M NaCl in order to prepare the working solution of ABTS radicals to an absorbance of 0.70±0.02 at 734 nm. A 65µl aliquot of sample solution was mixed with 910 µl of ABTS radical working solution, incubated for 10 min at room temperature in the dark, and absorbance was measured at 734nm. The percent reduction of ABTS* to ABTS was calculated according to the following equation:

ABTS (%) = $[(A_0 - A_1)/Ao] \times 100$ (4)

A_0 = absorbance of control, A_1 = absorbance of sample

2.2.8 Inhibition of lipid peroxidation

The reaction mixtures contained 0.2% lecithin in 0.1mol/l phosphate buffer solution (PBS)(pH 7.4), 25µl of extract was dissolved in 0.1mg/ L PBS (pH7.4) and 50µL 25mmol/L $FeSO_4.7H_2O$, the total volume was 1ml . The mixture was incubated at 37^0C in a water bath for 1 hour. Reaction was stopped by adding 250µl 20% TCA. Followed by addition of 250µl of 0.8% TBA, the tubes was placed in boiling water bath for 20 minutes and then crushed in ice bath before centrifuging at 2500rpm for 10mins. The rate of inhibition of the sample was assessed by measuring optical density of the supernatant at 532nm using spectrophotometer.

The antioxidative activity at the end of the assay time was expressed as reduction percent for peroxidation (RP %).

RP% = [(peroxidation indicator value without antioxidant) – (peroxidation indicator value with antioxidant) / (peroxidation indicator value without antioxidant)] x 100. (5)

2.3 Statistical Analysis

Data were entered and analyzed using SPSS 21 for Window software (SPSS Inc., Chicago, IL). Mean value of Polyphenols mg/g Dry Weight and Flavonoids mg/g Dry Weight were compared using independent t-test. Comparison of Anti-oxidant properties of Vitamin C, *Cajanus cajan*, and *Moringa oleifera* using different antioxidant Assay was done using one way Analysis of variance (ANOVA). *P*-value less than 0.05 were considered statistically significant.

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3. RESULTS AND DISCUSSION

3.1 Extract yield, total Phenolic and total flavonoids

The methanolic extraction yielded 21.24% and 26.66% dry weight for *Cajanus cajan* and *Moringa oleifera* respectively.
Table [1] shows the content of phenolic compounds that were measured in terms of gallic acid equivalents. The total phenolic contents in the *Cajanus cajan* and *Moringa oleifera* was 83.0 ± 0.019 and 541.0 ± 0.021 mg Gallic acid equivalents per gram of extract respectively.
The flavonoids content of *Cajanus cajan* and *Moringa oleifera* was 46.0 ± 0.009 and 645.0 ± 0.101mg pyrocatechol equivalent/g DW respectively. The higher total phenolic content and total flavonoids content were obtained from *Moringa oleifera* leave extract.

Table 1. Polyphenols and flavonoids contents of methanolic extracts of the leaves of *Moringa oleifera* and *Cajanus cajan* seeds.

Samples	**Polyphenols mg/gDry Weight** **Mean ± Standard Deviation**	**Flavonoids mg/g Dry Weight** **Mean ± Standard Deviation**	**p value**
Moringa oleifera leaves	541.0 ± 0.021	645.0 ± 0.101	<0.001
Cajanus cajan seeds	83.0 ± 0.019	46.0 ± 0.009	<0.001

3.2 Antioxidant assays

3.2.1 <u>DPPH free radical scavenging activity</u>
The DPPH assay results showed that the antioxidant property of Moringa oleifera is significantly different from that of *Cajanus cajan* as observed in Table 2. Methanol extract of Moringa leaves exhibited higher radical scavenging potential than the seed of *Cajanus cajan* (67.157± 0.081 and 26.440 ± 5.068% inhibition). The positive control, ascorbic acid showed maximum scavenging effect, (73.863 ± 0.541% inhibition).
3.2.2 <u>Hydroxyl (OH^-) radical scavenging activity</u>
Hydroxyl (OH^-) free radical scavenging potential of the *Moringa oleifera* extracts was found to be higher than that of the reference compound ascorbic acid and *Cajanus cajan*. The EC50 values of ascorbic acid, *Moringa oleifera* and *Cajanus cajan* were 168.35± 13.41 and 428.37 ± 17.46 and 60.0± 6.41 mg/ml, respectively.
3.2.3 <u>Total ascorbic acid content</u>
Table [2] showed that total ascorbic acid content in *Moringa oleifera* extract (28.02 ± 0.136 mg/g DW) was higher than what was obtained in *Cajanus cajan* (12.48 ± 0.591mg/g DW) while reference vitamin C (100 ±0.00 mg/g) had maximum reducing ability.
3.2.4 <u>Ferric reducing antioxidant power</u>
In the reducing power assay, the presence of reducing agents in the sample resulted in the reduction of Fe^{3+} to Fe^{2+} by donating an electron. Amount of Fe^{2+} complex was monitored by

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measuring the formation of Perl's blue at 700 nm. The extracts could reduce the most Fe^{3+} ions, as indicative from the results. The values for ferric reducing antioxidant power revealed that the moringa extract (34.70 ± 0.057) had a significantly higher reducing power than *Cajanus cajan* (33.0± 000) (P<0.05).The reference compound ascorbic acid had the highest reducing ability (50.00 ± 0.110).

3.2.5 **Antioxidant activity determined by the ABTS method**

ABTS assay of extracts demonstrated antioxidant capacities to scavenge radical action. The ABTS activity revealed that *Moringa oleifera* (99.860± 0.00 mg/L) had very close antioxidant activity to *Cajanus cajan* (99.587± 0.265mg/L) and vitamin C (69.57± 69.57mg/L).

3.2.6 Inhibition of lipid peroxidation

The result of inhibition of lecithin revealed that *Moringa oleifera* had higher antioxidant ability (3.458±2.37) than *Cajanus cajan* (9.631±2.65) while the reference ascorbic acid had the least inhibition (44.173±2.50). The lower the value obtained the higher the antioxidant activity**.**

Table 2. Comparison of Antioxidant properties of Vitamin C, *Cajanus cajan* and *Moringa oleifera* using different Anti-oxidants assay

	Mean ± Standard Deviation			
Anti-oxidant Assay*	**Vitamin C**	**Cajanus cajan**	**Moringa oleifera**	**p value**
DPPH	73.863 ± 0.541	26.440 ± 5.068	67.157 ± 0.081	<0.001
HO*	168.35 ± 13.41	60.00± 6.41	428.37 ± 17.46	0.043
ASCOBIC ACID	100.00 ± 0.000	12.48 ± 0.591	28.02 ± 0.136	<0.001
ABTS	69.570± 0.945	99.587 ± 0.248	99.860 ± 0.000	<0.001
LIPID PEROXIDATION	44.173 ± 2.509	9.631 ± 2.646	3.458 ± 2.370	< 0.001
FRAP	50.00 ± 0.110	33.00 ± 000	34.700 ± 0.057	<0.001

**DPPH: 2, 2-diphenyl-2-picrylhydrazyl*

ABTS: 2, 2-azino-bis (3-ethylbenzthiazoline-6- sulphonic acid

FRAP: Ferric Reducing Antioxidant Power

3.3 DISCUSSION

This study indicated that *Moringa oleifera* leave and *Cajanus cajan* seed extracts studied had antioxidant properties. The *in vitro* total antioxidant assays of the plant extracts revealed appreciable antioxidant potential compared with the standard ascorbic acid. However, a higher antioxidant property was found in *Moringa oleifera* leave. The extent of the antioxidant properties seems to be due to the presence of polyphenols and flavonoid that may act by donating electrons which help to scavenge free radicals.

Polyphenols and flavonoids have been shown to have potent antioxidant activity. [19-21] Phenolic compounds like flavonoids have been reported to possess significant antioxidant activities.[22] The phenolic content values obtained in this work shows that Moringa oleifera

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leaves and Cajanus cajan seeds had higher values than previously reported.[13]. Flavonoids in the leaves of Cajanus cajan was previously estimated.[7, 13] It was reported that different solvents yielded certain degree of flavonoids. Phenolic compounds have been found to be one of the most effective antioxidant constituents in plant foods, including fruits, vegetables and grains. [24] Similar antioxidant activities has been reported for vegetables.[25]
The reducing power of bioactive compounds has been reported to be associated with their antioxidant activity.[26] Among the oxygen radicals specifically, the hydroxyl radical is the most reactive which severely damages adjacent biomolecules such as proteins, DNA, nucleic acid and almost any biological molecule it touches. This damage causes aging, cancer and several diseases. In addition; this radical species is considered as one of the quick initiators of the lipid oxidation process, abstracting hydrogen atoms from unsaturated fatty acids.[26, 27] Therefore, the removal of hydroxyl radical is probably one of the most effective defenses of a living body against various diseases. The result of the DPPH scavenging activity assay in this study indicates that the extracts were potently active.

4. CONCLUSION

It was observed that *Moringa oleifera leaves* extract has higher antioxidant potentials than the *Cajanus cajan* seeds extract. The antioxidant activity of these extracts were directly proportional to the phenolic contents indicating that phenolic compounds are the major contributors to the antioxidant properties of *Moringa oleifera leaves* and *Cajanus cajan* seeds extract. Further research in determining the benefits of *Moringa Oleifera* leaves and *Cajanus cajan seeds* extracts due to their antioxidant potential is therefore recommended

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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