Free Radical Scavenging Activity and Antioxidant Potential of the Medicinal Plant *Moringa Oleifera*

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Abstract

The phytochemical analysis of the medicinal plant Moringa Oleifera was carried out to evaluate its antioxidant potential. Alcoholic and aqueous extracts of the leaves were subjected to in vitro antioxidant activity screening models such as inhibition of metal ion chelating activity, DPPH and ABTS scavenging activity. EDTA is used as a standard for metal ion chelating activity, Ascorbic acid is used as a standard. It can be deduced from the results obtained that the leaf extracts of Moringa Oleifera contains antioxidants capable of protecting normal cells from oxidative death, while rendering the cancer cells more susceptible to the cytotoxic action of the chemotherapeutic drug, etoposide, suggesting that the leaves of this plant can be administered as a supportive therapy during cancer chemotherapy, to minimize the toxic side effects to non-cancerous cells and to maximize the anticancer drug action. In all the models studied, the extracts showed potent antioxidant activity, thereby augmenting it into the present day system of medicine.

Key words: Phytochemical, antioxidant potential, chelating activity, supportive therapy.

1. Introduction

Plants play a significant role in the development of new drugs and in many developing countries attention has been paid to explore natural substances as substitutes for synthetic compounds. Products of secondary metabolism are specific species and give the plant its particular characteristics. They include plant pigments, flavors, and compounds that serve to protect the plant. Some of these secondary metabolic products impart toxicity to the individual when taken orally. These substances may be growth inhibitors, neurotoxins, carcinogens, and teratogens (Iweala *et al*., 2015). The presence of various potentially important compounds justifies exploration of its medicinal qualities reinforced by its importance in indigenous herbal and

conventional medicines (Entezari *et al*., 2014). Since ancient times man has made use of plant products for medicinal purposes. The rapidly ‘increasing number of potential therapeutic targets is leading the pharmaceutical industry to radically alter its drug discovery strategy (Solowey *et al*., 2014).

Foods rich in antioxidants play an essential role in the prevention of chronic and degenerative diseases such as cardiovascular diseases and cancer (Ames *et al*., 1993). Plant foods contain an array of bioactive compounds which are actively being researched for their health care potential, including flavonoids, plant sterols/stanols, salicylates and glucosinolates. Although to date, most research on the health benefits of plant-rich diets has focused on the established vitamins, the available data are not convincing and the drive continues to identify other additional components of a healthy diet which may reduce the risk of chronic diseases (Hoper and Cassidy, 2006). There is overwhelming epidemiological evidence that diets rich in fruit and vegetables are associated with a lower incidence of cancer, cardiovascular, and other degenerative diseases (Ames *et al*., 1993). The main characteristic of an antioxidant is its ability to trap free radicals. Free radicals can be described as any species, which is capable of independent existence and contained one or more unpaired electrons, which makes them highly reactive. They promote beneficial oxidation to generate energy and kill microbial invaders. But in excess they cause harmful oxidation that can damage cell membrane and even cell death. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidant nutrients have the ability to scavenge free radicals in the system and neutralize them before they do any damage to body cells. Most plants have protective biochemical functions of naturally occurring antioxidants in the cells. Many secondary compounds and enzymes of higher plants have been demonstrated with in vitro experiments to protect against oxidative damage by inhibiting or quenching free radicals and reactive oxygen species. Naturally occurring antioxidants in plant cells include i) enzymatic and peptide defense mechanisms (catalases, peroxidases, superoxide dismutase, glutathione and other proteins), ii. Non-enzymatic mechanisms, phenolic defense compounds (vitamin E, flavonoids, phenolic acids and other phenols); nitrogen compounds (alkaloids, amino acids and amines), carotenoids and chlorophyll derivatives. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as

peroxide, hydro peroxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases (Prasad *et al*., 2014).

Moringa Oleifera contains ascorbic acid and phenolics, both of which are powerful antioxidants. The plant has also been widely studied for their various pharmacological activities like antihypertensive, antioxidant, cardiovascular, anti-platelet aggregation activity, antimicrobial and antiviral activity (Huang *et al.*,2015). More clinical trials are required to be conducted to support its therapeutic use of the plant. It is also important to recognize that extracts of the plant may be effective not only when used singly, but may actually have a modulating effect when given in combination with other herbs or drugs (Ashok *et al*.,2010).

Studies have shown that their extracts suppress the development of blood vessels required for tumor growth and metastasis (Matsubara *et al*., 2005), and inhibit inflammation that can lead to atherosclerosis (Han *et al*., 2003). Extracts from some fruit skins have been demonstrated to possess high capacity in scavenging of superoxide free radicals and inhibition of hydroxyl radical generation by chelating ferrous iron (Kaneyuki *et al.*, 1999; Noda *et al*., 2000). Superoxide radicals generated in vivo are usually converted into hydrogen peroxide, and like other free radicals, can damage lipids, proteins, and DNA (Halliwell *et al*., 1995).

2. Experimentation and Methods

The sample of the plant is sourced in Ilorin, Kwara State, North Central Nigeria, where it grows as an ornamental plant. The chemical substances used in the experiments were all of analytical reagent grade. The visible spectra and absorption measurements were recorded in matched cuvettes using *Genesys 10S UV-VIS* spectrophotometer. Fresh leaves of *Moringa Oleifera* were collected and dried under shade. 1g of the leaves was homogenized in 10ml of the solvent. The residue was weighed and dissolved in dimethyl sulfoxide (DMSO) to obtain the desired concentration. Aqueous extracts were prepared fresh. Stock solutions were prepared by dissolving a weighed amount in a total volume of 10ml ethanol to give 10mM concentration. Working solutions of 1mM and 2mM concentrations were prepared from the 10mM stock solutions for each assay (Gulcin, 2006; Adjimani *et al*., 2015). All other reagents used were prepared by accurate dilutions

from stock solutions. Reactions were carried out in triplicate. The non-enzymic antioxidants analyzed include, total anthocyanin, β-Carotene, lycopene, total phenols, and chlorophyll.

Chlorophyll is estimated following the method of Arnon (1949) and Hartmut and Buschmann, 2001).

Total chlorophyll mg/g tissue=$20.2(A_{645}) +8.02(A_{663})$ x V/1000 x W

where A=Absorbance at specific wavelength, V=Final volume of chlorophyll extract in 80% acetone, W=Weight of tissue extracted.

Lycopene and β-carotene are determined according to procedures of Barros *et al*., 2007) and Hartmut and Buschmann, 2001).

Lycopene (mg/100 ml) = $-0.0458 \times A_{663} \times A_{505} - 0.0806 \times A_{453}$

β-Carotene (mg/100 ml) = $0.216 \times A_{663} - 0.304 \times A_{505} + 0.452 \times A_{453}$

The results are expressed as μg carotenoid/g dry tissue.

Total phenolic content is determined using Folin–Ciocalteu reagent and expressed as Gallic acid equivalents (GAE) (Singleton and Ross, 1965, Hartmut and Buschmann, 2001). Folin–Ciocalteu reagent contains metals like polytungston. Phenol content from the sample will reduce the metal and change the color from yellow to Prussian blue. The intensity of the color is directly proportional to the phenolic content.

Total anthocyanins are measured according to a modification of the method described by Fuleki and Francis (1968) and Lee *et al*., (2005). The concentration (mg/l) of each anthocyanin is calculated and expressed as Cyanidin-3-glucoside (Cy-3-glc) equivalent. The scavenging effects of leaf extracts would be evaluated against DPPH, ABTS, hydrogen peroxide, superoxide, nitric oxide and hydroxyl radicals.

Free radical scavenging activity of the sample extracts is determined by using a stable 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) (Brand Williams *et al*., 1995). DPPH is a free radical

of violet color. The antioxidants in the sample scavenge the free radicals and turn it into yellow color (Matthaüs, 2002).

The method of ABTS radical cation decolorization which is similar in principle to ORAC, uses a diode-array spectrophotometer to measure the loss of color when an antioxidant is added to the blue-green chromophore $ABTS^+$, 2, 2´-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (Prior *et al*., 2005). The antioxidant reduces $ABTS^+$ to ABTS, decolorizing it. $ABTS^+$ is a stable radical not found in the human body (Pollyanna *et al.*, 2014).

ABTS radical cations are prepared by adding solid manganese dioxide (80mg) to a 5mM aqueous stock solution of ABTS (20mL using a 75mM Na/K buffer of pH 7). Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) and a water-soluble analog of vitamin E, are used as an antioxidant standard. A standard calibration curve is constructed for Trolox at 0, 50,100, 150, 200, 250, 300, and 350µM concentrations (Schlesier *et al.*, 2002). Samples are diluted appropriately according to antioxidant activity in Na/K buffer pH 7. Diluted samples are mixed with 200µL of $ABTS^+$ radical cation solution in 96-well plates, and absorbance is read (at 750nm) after 5minutes in a microplate reader. TEAC values are calculated from the Trolox standard curve and expressed as Trolox equivalents (in mM) (Akinboro *et al.*, 2014).

The Ethanolic and Aqueous extracts of the leaves of *M.Oleifera*, were screened for the presence of phytochemicals according to the method of Adelman *et al*., (1998). Detection of the following are attempted; alkaloids by Mayer's test, Dragendroff's test, and/or Wagner's test; phenolic compounds by Ferric chloride test and Lead acetate test; flavonoids by aqueous NaOH test, concentrated H_2SO_4 test and Schinda's test; saponin by Foam test and Hemolytic test.

3. Experimental Results

ABTS assay pattern exhibited in Table 1 is illustrated in Figure 1.

Table 1: ABTS scavenging activity of leaf extract of *M.Oleifera*.

Percentage Inhibition	Plant Extract (µg/Assay)

14	0.04
25	0.08
34	0.12
48	0.16
65	0.20

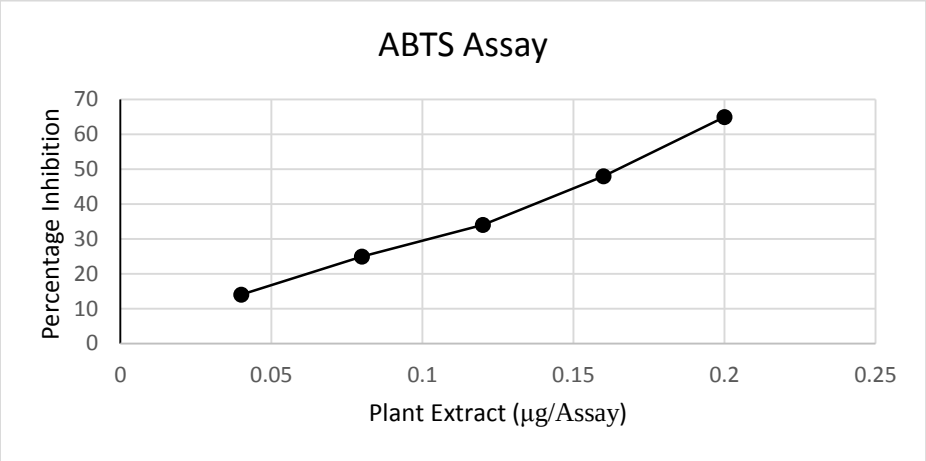


Figure1: Curve of the ABTS assay inhibition of the *Moringa* leaf extract

Reducing Power pattern exhibited in table 2 is illustrated in figure 2.

Table 2: Absorbance value of the Reducing power of *Moringa* leaf extract

Absorbance at 700nm	Plant Extract (µg/Assay)
0.35	0.04
0.50	0.08
0.80	0.12
1.00	0.16
1.22	0.20

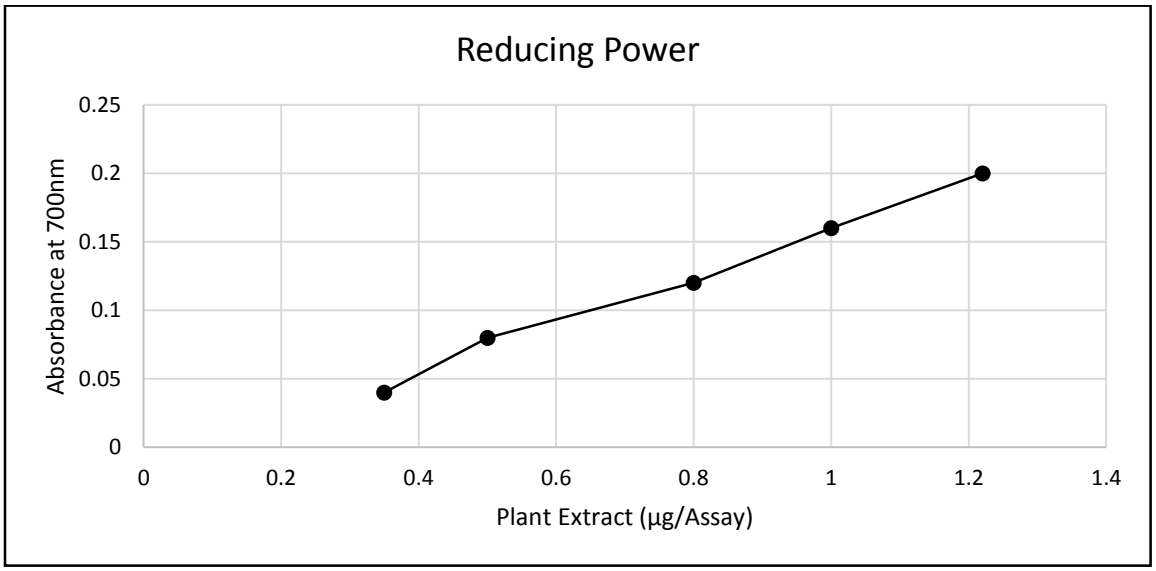


Figure 2: Absorption curve of the reducing power of *Moringa* leaf extract

Phytochemical Assay, the anti-oxidative, properties exhibited by the plant extract under study are presented in tables 3-6 below.

Table 3: Aqueous extract

P1	P2	F	A	T	S
+	+	+	–	–	–

Table 4: Ethanol extract

P1	P2	F	A	T	S
+++	++	++	++	–	–

The results obtained by color reactions and qualitative comparisonare recorded in tables 3 and 4, where. P1, $FeCl_3$-reacting-polyphenolic compounds; P2, $KMnO_4$-reacting polyphenolic compounds; F, flavonoids; A, alkaloids; T, tanins; S,saponins; Negative detection is indicated by -; Positive reaction is indicated by +, ++ and +++. With the levels in table 5, the total phenolic content in the leaf extract of *Moringa oleifera* leaves (120 mg/g), the extract was associated with TAA value (0.745 µmol Trolox/ mg) in table 6.

Table 5: Levels of Non-Enzymatic Antioxidants

Parameter	Value (μg/dw)
Chlorophyll	85.27
Lycopene	86.71
β-Carotene	33.73

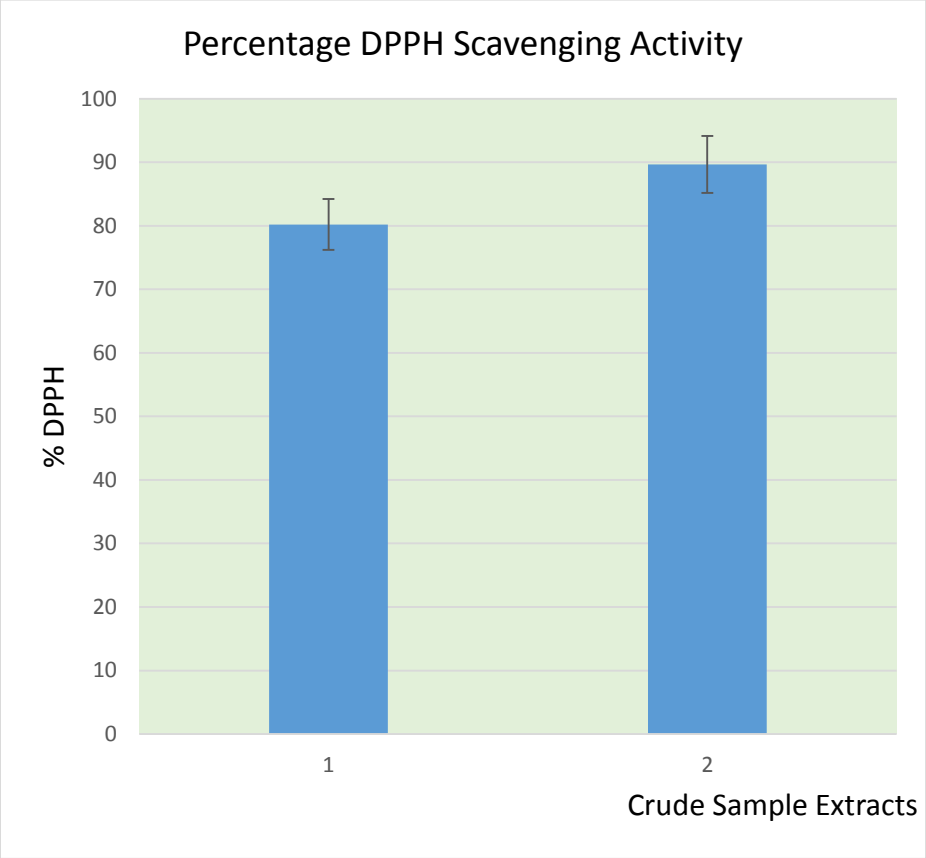
Table 6: Total Phenolics / Total Antioxidant Activity (TAA).

Total Phenolics (Gallic Acid equiv.) (mg/g)	Total Antioxidant Activity(μmol Trolox/g)
120±3	0.745±0.023

In table 7, the scavenging capacity was expressed as the percentage of DPPH' inhibition and illustrated as in figure 3. Values are the mean ± 1 standard deviation of n=3.

Table 7: Percentage DPPH scavenging activity of Aqueous and Ethanolic Extract

Aqueous extract	Ethanolic extract
80.20 ± 0.001	89.67 ± 0.750



Key:1= Aqueous extract, 2= Ethanolic extract

Figure 3: Antioxidant (DPPH scavenging) potential of the crude *Moringa* leaf extract

4. Discussion of Results

Phenolic compounds are phytochemicals usually relevant for therapeutics and nutraceutical applications due to several biological activities such as antioxidant potential and radical scavenging properties. In this sense, DPPH˙ radical scavenging was carried out on *M. oleifera* leaf extract with a view of determining the best solvent for extraction. *Moringa oleifera* plant is a valuable food source for humans (Adedapo *et al*., 2009). Thus the knowledge of the bioactive components of the whole plant, and their relative abundance with their potential biological effects could be a lead in the design of strategies with focus on its appropriate utilization. The present study contributes to support the ideas of alternative uses, genetic improvement, and the study of defense strategies to infer ecological interactions and adaptations. It is worthy of note that the presence of $KMnO_4$ -reacting polyphenolic compounds and flavonoids was registered. These compounds have the ability to protect the foliage area from abiotic and biotic stresses. The

presence and accumulation of alkaloids and polyphenols in foliage is implicated in the ability to confer deterrent and potential toxic effects to herbivores and insects (Cook and Evans, 2005; Duthie *et al*., 1996).

Usually, antioxidant properties of the plant extracts have been attributed to the presence of polyphenolic compounds, which have great potential as antimicrobial agents. Thus measurement of antioxidant activity is the most widely accepted analysis to attribute the several benefits of phenolic compounds (Hofer *et al*., 2006; Fulda 2015). Leaves of the plant show the highest antioxidant potential, coinciding with the positive signals obtained for polyphenolic and flavonoid compounds and are probably responsible for such antioxidant activity. Similar findings have been reported by Duarte *et al*., (2005) on juices from vegetables, pineapple and apple. As a result, the leaf could be considered the most relevant part of *M. Oliefera* as antioxidant source. The fact of its high antioxidant activity is in line with findings in previous reports (Vinson *et al*., 1998; Anwar *et al*., 2007; Akinboro *et al*., 2014). Evidently, more studies on the general biochemical and molecular biology for this plant species will lead to new and directed applications of the resource, and a more comprehensive study of metabolites, enzymes and other compounds in *M. Oleifera*. Variation through time, developmental stage, or as a particular metabolic state in each organ could lead to improve understanding of plant responses with physiological and ecological implications, looking for a better use of the resource (Samavati *et al*., 2014; Elumalai *et al*., 2014). Since this plant naturally occurs in varying habitats, it is naïve to expect a great magnitude of variation in the concentration and composition of chemical ingredients in different origins of the plant. However, the extent to which the chemical composition varies in populations adapted to varying habitats is not known. Thus, detailed studies are required to examine this aspect.

5. Conclusion

Recently, there has been an increased global interest in the identification of pharmacologically potent antioxidant compounds from plant sources, with low or no side effects. Increased use of different chemicals, pesticides, pollutants, smoking and alcohol intake and even some synthetic medicines increase the chance of free radical-based diseases. Plants produce large amounts of antioxidants that may prevent oxidative stress, thus presenting a potential source of new compounds with antioxidant activity. Increasing knowledge of antioxidant phytochemicals and

their inclusions can sufficiently support the human body's ability to fight diseases. Phytochemicals and herbal medicines are also important in managing pathological conditions of diseases caused by free radicals. Therefore, it is time, to explore and identify our traditional therapeutic knowledge of plant sources and interpret according to recent advancements at fighting against oxidative stress in order to accord it a deserving place.

The most effective way to eliminate free radicals responsible for oxidative stress is with the help of antioxidants. Antioxidants prevent free radical-induced tissue damage by preventing the formation of radicals, scavenging them, or by promoting their decomposition. Many synthetic drugs protect against oxidative damage, but they have adverse side effects. An alternative solution to the problem is the consumption of natural antioxidants from food supplements and traditional medicines. In almost all the traditional systems of medicine, medicinal plants play a major role and constitute their backbone. Prevention is a more effective strategy than treatment of chronic diseases. The incorporation of herbs into everyday meals may be beneficial as a diet in which culinary herbs are included to generously provide a variety of active phytochemicals that could promote good health by protecting tissues against O_2-induced damage thereby preventing the onset of chronic diseases. Medicinal plants are the most important source of life saving drugs for the majority of the world's population. Our results show that the leaves of *Moringa Oleifera* possess considerable quantities of non-enzymic antioxidants. Extracts of the leaves effectively scavenged or inhibited all the radicals tested. It is evident from the study that extracts of leaves of the plant exhibit effective antioxidant and radical scavenging activity *in -vitro,* implying they can be used in pharmacological and food industries due to the antioxidant properties.

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