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Comparative Evaluation of *in vitro* Antioxidant Properties of *Cajanus cajan* Seed and *Moringa oleifera* Leaf Extracts

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Authors' contributions

This work was carried out in collaboration between all authors. Author AOA designed the study, author IFF managed the analyses of the study and wrote the first draft of the manuscript. Author IOS performed the statistical analysis and managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: This study was carried out to compare the antioxidant potentials of hydro-methanolic extracts of seed of *Cajanus cajan* and the leaf of *Moringa oleifera*. These plants have been associated with alleviating oxidative stress related conditions.
Place and Duration of Study: Department of Biochemistry, Federal University of Agriculture, Abeokuta and Covenant University Sango, Ogun state Nigeria, between August 2012 and September, 2012.
Methodology: Different analyses for determining antioxidant potentials were used to compare the antioxidant properties of the plants: hydroxyl radical scavenging test, ascorbic acid value, total polyphenols, total flavonoids content, ferric reducing antioxidant power, 2, 2-azino-bis (3-ethylbenzthiazoline-6- sulphonic acid (ABTS) scavenging test, 2,2-diphenyl-2-picrylhydrazyl (DPPH) and inhibition of lipid peroxidation (lipoprotein).

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Results: The total phenolic contents of *Cajanus cajan* and *Moringa oleifera* were 83.0 ±0.02 and 541.0 ± 0.02 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±.01 and 645.0±0.10 mg pyrocatechol equivalent per gram dry weight respectively. It was observed that *Moringa oleifera leaf* extract has significantly higher antioxidant potentials than the *Cajanus cajan* seeds extract (p<.05)
Conclusion: Data suggest that *Moringa oleifera leaf* extract had higher antioxdant potentials in comparison with that of *Cajanus cajan* seeds. Further research in determining the benefits of *Moringa Oleifera* leaf and *Cajanus cajan seed* extracts due to their antioxidant potential in animal model is therefore recommended.

Keywords: Antioxidant; Moringa oleifera; Pigeon pea; flavonoids.

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 phytochemical compositions of its various parts; the roots, bark, leaf, flowers, fruits, and seeds [4,5]. Though, the dry leaf of *Moringa oleifera* is 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 salads, in vegetable curries, and as pickles. The leaf can be eaten fresh, cooked, or stored as dried powder for many months without refrigeration, and reportedly without loss of nutritional values [3].

Cajanus cajan millsp. (Pigeon pea), an herbaceous plant of the botanical family Fabaceae occurs in several varieties. It is known as pigeon pea (English), Otili (Yoruba), and Waken turawa (Hausa). Other names of *Cajanus cajan include,* Pigeon pea, red gram, Congo pea, gungo pea, no eye pea, dhal, gandul, gandure, frijol de árbol, and pois cajan. In developing tropical countries Pigeon peas are widely consumed as an excellent source of protein. The seeds and the pods are eaten as vegetables and additives in soups. Preparations of the leaf are used to treat jaundice and the extracts of pigeon pea are commonly used all over the world for the treatment of diabetes, dysentery, hepatitis, measles and 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 leaf is rich in flavonoids and stilbenes, which are considered to be responsible for its beneficial efficacies. [9,10].

Moringa oleifera leaf 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 leaf and *Cajanus cajan* seed extracts have been documented [13]. It was suggested that pigeon pea leaf extracts may be valuable natural antioxidant sources and are potentially applicable in both medicine and food industry [13]. The extent of the antioxidant properties of both *Moringa oleifera* leaf 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 and Moringa oleifera leaf extracts with antioxidant properties of Ascorbic acid.

2. MATERIAL AND METHODS

2.1 Plant Materials

The leaf 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 were purchased from Bodija Market Ibadan. The plant materials were identified and authenticated by taxonomists. (Ugbogu O.A. & Shosanya O. S. Voucher Specimen no.108486) at Forestry Research Institute of Nigeria, Ibadan, Nigeria.

2.1.1 Chemicals

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

2.1.2 Preparation of plant extracts

Fresh leaf 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. 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 Analysis

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* leaf and *Cajanus cajan* seed using pyrocatechol as standard[15]. Briefly, 0.5mL aliquot extract

was added to 75µL of 5% $NaNO_2$ (sodium nitrite) 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 molar 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 ascorbic acid content (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 37ºC after which 500 µl of 65% (v/v) H_2SO_4 was added. Absorbance was read at 520nm and ascorbic acid capacity was calculated from the formula:

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

2.2.4 Ferric reducing antioxidant potential (FRAP) test

The FRAP test 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% potassium ferricyanide was added. The mixture was incubated at 50ºC 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 500 µl 0.1% $FeCl_3$. Absorbance of the reaction mixture was measured at 700 nm 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 Potentials

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 potential.

2.2.6 Hydroxyl Radical Scavenging potential

Hydroxyl radical scavenging potential 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 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 potential was calculated according to the following equation and compared with ascorbic acid as standard:

$$\% \text{ Inhibition} = [(AB - AE)/AB] \times 100 \quad (3)$$

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

2.2.7 ABTS radicals scavenging potentials

ABTS radical scavenging potential 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:

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

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 potential at the end of the analysis 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 Ascorbic acid, *Cajanus cajan*, and *Moringa oleifera* using different antioxidant Assay was done using one way analysis of variance (ANOVA). *P-value* less than .05 were considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Extract Yield, Total Polyphenolic 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 ± 0.02 and 541.0 ± 0.02 mg Gallic acid equivalents per gram of extract respectively.

The flavonoids content of *Cajanus cajan* and *Moringa oleifera* was 46.0 ± 0.01 and 645.0 ± 0.10mg pyrocatechol equivalent/g DW respectively. The higher total phenolic content and total flavonoids content were obtained from *Moringa oleifera* leaf extract.

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

Samples	Polyphenols mg/g Dry Weight Mean ± Standard Deviation	Flavonoids mg/g Dry Weight Mean ± Standard Deviation	p-value
Moringa oleifera leaf	541.0 ± 0.02	645.0 ± 0.10	<0.001
Cajanus cajan seeds	83.0 ± 0.02	46.0 ± 0.01	<0.001

3.2 Antioxidant Analysis

3.2.1 DPPH free radical scavenging Potential

The DPPH analysis 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 leaf exhibited higher radical scavenging potential than the seed of *Cajanus cajan* (67.157± 0.08 and 26.440±5.07% inhibition). The positive control, ascorbic acid showed maximum scavenging effect, (73.863±0.54% inhibition).

3.2.2 Hydroxyl (OH^-) radical scavenging potential

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, 428.37±17.46 and 60.0±6.41 mg/ml, respectively.

3.2.3 Total ascorbic acid content

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 ascorbic acid (100±0.00 mg/g) had maximum reducing ability.

Table 2. Comparison of Antioxidant properties of Ascorbic acid, *Cajanus cajan* and *Moringa oleifera* using different Anti-oxidants parameters

	Mean±Standard Deviation			
Anti-oxidant parameters*	**Ascorbic acid**	**Cajanus cajan**	**Moringa oleifera**	**p value**
DPPH	73.863±0.54	26.440±5.07	67.157±0.08	<0.001
HO*	168.35±13.41	60.00±6.41	428.37±17.46	0.043
Total Ascorbic Acid	100.00±0.00	12.48±0.59	28.02±0.14	<0.001
ABTS	69.570±0.95	99.587±0.25	99.860±0.00	<0.001
LIPID PEROXIDATION	44.173±2.51	9.631±2.65	3.458±2.37	< 0.001
FRAP	50.00±0.11	33.00±00	34.700±0.06	<0.001

**DPPH: 2, 2-diphenyl-2-picrylhydrazyl.*
HO: Hydroxyl radical scavenging potential*
ABTS: 2, 2-azino-bis (3-ethylbenzthiazoline-6- sulphonic acid
FRAP: Ferric Reducing Antioxidant Power

3.2.4 Ferric reducing antioxidant potentials

In the reducing potential determination, 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 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±0.06) had a significantly higher reducing power than *Cajanus cajan* (33.0±00) (P<0.05).The reference compound ascorbic acid had the highest reducing ability (50.00±0.11).

3.2.5 Antioxidant potential determined by the ABTS method

ABTS analysis of extracts demonstrated antioxidant capacities to scavenge radical action. The ABTS analysis revealed that *Moringa oleifera*, Cajanus cajan and Ascorbic acid had (99.86±0.00 mg/L), (99.587±0.27mg/L) and (69.57±69.57mg/L) antioxidant values respectively.

3.2.6 Inhibition of lipid peroxidation

The result of inhibition of lecithin revealed that *Moringa oleifera* had lower lipid peroxidation inhibition value (3.458±2.37) compared to either *Cajanus cajan* (9.631±2.65) or the reference ascorbic acid (44.173±2.50).

3.3 DISCUSSION

This study compared the antioxidant properties of *Cajanus cajan* seed extracts and *Moringa oleifera* leaf extracts with antioxidant properties of Ascorbic acid. The *in vitro* total antioxidant analysis of the plant extracts revealed appreciable antioxidant potential compared with the standard ascorbic acid. However, a higher antioxidant property was found in *Moringa oleifera* leaf. The extent of the antioxidant properties seems to be due to the presence of polyphenols and flavonoids.

Phenolic compounds like flavonoids have been reported to possess significant antioxidant properties [19-22]. The phenolic content values obtained in this work shows that *Moringa*

oleifera leaf and *Cajanus cajan* seeds were similar to previous study [23]. Flavonoids in the leaf of *Cajanus cajan* was previously estimated [7,13]. Phenolic compounds have been found to be one of the most effective antioxidant constituents in plant foods, including fruits, vegetables and grains [24,25].

Among the oxygen radicals, 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.

4. CONCLUSION

It was observed that *Moringa oleifera leaf* extract has higher antioxidant potentials than the *Cajanus cajan* seed extract.The antioxidant potentials 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 leaf* and *Cajanus cajan* seeds extract. Further research in determining the benefits of *Moringa oleifera* leaf and *Cajanus cajan seed* extracts due to their antioxidant potential in animal model is therefore recommended.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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