

Comparison of Antioxidant and Anti-hemolytic Activity of Aqueous Leaf Extracts of *Adansonia digitata* (Baobab) and *Moringa oleifera* (Moringa)

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Abstract

Plants contain biologically active compounds with properties such as antioxidant activity, anti-hemolytic activity, antimicrobial effect etc. However, these properties vary from plant to plant. This study aims to compare the *in vitro* antioxidant and anti-hemolytic activity of aqueous leaf extracts of *Adansonia digitata* and *Moringa oleifera*, two commonly consumed vegetables in Northern Nigeria. The antioxidant activity was assessed using DPPH radical scavenging and FRAP assays, while anti-hemolytic activity was evaluated in comparison to quercetin as standard. The percentage inhibition of DPPH radical scavenging activity of the extracts and the standard ascorbic showed that the aqueous leaf extract of *M. oleifera* exhibited higher antioxidant activity than *A. digitata* extract. The IC₅₀ of *M. oleifera* (3.01 mg/ml) was lower than *A. digitata* (3.48 mg/ml) with respect to the standard ascorbic acid (2.32 mg/ml). The FRAP assay also showed that extract of *M. oleifera* exhibited better antioxidant capacity than the *A. digitata* extract. The IC₅₀ of *M. oleifera* (2.96 mg/ml) was lower than *A. digitata* (3.36 mg/ml) in comparison to ascorbic acid (2.02 mg/ml). Also, percentage inhibitions of anti-hemolytic activity of aqueous leaf extracts of *A. digitata* and *M. oleifera*; and the standard quercetin showed that the aqueous leaf extract of *M. oleifera* exhibited higher anti-hemolytic activity than *A. digitata* extract. The IC₅₀ of *M. oleifera* (3.35 mg/ml) was lower than *A. digitata* (4.16 mg/ml) in comparison to quercetin (1.92 mg/ml). These findings suggest that *Moringa oleifera* may serve as a more suitable and potent agent against free radical mediated and hemolytic diseases.

Keywords: *Adansonia digitata*, Anti-hemolytic, Antioxidant, *Moringa oleifera*

1.0 Introduction

Plants have been used throughout history to provide nutrition, shelter, heating, wound healing, and disease treatment [1, 2]. A number of plants have been documented to have been used for medicinal purpose as far back as the 5000s B.C. Assyrians, Sumerians, Hittites, Egyptians, and Mesopotamians have long utilized herbs to treat various conditions [3, 4]. Before the advent of modern medicine, phytomedicines were utilized for centuries to treat a variety of ailments in Nigeria and other nations [5, 6]. Edible fruits, flowers, seeds, and leaves contain a variety of phytochemicals [7, 8] that have properties that protect the neurological, hepatic, cardio, and gastrointestinal systems [9, 10] and are linked to

health-promoting benefit like antioxidants, anti-inflammatory, anti-cancer, hypoglycemic, and anti-obesity compounds [11]. Numerous animal model studies have demonstrated the effectiveness of plant extracts in treating an array of diseases. New uses of these molecules appear as technology advances and scientific understanding of their mechanisms of action deepens. In all, available data indicates that although there are indications of the likely benefits of plant extracts, more research is necessary to ratify a more factual cause-and-effect relationship [12].

A key plant species in African ethnomedicine, *Adansonia digitata*, also called the baobab tree, has significant pharmacognostic potential because of its varied phytochemical composition and traditional

therapeutic uses. The tree is a fruit-producing member of the Bombacaceae family [13]. The baobab has an extremely wide range of uses, including food and beverages [14]. The leaves of this plant are especially rich in bioactive compounds like flavonoids, alkaloids, saponins, tannins, and essential micronutrients, which contribute to their hepatoprotective, anti-inflammatory, and antioxidative properties [15, 16]. Significant antimicrobial, antidiabetic, and immunomodulatory activities have been shown in recent scientific studies; this supports the traditional use of the plant preparations to treat microbial infections, malaria, diarrhea, and asthma [17]. The use of baobab extracts in chronic inflammatory and degenerative disorders has been further supported by the discovery of its strong radical scavenging activity and protective benefits against oxidative stress-induced cellular damage [17].

Moringa oleifera, also known as the "miracle tree," has been extensively recorded in both ethnopharmacological literature and current biomedical research due to its astonishing range of therapeutic capabilities. The leaves contain high concentrations of phytochemicals such as quercetin, kaempferol, chlorogenic acid, glucosinolates, and a variety of vital amino acids, vitamins, and minerals [18]. *M. oleifera* has traditionally been used to treat malnutrition, infections, and chronic inflammatory illnesses, and its pharmacological validation includes powerful antioxidative, anti-inflammatory, antidiabetic, antibacterial, and anticancer properties [19, 20]. Recent research has underlined its protective benefits on hepatic, renal, and cardiovascular tissues via regulating redox state and pro-inflammatory cytokines [21, 22].

Antioxidants are defined as substances that are capable of substantially inhibiting or retarding the oxidation of a substrate when present in low concentrations in relation to the oxidized substrate. Antioxidants do not become free radicals through the donation of electrons, because they are stable in both forms [23]. Antioxidants protect cells against damage caused by free radicals. The antioxidant effects in plants are mainly due to the presence of phenolic compounds such as flavonoids, phenolic acids, tannins and phenolic diterpenes [24]. Oxidative damage is implicated in most disease processes such as cardiovascular disease, cancer, inflammatory conditions, asthma, liver disease and muscular degeneration [25].

Hemolysis, the destruction of red blood cells (RBCs), poses significant health risks, including anemia and various organ dysfunctions [26]. The search for natural compounds to mitigate hemolytic conditions has garnered considerable interest, particularly in traditional medicine. Plant extracts, with their rich phytochemical profiles, offer a promising avenue for discovering anti-hemolytic agents [27]. Hemolysis can occur through various mechanisms, including oxidative stress, mechanical damage, and immune-mediated processes. Oxidative stress, characterized by an imbalance between reactive oxygen species (ROS) and the antioxidant defense system, is a major contributor to hemolytic anemia. The production of ROS leads to lipid peroxidation and subsequent damage to RBC membranes, causing hemolysis [28]. Thus, substances that can enhance antioxidant defense or directly scavenge ROS are of significant therapeutic interest. Many plants are known for their medicinal properties, particularly their ability to combat oxidative stress. Various studies have documented the efficacy of specific plant extracts in mitigating oxidative stress and protecting RBCs [29]. This study aims to compare the *in vitro* antioxidant and anti-hemolytic activity of aqueous leaf extracts of *A. digitata* and *M. oleifera* which are both commonly consumed vegetables in Northern Nigeria

2.0 Materials and methods

2.1 Collection and Identification of Plant Materials

Fresh leaves of *A. digitata* and *M. oleifera* were collected from Jimeta Metropolis, Yola, Adamawa State Nigeria. The plant samples were identified at the Herbarium section of Ahmadu Bello University, Zaria, Kaduna State and samples of the authenticated materials with the voucher numbers ABU02516 for *A. digitata* and ABU0517 for *M. oleifera* were deposited. Leaves of the plants were rinsed gently in clean water to remove impurities, shade dried for 14 days, pulverized and stored in airtight containers pending the commencement of the experiment.

2.2 Preparation of Aqueous Leaf Extracts of *A. digitata* and *M. oleifera*

Aqueous leaf extracts of *A. digitata* and *M. oleifera* were prepared by separately soaking 500g of the powdered plant material in 1500ml of distilled

water for 72 hours. They were then filtered using Whatman no. 4 filter paper. The extracts were then concentrated using a rotary evaporator at 40°C and then oven dried to a constant weight. The extracts were then stored in the refrigerator until further use.

2.3 Antioxidant Activity Determination

2.3.1 2, 2-diphenyl-1-picrylhydrazyl Free Radical Scavenging Activity

Free radical scavenging ability of the extracts was tested by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay as described by Blois [30] and Desmarchelier[31]. The hydrogen atom donating ability of the plant extractives was determined by the decolorization of methanol solution containing DPPH. DPPH produces violet/purple color in methanol solution and fades to shades of yellow color in the presence of antioxidants. A solution of 0.1 mM DPPH in methanol was prepared, and 2.4 mL of this solution was mixed with 1.6 mL of extract in methanol at different concentrations (20–100 mg/mL). The reaction mixture was vortexed thoroughly and left in the dark at RT for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid was used as reference. Percentage DPPH radical scavenging activity was calculated by the following equation:

$$\% \text{ DPPH radical scavenging activity} = \{(A_0 - A_1)/A_0\} \times 100$$

Where A_0 is the absorbance of the control, and A_1 is the absorbance of the extract/standard. Then % of inhibition was plotted against concentration, and from the graph IC_{50} was calculated. The experiment was repeated three times at each concentration.

2.3.2 Ferric Reducing Antioxidant Power (FRAP) Assay

The Ferric Reducing Antioxidant Power (FRAP) assay was carried out using a modified method of Benzie and Strain [32]. The assay is based on the ability of antioxidant to reduce Fe^{3+} to Fe^{2+} in the presence of 2, 4, 6-tri(2-pyridyl)-s-triazine (TPTZ), forming an intense blue Fe^{2+} -TPTZ complex with an absorption maximum at 593 nm.

Procedure

1.5 mL of freshly prepared FRAP solution (25 mL of 300 mM acetate buffer pH 3.6, 2.5 mL of 10mM

2,4,6-tripyridyl-s-triazine (TPTZ) in 40mM HCl, and 2.5 mL of 20 mM ferric chloride ($FeCl_3 \cdot 6H_2O$) solution) was added to 1mL of the extracts at varying concentrations. The reaction mixture was incubated at 37°C for 30 min and the increase in absorbance at 593nm was measured. Ascorbic acid served as the control. Percentage FRAP scavenging activity was calculated by the following equation:

$$\% \text{ FRAP scavenging activity} = \{(A_0 - A_1)/A_0\} \times 100$$

Where A_1 = absorbance of the control, and A_0 = absorbance of test sample mixture.

2.4 Determination of Anti-hemolytic Activity

2.4.1 Preparation of Erythrocyte Suspension

Method: Erythrocyte suspension was prepared according to the method described in Shinde et al.[33] with some modifications.

Procedure: Whole blood was collected from a healthy human subject. The blood was centrifuged at 3000rpm for 5 minutes in heparinized centrifuge tubes, and washed three times with equal volume of normal saline (0.9% NaCl). After the centrifugation, the blood volume was measured and reconstituted as a 10% (v/v) suspension with isotonic buffer solution (10 mM sodium phosphate buffer pH 7.4). Composition of the buffer solution (g/L) used are NaH_2PO_4 (1.15) and NaCl (9.0).

2.4.1 Heat-Induced Hemolysis

Method: this test was carried out based on the method described by Okoli et al. [34], with some modifications as described in Gunathilake et al. [35].

Procedure: exactly 0.05mL of blood cell suspension and 0.05mL extract was mixed with 5mL phosphate buffer (pH 7.4). The mixture was incubated at 54 °C for 20 minutes in a shaking water bath. After the incubation, the mixture was centrifuged at 2500rpm for 3 minutes and the absorbance of the supernatant was read at 540nm. Phosphate buffer solution was used as a control for the experiment. The level of hemolysis was calculated using the following equation;

$$\text{Hemolysis (\%)} = [(A_1 - A_2)/A_1] \times 100$$

Where A1 = absorbance of the control, and A2 = absorbance of test sample mixture.

2.5 Data analysis

Values were expressed as Means $\pm$ standard deviation (SD). The differences between experimental groups were compared by using one way analysis of Variance (ANOVA) using the software Graph Pad prism (version 22).

3.0 Results

3.1 2, 2-diphenyl-1-picrylhydrazyl Radical Scavenging Assay

Figure 1 shows the percentage inhibitions of DPPH radical scavenging activity of aqueous leaf extracts of *A. digitata* and *M. oleifera*; and the standard ascorbic acid. The percentage inhibitions increase with increase in concentrations of both the extracts and ascorbic acid. The IC_{50} (50% inhibitory concentration) are 3.48 mg/ml for the *A. digitata*, 3.01 mg/ml for the *M. oleifera* extract and 2.32 mg/ml for ascorbic acid.

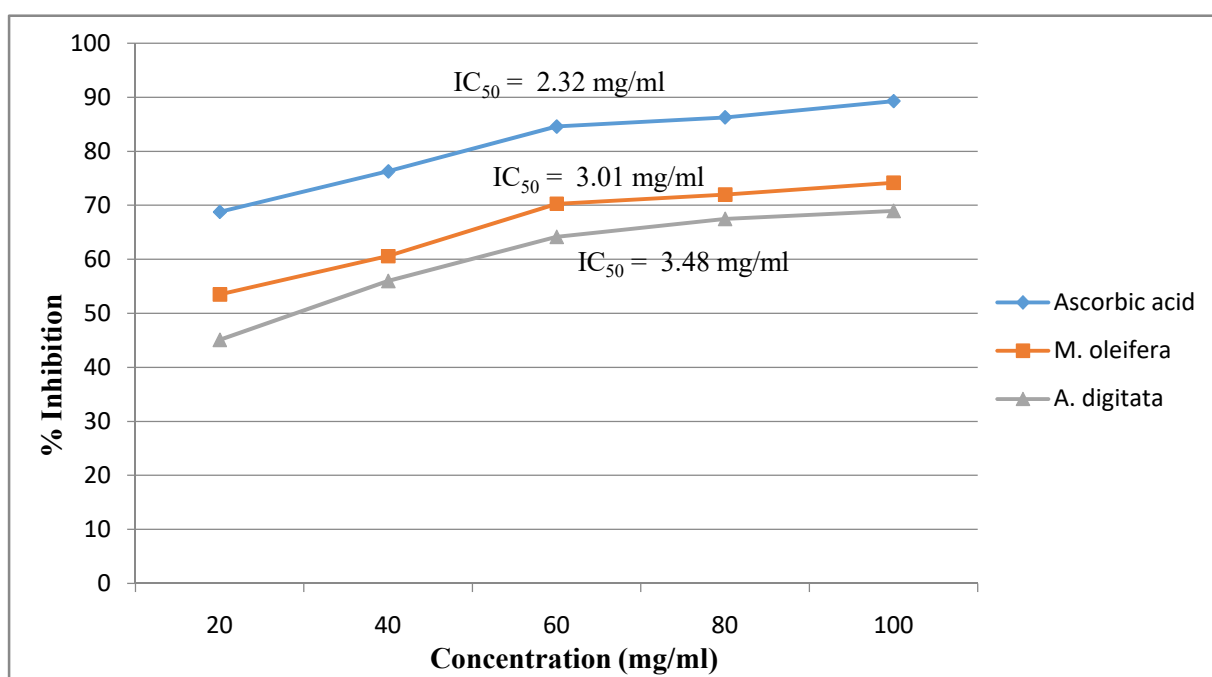


Figure 1: Percentage inhibitions of DPPH radical scavenging activity of aqueous leaf extracts of *A. digitata* and *M. oleifera*, and ascorbic acid.

3.2 Ferric Reducing Antioxidant Power Scavenging Assay

Figure 2 shows the percentage inhibitions of FRAP activity of aqueous leaf extracts of *A. digitata* and *M. oleifera*; and the standard ascorbic acid. The

percentage inhibitions increase with increase in concentrations of both the extracts and ascorbic acid. The IC_{50} is 3.36 mg/ml for the *A. digitata*, 2.96 mg/ml for the *M. oleifera* extract and 2.02 mg/ml for ascorbic acid.

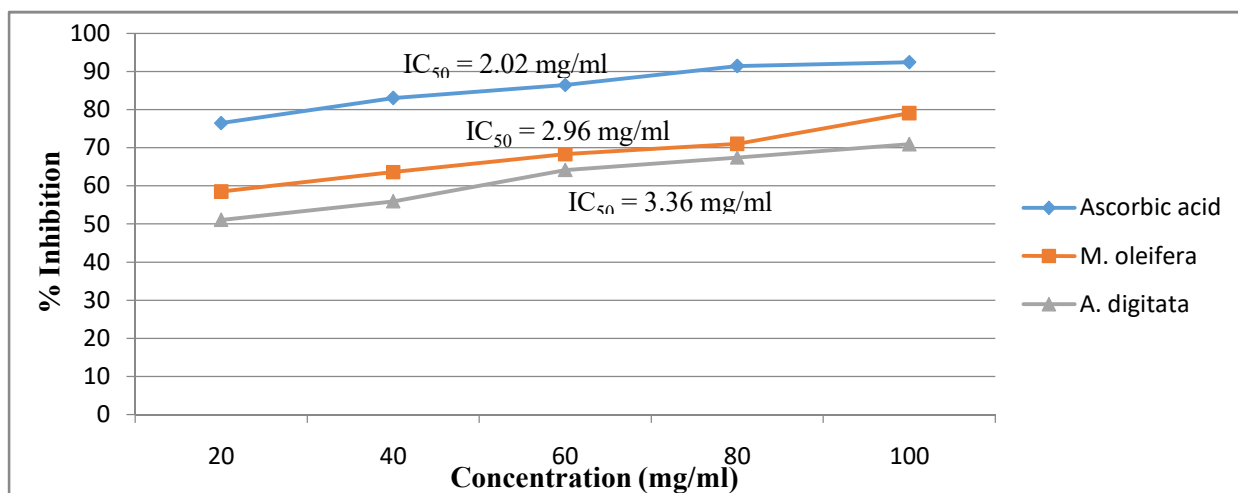


Figure 2: Percentage inhibitions of FRAP scavenging activity of aqueous leaf extracts of *A. digitata* and *M. oleifera*, and ascorbic acid

3.3 Anti-hemolytic Activity Assay

Figure 3 shows the percentage inhibitions of anti-hemolytic activity of aqueous leaf extracts of *A. digitata* and *M. oleifera*; and the standard quercetin.

The percentage inhibitions increase with increase in concentrations of both the extracts and ascorbic acid. The IC_{50} is 4.16 mg/ml for *A. digitata* extract, 3.35 mg/ml for *M. oleifera* extract and 1.96 mg/ml for quercetin.

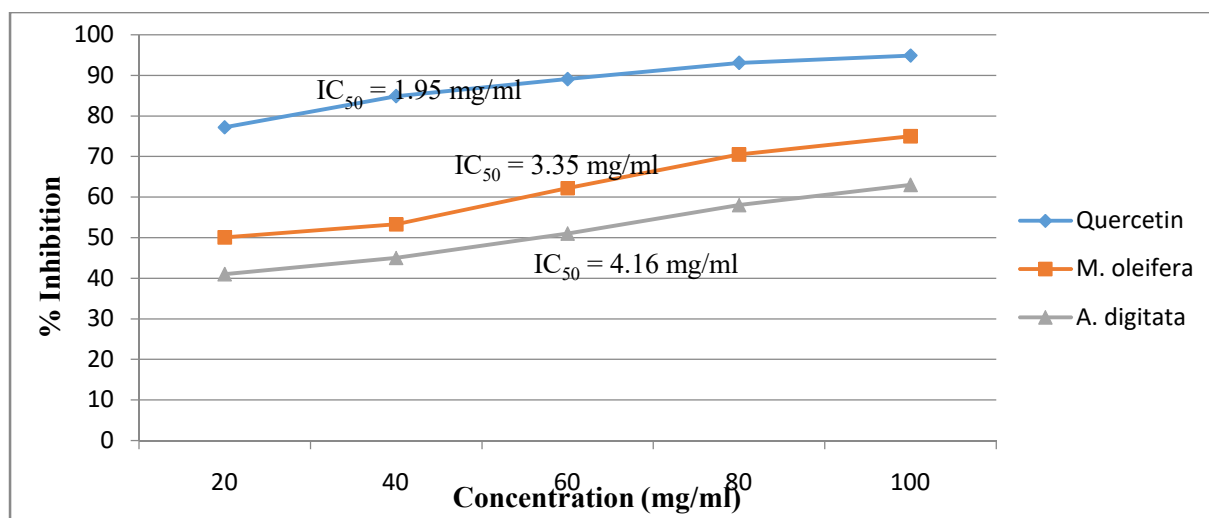


Figure 3: Percentage inhibitions of anti-hemolytic activity of aqueous leaf extracts of *A. digitata* and *M. oleifera*, and ascorbic acid.

4.0 Discussion

Antioxidants are substances that prevent and stabilize the damage caused by free radicals by supplying electrons from antioxidants to these damage cells. Antioxidants also turn free radicals into waste by-products, which are eliminated from the body [36]. Consumption of antioxidant enriched

plants is known to lower the risk of several diseases such as cancer, autoimmune disorders, cataract, rheumatoid arthritis, cardiovascular and neurodegenerative diseases [37, 38]. Such health benefits are mainly due to the presence of phytochemicals such as polyphenols, carotenoids, and vitamin E and C [39, 40].

DPPH free radical scavenging is an accepted mechanism for screening the antioxidant activity of plant extracts. The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability [41]. In the DPPH assay, violet color DPPH solution is reduced to yellow colored product, diphenylpicryl hydrazine, by the addition of the extract in a concentration dependent manner. This method has been used extensively to predict antioxidant activities because of the relatively short time required for analysis [42]. From this study, the aqueous leaf extracts of *A. dansonii digitata* and *M. oleifera* both had a similar DPPH radical scavenging activity when compared with standard ascorbic acid. Also the free radical scavenging activity increases with increase in concentration. However *M. oleifera* exhibited a higher antioxidant capacity which is evident from a lower IC₅₀ in comparison to *A. dansonii*.

IC₅₀ is a measure of the potency of a substance in inhibiting a specific biological or biochemical function [43]. It is a quantitative measure that indicates how much of a particular inhibitory substance is needed to inhibit, *in vitro*, a given biological process or biological component by 50%. The lower the IC₅₀ value, the higher the inhibition capacity [44].

The presence of reductants (antioxidants) in the plant extract causes the reduction of Fe³⁺/Ferricyanide complex to ferrous form. Therefore Fe²⁺ can be monitored by measuring the formation of Perl's Prussian blue spectrophotometrically [45]. The results from this study revealed that aqueous leaf extracts of *A. digitata* and *M. oleifera* both had a similar FRAP scavenging activity similar to the standard ascorbic acid. Also the free radical scavenging activity increases with increase in concentration. This is consistent with other documented findings [46, 47]. But *M. oleifera* exhibited a higher antioxidant capacity than *A. dansonii* with a lower IC₅₀ closer to the standard.

Hemolysis represents the rupture or alteration of the integrity of the red blood cell membrane, causing the release of hemoglobin [48]. In the physiological environment, the red cell membrane rupture allows the cell contents to escape into the plasma. The most abundant cytosolic content of red blood cells is hemoglobin, which comprises 97% of the red blood cells. Hemoglobin is specialized in the transport of blood gases in animals; therefore,

significant hemolysis compromises all tissue functionality [49]. This study shows that, the aqueous leaf extracts of *A. digitata* and *M. oleifera* both had a similar anti-hemolytic activity when compared with standard quercetin. The anti-hemolytic activity also increases with increase in concentration. These findings are in agreement with other studies [50, 51]. However *M. oleifera* exhibited a higher anti-hemolytic activity which is evident from a lower IC₅₀ closer to the standard in comparison to *A. dansonii*.

5.0 Conclusion

This study revealed the aqueous leaf extracts of *A. digitata* and *M. oleifera* both have high *in vitro* antioxidant (free radical scavenging) and anti-hemolytic activity. However the aqueous leaf extract of *M. oleifera* is shown to have a higher *in vitro* antioxidant and anti-hemolytic activity than *A. digitata*. *M. oleifera* extract could therefore potentially serve as a more suitable and potent agent against free radical mediated and hemolytic diseases.

6.0 Conflict of Interest

The authors have none to declare.

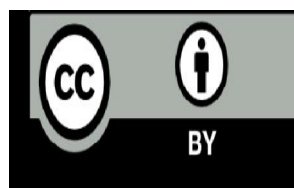
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