Research Article:

Evaluation of the Antioxidant Potential of Aqueous Extracts of *Moringa oleifera* Leaf and *Cocos nucifera* Husk: A Comparative Analysis

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ABSTRACT

Background: Oxidative Stress (OS) can result in several diseases, such as cancer or neurodegenerative illnesses. Plant antioxidants can supplement the body's antioxidant system, thereby reducing cell oxidation resulting from OS.

Objectives: In this research, the antioxidant potential of aqueous husk extract of *Cocos nucifera* and aqueous leaf extract of *Moringa oleifera* was evaluated and compared.

Methods: Total Phenolic Contents (TPCs), iron-chelating ability, Ferric Reducing Antioxidant Power (FRAP), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging antioxidant activity of aqueous husk extract of *Cocos nucifera* and aqueous leaf extract of *Moringa oleifera* are determined spectrophotometrically at varying concentrations (25, 50, 75, 100, 125µg/mL) and 1-sample t test statistical analysis was done using GraphPad Prism. The statistical significance was set at $P<0.05$.

Results: The aqueous husk extract of *Cocos nucifera* and aqueous leaf extract of *Moringa oleifera* possess antioxidant activities at all tested concentrations. Significant increases were observed in TPCs, iron-chelating ability, and FRAP of aqueous leaf extract of *Moringa oleifera* compared with aqueous husk extracts of *Cocos nucifera* at the same concentration. In contrast, a significant decrease in DPPH scavenging activities was observed.

Conclusion: Both aqueous husk extract of *Cocos nucifera* and aqueous leaf extract of *Moringa oleifera* are potent antioxidant agents and could be useful in supplementing the endogenous antioxidant system. Albeit, the aqueous leaf extract of *Moringa oleifera* is a more powerful antioxidant agent.

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Introduction

Reactive Oxygen Species (ROS) production is an inevitable phenomenon associated with aerobic metabolism; however, exposure to several exogenous sources of oxidant molecules can increase ROS production. Reactive oxygen species participate in different cellular processes at a relatively low level but are extremely harmful to organisms at high concentrations. A cell is said to be in a state of Oxidative Stress (OS) when the concentration of ROS overwhelms the body's antioxidant system. This state can be detrimental to the body system as it can destroy major body biomolecules (protein, lipid, and nucleic acid), triggers enzyme inhibition, and commit cell suicide [1-8]. Substances capable of inhibiting this detrimental effect are called antioxidants. One of the mechanisms through which antioxidants fulfill their duties is donating electrons to stabilize ROS [9]. Antioxidants can be classified as endogenous (natural) and exogenous (synthetic) [10]. The possible carcinogenic effects of synthetic antioxidants have drawn the attention of researchers toward natural antioxidants [11-13]. Over the years, plants have been established as a good source of exogenous antioxidants, with two-thirds of the world's plant species proven to have potential antioxidant agents [14]. The interest in the exogenous plant antioxidants was first evoked by discovering and subsequent isolation of ascorbic acids from plants [15]. Phytochemicals characterized by antioxidant activity remove free radicals and inhibit oxidative reactions by oxidizing [16]. These antioxidants are polyphenolic compounds found in all plants and their parts (tree bark, stalks, leaves, fruits, roots, flowers, pods, and seeds) [17]. Among the plants' antioxidants are ascorbic acid (vitamin C), tocopherol (vitamin E), carotene (vitamin A), polyphenol metabolites [18], and microelements such as Se, Zn, Mn, and Cu [19]. In recent years, interest has grown in using natural antioxidants to prevent or treat different diseases resulting from OS [20, 21].

Moringa oleifera is the most cultivated plant in the Moringaceae family. It is cultivated chiefly for its nutritional and medicinal properties. The plant is native to tropical, subtropical, and semi-arid climates, where it naturally grows [22]. It is commonly called the "tree of life" due to its numerous nutritious, medicinal, and industrial benefits [23, 24]. The plant is becoming increasingly more appreciated worldwide, as almost every part of the tree (pods, seeds, leaves, barks, roots, flowers) can be used for medicinal purposes [25, 26]. These therapeutic properties are generally attributed to the rich phytochemical composition of *Moringa oleifera*. Phytochemicals such as vanillin, phenolic acids flavonoids, carotenoids, ascorbates, tocopherols, β-sitosterol, kaempferol, moringin, omega fatty acids, alkaloids, volatile oil, saponin, phenol, tannin, and flavonoid have been reported to be found in its different parts [22, 27]. *Moringa oleifera* is a good source of antioxidants [27-29].

Cocos nucifera l., popularly called coconut tree, is a perennial monocot tree belonging to the Arecaceae family. The tree, native to southeast Asia and Melanesia, is distributed throughout the tropics and sub-tropics of the world [30]. Its uses range from domestic to therapeutic [31]. However, the therapeutic properties are a function of the various phytochemicals present in the plant [31]. Dyana and Kanchana [32] found tannins, alkaloids, flavonoids, phenols, carbohydrates, amino acids, and phytosterols in the various *Cocos nucifera* extracts. The presence of polyphenolic compounds in the plant suggests its antioxidant properties.

So far, there is no information regarding the comparative study of the antioxidant potential of these plants. Therefore, this research compares the antioxidant potential of the aqueous leaf extract of *Moringa oleifera* and the aqueous husk extract of *Cocos nucifera*.

Materials and Methods

Chemicals and reagents

Tris-HCl, sodium hydroxide, iron (II) sulfate, 1,10-phenanthione, Folin-Ciocalteu, methanol, sodium bicarbonate Tripyridyl Triazine (TPTZ), sodium acetate, iron (III) chloride, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were prepared from Sigma Aldrich Chemic. Co. (GmbH Germany).

Plants

Fresh *Moringa oleifera* and *Cocos nucifera* plants were obtained at Aroje, Ogbomoso, Nigeria, and identified at the Department of Biology, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. The plants' herbarium numbers are LHO 579 and LHO 580, respectively.

The leaves of *Moringa oleifera* were air-dried and soaked (1 g/20 mL) in distilled water for 24 h; it was then sieved to obtain the aqueous extract. Similarly, the husk of *Cocos nucifera* was air-dried, ground, and soaked (1 g/20 mL) for 24 h, after which it was sieved to obtain the aqueous extract.

Methodology

Iron-chelating ability

The iron-chelating ability of the extracts was determined according to the modified method of Minotti and Aust [33]. About 0.7 mL of Tris-HCl was added to 100 µL of the extract. Then, 0.9 mL of 0.9% saline was added to the mixture. Next, 0.06 mL of iron (ii) sulfate was added and then incubated for five minutes at room temperature. Afterward, 2 drops of 0.25% 1,10-phenanthroline were added, and the absorbance rate was measured at 510 nm.

$$\text{Iron-chelating ability (\%)} = \frac{(Abs\ of\ Control - Abs\ of\ Sample) \times 100)}{Abs\ of\ Control}$$

DPPH scavenging antioxidant activity

The antioxidant activity of the extract against DPPH was determined using the modified method of Gyamfi et al. [34]. About 1 mL of 0.2 mM 70% methanol solution of DPPH (Sigma Aldrich) was added to 1 mL of the extract and left for 30 min at room temperature in the dark. The absorbance of the resulting yellow-colored solution was read at 517 nm. The control was carried out using 2 mL DPPH solution without the test samples. The DPPH free radical scavenging ability was subsequently calculated.

$$\text{DPPH scavenging ability (\%)} = \frac{Abs.of\ DPPH\ alone - Abs.of\ sample\ alone}{(Abs.of\ DPPH\ alone) \times 100}$$

Determination of total phenolic contents

The total phenolic concentration of the samples was estimated spectrophotometrically according to the method of Singleton et al. [35]. About 100 µL of the extract was added to 0.4 mL of 7.5% sodium carbonate. Then, 0.5 mL of dilute 10% Folin-Ciocalteu reagent was added to the solution, and the absorbance of the resulting blue-colored solution was read after 30 min of incubation at 680 nm.

Determination of Ferric Reducing Antioxidant Power (FRAP)

The Ferric Reducing Antioxidant Power (FRAP) was determined according to the Oyaizu method [36]. About 1.5 mL of distilled water was added to 0.5 mL of the extract. Next, 0.8 mL of prepared FRAP reagent (a mixture of tripyridyl triazine, iron (iii) chloride, and sodium acetate) was added to the mixture.

Statistical analysis

Graphical representation was done using Microsoft Excel 2010, while 1-sample t test statistical analysis was done with GraphPad Prism 5.0. Differences between means were determined at a $P<0.05$.

Results

As shown in Figure 1, at all tested concentrations, the aqueous leaf extract of *Moringa oleifera* and aqueous husk extract of *Cocos nucifera* exhibit iron-chelating ability with the highest abilities of 47.71% and 40.94% at 125 µg/mL and 100 µg/mL, respectively.

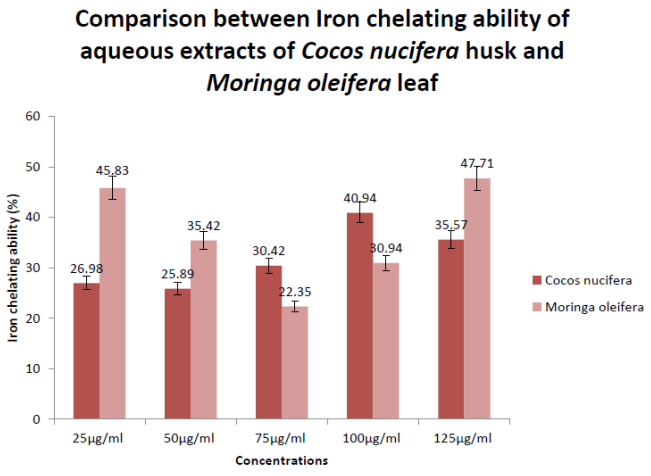

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Figure 1. Comparison between Iron chelating ability of aqueous extracts of Cocos nucifera husk and Moringa oleifera leaf.

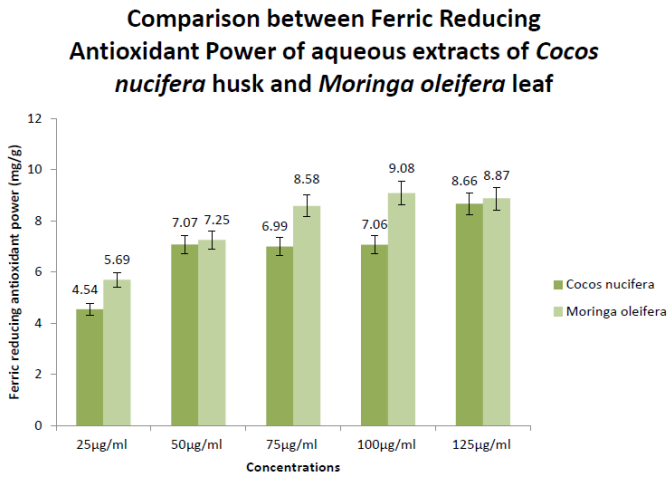

Figure 2. Comparison between Ferric Reducing Antioxidant Power of aqueous extracts of Cocos nucifera husk and Moringa oleifera leaf.

Figure 2 shows a somewhat concentration-dependent increase in ferric reducing antioxidant power of the aqueous leaf extract of *Moringa oleifera* and husk aqueous extract of *Cocos nucifera* with the highest ferric reducing antioxidant power observed at 100 µg/mL and 125 µg/mL, respectively.

According to Figure 3, both aqueous husk extract of *Cocos nucifera* husk and aqueous leaf extract of *Moringa oleifera* showed DPPH radical scavenging ability. While a concentration-dependent increase was observed in aqueous husk extract of *Cocos nucifera* with the highest DPPH radical scavenging ability of 97.94%, a somewhat concentration-dependent rise was observed in aqueous leaf extract of *Moringa oleifera* with the highest DPPH radical scavenging ability of 94.14%

As depicted in Figure 4, a concentration-dependent increase in Total Phenolic Contents (TPCs) is observed in aqueous husk extract of *Cocos nucifera*, with the highest TPCs of 15.22 mg/g. In comparison, the highest total phenolic contents observed in aqueous leaf extract of *Moringa oleifera* is 21.36 mg/g (at 100 µg/mL).

Discussion

Recently, interest has grown in using natural antioxidants to prevent or treat different diseases related to oxidative stress [20]. In this regard, plant-based antioxi-

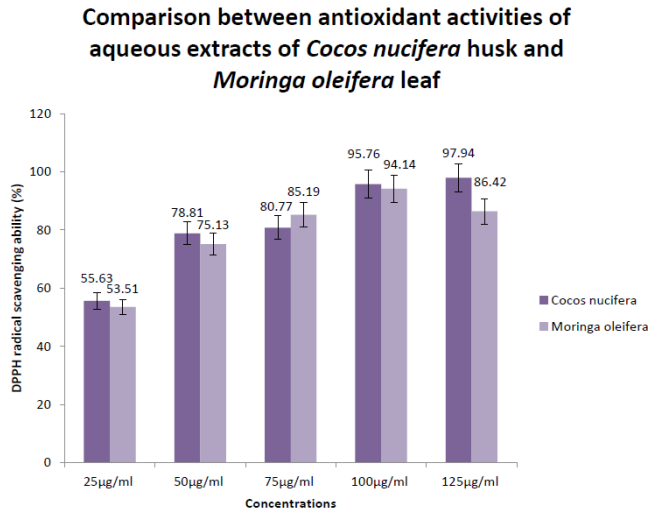

Figure 3. Comparison between antioxidant activities of aqueous extracts of Cocos nucifera husk and Moringa oleifera leaf.

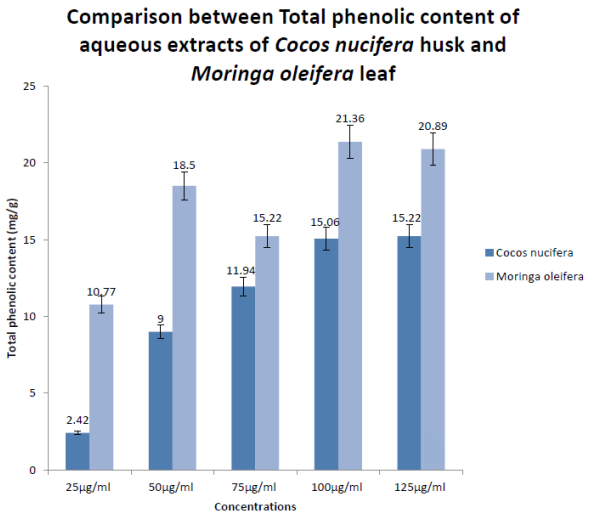

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Figure 4. Comparison between Total phenolic content of aqueous extracts of Cocos nucifera husk and Moringa oleifera leaf.

dants origins are of great interest because they contain significant antioxidant activity [37]. It is believed that two-thirds of the world's plant species have medicinal benefits, and almost all have the excellent antioxidant potential [14]. Thus, plants are a good source of antioxidant supplements that can be of great use in alleviating or preventing diseases resulting from an oxidative imbalance in living creatures.

Figure 1 shows that aqueous leaf extract of *Moringa oleifera* and aqueous husk extract of *Cocos nucifera* possess iron-chelating ability at all tested concentrations with *Moringa oleifera* showing significant increases ($P<0.05$) at 25, 50, 125 µg/mL and significant decreases ($P<0.05$) at 75 and 100 µg/mL when compared with *Cocos nucifera*. The use of iron chelation is a popular therapy for managing Fe^{2+}-associated oxidative stress in the brain. The ability of agents to chelate transition metals, specifically Fe^{2+}, has been considered a defense mechanism of antioxidant agents [38]. One of the ways antioxidant protects the cells is by reducing/chelating the transition metal composition of foods [39, 40]. The iron-chelating ability of plants is also an indicator of the neuroprotective property of the plants as iron is involved in the pathogenesis of Alzheimer disease and other diseases by multiple mechanisms [38].

Leaf aqueous extract of *Moringa oleifera* showed significant increases ($P<0.05$) in FRAP at all tested concentrations when compared to aqueous husk extract of *Cocos nucifera* (Figure 2). The ability of the plant extract to reduce Fe^{3+} to Fe^{2+} is suggested to be a novel antioxidant defense mechanism [41]. Therefore, this finding confirms that the plants considered are potent antioxidant agents.

The antioxidant activities of these plants were also assessed by their DDPH scavenging ability. As shown in Figure 3, aqueous husk extract of *Cocos nucifera* significantly increased ($P<0.05$) DPPH scavenging ability at all tested concentrations except at 75 µg/mL, where it shows a significant decrease ($P<0.05$) when compared with aqueous leaf extract of *Moringa oleifera*. DPPH has been reported to have antioxidants that scavenge free radicals and prevent stress. Due to the antioxidant activity, DPPH fights against chronic diseases caused by oxidative diseases. It prevents degenerative disorders like mutagenesis, carcinogenesis, and cardiovascular disturbances [42]. It also prevents proteins, lipids, and DNA damage [43].

According to Figure 4, at all tested concentrations, the aqueous leaf extract of *Moringa oleifera* shows significant increases ($P<0.05$) compared with the aqueous husk extract of *Cocos nucifera*. Phenolics are diverse secondary metabolites (flavonoids, tannins, hydroxycinnamate esters, and lignin), which possess antioxidant properties. Polyphenols contain an aromatic ring with –OH or OCH3 substituents that contribute to their biological activity, including antioxidant action. Polyphenols can chelate transition metal ions, directly scavenge molecular species of active oxygen, and inhibit lipid peroxidation by trapping the lipid alkoxyl radical. They also modify lipid packing order and decrease the fluidity of the membranes [44]. Phenolics are potent antioxidants and free radical scavengers [45, 46]. The antioxidant activity of

phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers [47-49]. They also protect DNA from oxidative damage, inhibit the growth of tumor cells, and possess anti-inflammatory and antimicrobial properties [50].

Conclusion

The results mentioned above confirm that aqueous husk extract of *Cocos nucifera* and aqueous leaf extract of *Moringa oleifera* are potent antioxidant agents and could be used in supplementing endogenous antioxidant systems as well as treating diseases caused by oxidative imbalance. The overall antioxidant activities of leaf aqueous extracts of *Moringa oleifera* are more significant than that of aqueous husk extract of *Cocos nucifera*.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Authors' contributions

Designed the work protocol and carried out the analyses: Olalekan Amos Akinyemi; Developed the manuscript and did the statistical analysis: Sunday Faith Oyelere; Oversee the plant identification process: Adeola Folasade Ehigie and Adijat Funke Ogundola; Proofread and edited the manuscript: Titilayo Eunice Ayoade; Approved the final manuscript for submission: All authors.

Conflict of interest

The authors declared no conflict of interest.

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