RESEARCH ARTICLE

Evaluation of Total Phenolic Content, Flavanoid Content and *In-vitro* Free Radical Scavenging Activity of *Moringa Oleifera* Lam

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ABSTRACT

Objective: To evaluate total phenolic contents, flavanoid contents and in-vitro free radical scavenging activity of aqueous and ethanolic extract of *Moringa oleifera* leaves.

Methods: Phytochemical screening, qualitative, quantitative determination of total phenol content (TPC), total flavonoid content (TFC), and in-vitro free radical scavenging activity such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydrogen Peroxide (H_2O_2) and reducing power assay (RPA) were performed using standard procedures.

Results: Phytochemical analysis of ethanolic and aqueous extracts revealed the availability of important classes of phytochemicals such as tannins, carbohydrates, alkaloids, terpenoids, glycosides, flavonoids, and others. In comparison to chloroform and pet ether, the total phenolic content of ethanolic and aqueous extracts of *M. oleifera* leaves was found to be the highest as 99.24 ± 0.42 and 43.13 ± 0.062 mg/gm of gallic acid, respectively. The total flavonoid content of ethanolic and aqueous extracts was found to be 91.72 ± 1.98 mg/gm and 45.76 ± 1.85 mg/gm, respectively. In *in-vitro* DPPH free radical scavenging analysis, the IC50 ethanolic and aqueous extracts was found to be 237.6 and 456.9 µg/mL as compared to standards Ascorbic acid (122.7 µg/mL) and BHT (167.81 µg/mL), Whereas in hydrogen peroxide scavenging analysis the IC50 value were found to be 14.30 and15.92 µg/mL for ethanolic and aqueous extracts whereas for standards it was found to be11.30 µg/mL and 13.67 µg/mL for Ascorbic acid and BHT, respectively.

Conclusion: According to our findings, the ethanolic extract of *M. oleifera* leaves is a more powerful antioxidant in compare with aqueous extract of *M. oleifera* leaves. Hence, this study suggested that *M. oleifera* Lam leaves are a potential source of natural antioxidants. More research is required to recognize the chemical compounds for various pharmacological attributes.

Keywords: Antioxidant, DPPH, Free radical, Hydrogen Peroxide, *Moringa oleifera,* Reducing power assay.

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INTRODUCTION

Traditional medicine is a centuries-old discipline that has been associated since very long with to humanity in the fight against disease and promoting good health. For decades, indigenous peoples have used the unique approach of their traditional systems of medicine, with the Chinese, Indian, and African systems of medicine being among the most well-known.[1] In many respects, medicinal plants have enormous importance in today's world. All plants have a long history of being used as a source of food, medicine, and daily necessities.[2]

There are around 80,000 plant species used for therapeutic purposes, while there are over 250,000 identified plant species on the planet. India has been one of the 12 biodiversity hotspots worldwide and has over 45,000 different plant species. A high medicinal value can be found in approximately 15,000–20,000 plants. Traditional communities consume only 7,000–7,500 types of plants on a regular basis for their therapeutic properties.[3]

Plants' therapeutic properties have also been studied because of their significant pharmacological properties, low

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toxicity, pharmaceutical efficacy, and economic feasibility. Many varieties of plants produce materials that are beneficial to human and animal life support and health.

In India, herbal remedies have been employed since ancient times by the Siddha, Unani, and Ayurveda systems of medicines. Ayurveda is the oldest of the conventional systems and is currently used successfully in China, India, Germany, Sri Lanka, Japan, Bhutan, America, and various other nations.[4] It has a comprehensive philosophical, practical, and a holistic approach to treating or treating ailments to preserve a healthy mind and body. Ayurveda's historical sources have described a variety of plant species and how they can be used to treat a variety of diseases. Several ancient texts, such as the Charak Samhita and the Sushrut Samhita, detailed the qualities and applications of 1,100 and 1,270 species, respectively.[3] Ayurveda has around 8,000 herbal medicines that have been defined till now.

A free radical is an unstable substance that causes oxidative stress in the body during normal cell metabolism. It is affected by multiple factors such as environmental, physical, air pollution, fatty foods, consuming alcohol, UV radiation, sunlight, poor nutrition, and some chemicals causing various reactive free radicals and subsequent damage to macromolecules such as proteins, lipids, and DNA, resulting in a variety of diseases like heart disease, cardiovascular disease, Alzheimer's disease, diabetes mellitus, atherosclerosis, parkinson's disease, cancer, inflammation, and ageing, among many others.[5]

The harmful effects of these free radicals, causing potential biological damage, are termed oxidative stress and occur when the production of these free radicals overwhelms the body's ability to defend against them.[6-8]

Superoxide dismutase, thioredoxin, catalase, glutathione, uric acid, and ascorbic acid are antioxidant defence systems that help the body mop up free radicals and stop the chain reactions they start.[9] In recent years, there has been a surge in interest in natural antioxidants that can fight free radicals and thereby reduce the risk of diseases linked to them. Plant-based products are one of the natural antioxidant reservoirs.

Carcinogenicity has been suspected in synthetic antioxidants such as tertiary butylated hydroxy quinine, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and gallic acid esters. As a result, their use has been severely restricted, and there is a current trend to replace them with naturally occurring antioxidants. Furthermore, these synthetic antioxidants have a lower solubility and have moderate antioxidant activity.[10] As a result the current stage of work providing information about the studies has seen a significant increase in the search for natural antioxidants.

Aromatic plants and their products offer a wide range of therapeutic properties and chemical elements that have been utilized for medical reasons across the world. The phyto-constituents found in plants work together to strengthen the immune system, improve cognition, and alleviate stress and weariness. Plant elements such as leaves, flowers, bark, seeds, and roots are naturally utilized to treat sickness. Around 80% of the world's population relies on herbal medicine as their major source of health care.[11]

Compounds like flavonoids and phenolics, which are found in a wide range of aromatic and medicinal plants and have significant pharmacological potential, can help avoid oxidative stress.[12]

Moringa oleifera Lam. (Moringaceae) is a tree mostly found in India, Africa, Arabia, Southeast Asia, South America, and the Pacific and Caribbean Islands that grows up to 12 metres tall and has an open umbrella-shaped crown.[13]

Horseradish tree, drumstick tree, bean oil tree, miracle tree, and "Mother's Best Friend" are all common names for the plant, which is grown in many tropical and subtropical climates across the world. Culturally, many portions of the plant are employed for their nutritional and therapeutic properties. The leaves can be consumed raw, roasted, or dried and used as a culinary flavouring or ingredient in the future.[14-20] *M. oleifera* Lam. leaves have wonderful nutritional value and anti-tumor, anti-inflammatory, anti-ulcer, anti-atherosclerotic, and anticonvulsant properties due to the presence of polyphenolic and flavanoids compounds such as Querectin, chlorogenic acid, kaempferol, beta-carotene, and amino acids, which contribute to their antioxidant property.[21,22]

M. oleifera (MO) contains high in antioxidants and has a wide range of medicinal properties. This plant is said to have analgesic, anti-diabetic, antispasmodic, diuretic, cholesterol-lowering, antioxidant, and antibacterial qualities and it is used in contemporary medicine.[23,24]

As a result, the current stage of work providing information about the studies has seen a significant surge in the hunt for natural antioxidants. The antioxidant activities of aqueous and ethanolic extracts of *M. oleifera* Lam. were extensively examined and also evaluated the phytochemical screening as well as total phenolic and flavonoid content of *M. oleafera* leaves.

As a result, the antioxidant activity of *Moringa oleifera* leaves was evaluated in order to confirm the plant's ethnopharmacological claim. Other than vitamin E, C, and carotenoid,[25] a broad range of naturally occurring items have been shown to possess a considerable amount of antioxidant phytochemical potentials. These antioxidants help to neutralize, delay, halt, or avoid oxidative chemical processes triggered by free radicals.[26] the presence of phenolic components such as phenolic acid, flavonoids, flavonols and diterpenes[27,28] may be responsible for herbal plants' antioxidant effects.

MATERIALS AND METHODS

Plant Materials

Fresh leaves of *M. oleifera* Lam. (Moringaceae) were collected in January 2020 from a locality in Moradabad, Uttar Pradesh, India, and the specimens (voucher no: NICAIR/ RHMD/ Consult/2020/3600-01) were authenticated by Dr. Sunita Garg (Emeritus Scientist), Raw Materials Herbarium & Museum Department (RHMD) of the National Institute of Science

Communication and Information Resources (NISCAIR), PUSA Institute, New Delhi 110012, India. These fresh plant components were sun-dried, then crushed with a disintegrator before being put in sealed polyethylene bags and kept in the fridge at room temperature at cool place.

Chemicals and Standard

Butylated hydroxytoluene (BHT), Ascorbic acid (AA) and I diphenyl-2-picrylhydrazyl (DPPH) were procured from Sigma Aldrich. Sodium carbonate, sodium phosphate, Methanol, Ethanol, Petroleum ether, Gallic acid, Potassium dihydrogen phosphate, Acetic acid (glacial), Cupric chloride, Concentrated H_2SO_4 (96%) and H_2O_2 (30%, v/v) were among the other compounds procured from (Merck KGaA, Darmstadt, Germany). Sodium nitroprusside, ascorbic acid, ferric chloride, naphthyl ethylenediamine dihydrochloride, and folin– ciocalteu's reagent were all provided by Loba Chemie Pvt. Ltd. of India. All solvents and reagents that have been utilized were of analytical grade.

Extraction

100 gm coarse powder of air dried *M. oleifera* leaves was wrapped in muslin fabric and subjected to soxhlet extraction for continuous hot extraction with petroleum ether (40-60) ^{o}C, chloroform, and ethanol (non-polar to polar).[29] To get dry extracts, each extract was filtered separately via filter paper and a rotatory evaporator was used to evaporate the solvents from the extract. The crude extracts were weighed after drying and stored in the refrigerator (0–4°C) for further use. For *M. oleifera* leaves aqueous extracts the extraction was performed by dissolving 10g dried powdered plant parts in 100 mL distilled water in a round bottom flask at room temperature for 72 hours in a mechanical shaker. Content was filtered via Whatman filter paper no. 1. The filtrate was concentrated and stored in durable glass bottle kept at 4ºC for further experimental use.

Phytochemical Screening

Standard qualitative test was used to determine the presence of alkaloids, tannins, saponins, carbohydrates, reducing sugars, proteins, flavonoids, and other phenolic components using simple chemical assays.[30,31]

Determination of Total Phenolic Content

Ainsworth et al. described the Folin-Ciocalteu reagent for determining total phenolic content in extracts.[32] One-mL of varied concentration plant extracts/standards was combined with 5 mL Folin-Ciocalteu reagent (previously diluted with water l:10 v/v) and 4 mL sodium carbonate (7.5 percent). The tubes were vortexed for a few seconds before being left at 20°C for 30 minutes to develop color. The absorbance of the samples and standard was measured using a spectrophotometer against a blank at 765 nm. The solvent used to dissolve the plant extract was present in a standard blank solution. The following equation was used to calculate the total amount of phenolic content as Gallic acid equivalents (GAE):

$$C = (c\ xV) / m$$

Where; C is Total content of phenolic compounds, mg/gm plant extract, in GAE, c: the Concentration of Gallic acid established from the calibration curve (mg/ml), V is the volume of extract in ml, m is the weight of crude plant extract in gm.

Determination of Total Flavonoid Content

Flavonoid determination was done using the aluminium chloride colorimetric technique.[33] One-mL of plant extract/ standard solution was combined with 3 mL of methanol, 0.2 mL of aluminium chloride, 0.2 mL of 1 M potassium acetate, and 5.6 mL of distilled water. After 30 minutes at room temperature, the absorbance of the reaction mixture was measured using a spectrophotometer at 415 nm against a methanol blank. The following equation was used to quantify the total concentration of flavonoid components in plant ethanol extracts as Quercetin equivalents:

$$C=(c\ xV/m$$

Where C is the total content of flavonoid compounds in mg/ gm of plant extract in Quercetin equivalents, c is the concentration of Quercetin determined from the calibration curve in mg/mL. of extract, V is the volume of extracts in mL, and m is the weight of crude plant extract in gm.

Free Radical Scavenging Antioxidant Activity

The DPPH radical scavenging effect of crude ethanolic and aqueous extracts of *M. oleifera* leaves was calculated using the technique reported by Jain *et al*.[34] A solution of 0.1-mM DPPH in methanol was prepared, and 3.0 mL of extract prepared in DPPH methanol containing various concentrations 0.001–0.2 mg/mL of the extract was combined with 1.0-mL of this solution. The reaction mixture was vortexed completely and kept at room temperature for 30 minutes in the dark. At 517 nm, the mixture's absorbance was determined spectrophotometrically. The reference standards used were ascorbic acid and BHT. The following formula was used to calculate the ability to scavenge DPPH radicals:

DPPH radical scavenging activity (%) =

$[(A0-A1)) / (A0)] \times 100$

Where A0 is the DPPH radical + methanol absorbance and A1 was the DPPH radical + sample extract / standard absorbance.

The 50 percent inhibitory concentration value (IC-50) is defined as the sample's effective concentration necessary to scavenge 50 percent of the DPPH free radicals present at the outset.

Scavenging of Hydrogen Peroxide

Hydrogen peroxide was used as free radical to test the extract's and sub-fractions' scavenging activities.[35] In a phosphate buffered saline solution, 1-mL of different concentrations of the extract, sub-fractions, and standards in ethanol were added to 2 mL of hydrogen peroxide solution (PBS, pH 7.4). After l0 minutes, the absorbance was measured at 230 nm. The standards were ascorbic acid and butylated hydroxy toluene (BHT). The absorbance of a control sample containing the same amount of extract as the standard was measured using a spectrophotometer at 230 nm.

Reducing Power Assay

The potassium ferricyanide technique was used to test the reducing power.[36] A 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide were combined with one ml of extract and its sub-fractions (final concentration: 5–200 µg/mL). The mixture was then incubated for 20 minutes at 50°C. 2.5 mL of trichloroacetic acid was added to the mixture, which was then centrifuged at 3000 rpm for 30 minutes. Finally, 2.5 mL of the supernatant solution was collected and mixed with 2.5 mL of distilled water and 0.5 mL of ferric chloride, and absorbance at 700 nm was measured. The standard used were ascorbic acid and butylated hydroxy toluene (BHT), respectively, whereas distilled water was used as blank.

Statistical Analysis

The experimental results were expressed as mean ± standard deviation of mean (SD) of three replicates.

RESULT AND DISCUSSION

Phytochemical Screening

Preliminary phytochemical screening of various extracts of leaves of *M. oleifera* Lam showed presence of various phytoconstituents. Ethanolic and aqueous extracts showed the presence of various phytoconstituents and significant phytochemicals like carbohydrate, tannins, alkaloids (ethanol only), terpenoids and flavonoids. Glycosides were also detected in pet ether, chloroform and ethanolic extracts very weak test for saponin glycosides showed the low conc. of phytoconstituents in sample. The observations were presented in Table 1. Studies showed that leaves of *M. oleifera* possess a plethora of phytochemicals.[37]

Total Phenol Contents

Using the Folin-Ciocalteu reagent, the total phenolic content of M. oleifera extracts was measured and represented as gallic acid equivalents (GAE)/gm of plant extract. Using the gallic acid standard curve (y = 0.0097x-0.0279; R2 = 0.9963), the total phenolic content of the test fractions was determined (Figure l). The highest level of phenolic content was present in an ethanolic extract of *M. oleifera* leaves. The extracts' phenolic contents decreased in the following order: Ethanolic extract (99.24 ± 0.42 mg/gm) > aqueous extract (43.13 ± 0.062 mg/gm) > chloroform extract (21.80 ± 0.98 mg/gm) > petroleum ether extract (11.05 ± 0.60 mg/gm). (Table 2). Phenolic chemicals, in general, can trap and neutralize free radicals, protecting our cells against the ageing process and various diseases. Moreover, high phenolic content in plants is associated with anticancer and disease-prevention abilities.[38] the ethanolic extracts of *M. oleifera* leaves had higher DPPH radical scavenging activities indicated with IC50 value of 237.6 µg/mL than aqueous (456.9 µg/mL) according to this study. The presence of several phenolic compounds and other phytochemicals, which are a rich source of antioxidant, was shown to be responsible for the therapeutic capabilities of the *M. oleifera* leaves.

Total Flavanoid Contents

The total flavonoid content of *M. oleifera* leaves is shown in Table 3. The total flavonoid content was calculated using a

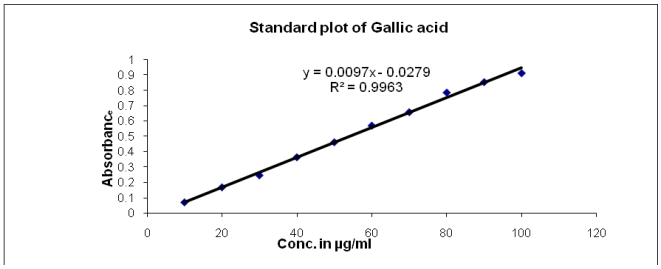


Figure 1: Calibration curve of Gallic acid for total phenolic content

Table 1: Preliminary phytochemical investigation of different extracts of *Moringa Oleifera* Lam Leaves

Phytochemicals	*Tests*	*I*	*II*	*III*	*IV*
Carbohydrate	Molisch's	-	-	+	+
	Fehling's	-	-	+	+
	Barfoed test	-	-	+	-
Tannin	Ferric chloride	-	-	+	+
	Gelatin	-	-	+	+
Alkaloid	Dragendorff's	-	+	+	+
	Hager's	-	+	+	-
	Mayer's	-	+	+	-
	Wagners	-	+	+	-
Terpenoids	Salkowski	-	-	+	+
Steroids	liebermann	+	+	-	-
Glycoside	Kellar killani	+	+	+	-
Saponin	Foam test	-	-	-	-
Flavonoid	Shinoda	-	-	+	+
	Lead acetate	-	-	+	+

I-Pet. Ether extracts, II-Chloroform Extracts, III-Ethanolic Extracts, IV-Aqueous Extract
- Abscent, + Present

Table 2: Total phenolic content of different extract of *M. olifera* Lam. leaves

MO Laeves Extract	*Total Phenolic Contents (mg/gm, Equivalent to Gallic Acid)*
Ethanolic Extracts	99.24 ± 0.42
Aqueous Extracts	43.13 ± 0.062
Chloroform Extracts	21.80 ± 0.98
Petroleum Ether Extracts	11.05 ± 0.60

Data represent Mean ± Standard deviation of triplicate analysis

Table 3: Total Flavanoid contents of different extract of *M. Olifera* Lam. leaves

MO Laeves Extract	*Total Flavanoid Contents (mg/gm, Equivalent to Quercetin)*
Ethanolic Extracts	91.72 ± 1.98
Aqueous Extracts	45.76 ± 1.85
Chloroform Extracts	10.40 ± 1.30
Petroleum Ether Extracts	3.36 ± 0.69

Data represent Mean ± Standard deviation of triplicate analysis

Table 4: Percentage inhibition of free radical scavenging activity by test and standard drugs and their IC50

DPPH % Scavenging Activity											
Conc. (µg/mL)	*% inhibition*	*IC 50 value*	*Conc. (µg/mL)*	*% inhibition*	*IC 50 value*	*Conc. (µg/mL)*	*% inhibition*	*IC 50 value*	*Conc. (µg/mL)*	*% inhibition*	*IC 50 value*
Ascorbic Acid			*BHT*			*MOAE*			*MOEE*		
31.25	11.72		31.25	11.52		31.25	10.35		31.25	11.23	
62.5	47.17		62.5	46.09		62.5	35.45		62.5	42.58	
125	60.55	122.7 µg/mL	125	58.50	167.8 µg/mL	125	44.43	456.9 µg/mL	125	55.57	237.6 µg/mL
250	80.37		250	74.12		250	55.47		250	69.34	
500	79.98		500	76.95		500	60.84		500	71.09	
1000	83.69		1000	76.86		1000	63.09		1000	74.80	

MOAE-Moringa oleifera Aqueous Extract, MOEE-Moringa oleifera Ethanolic Extracts

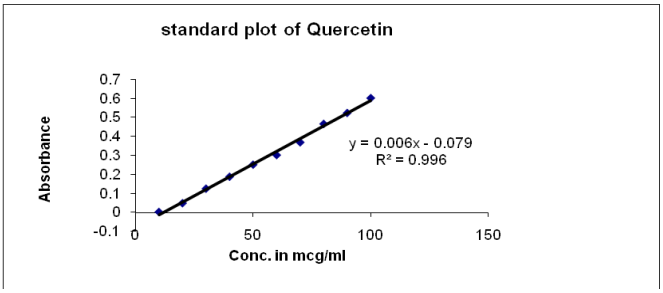


Figure 2: Calibration curve of quercetin for total flavonoid content

standard curve (y = 0.006x-0.079; R2 = 0.996) and expressed as mg of quercetin equivalent/g of extract (Figure 2). The highest quantity of flavonoid content was detected in the ethanolic extract of *M. oleifera* leaves. The flavonoid concentration of the extracts was shown to decrease in this order: Ethanolic extract (91.72 ± 1.98 mg/gm) > aqueous extract (45.76 ± 1.85 mg/gm) > chloroform extract (10.40 ± 1.30 mg/gm) > petroleum ether extract (3.36 ± 0.69 mg/gm) (Table 3) The ethanol soluble component of the plant contained more flavonoids than the aqueous soluble fraction, according to the overall flavonoid content. Depending on the phenolic content of extracts, solvent extraction has been shown to play a significant role in their antioxidant potential.[39] The presence of hydroxyl (OH) groups in phenolic compounds may contribute directly to their antioxidant activity and be a significant predictor of their radical scavenging ability.[40]

DPPH Free Radical Scavenging

The DPPH radical scavenging activity of *M. oleifera* leaves extracts revealed a significant and concentration-dependent increase in scavenging impact, with the ethanolic fraction of the leaves being the most active. The capacity of various extracts to diminish DPPH, a stable free radical, was investigated. Any molecule that can contribute an electron or hydrogen to DPPH can react with it and therefore bleach the DPPH absorption. DPPH is a purple-colored dye with a maximum absorption wavelength of 517 nm. When it reacts with a hydrogen donor, it converts to 2, 2-diphenyl-lpicryl hydrazine, which results in a lowering in absorbance.[41] we chose ethanol and aqueous extract for antioxidant properties among based on quantitative analyses. At 1000 g/mL, the ethanol extract had the highest

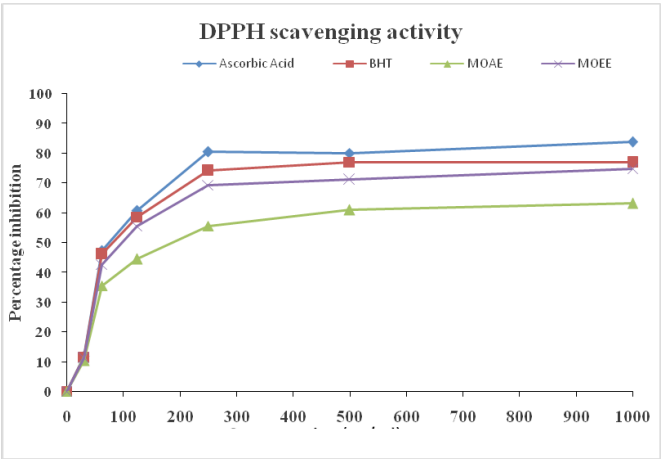
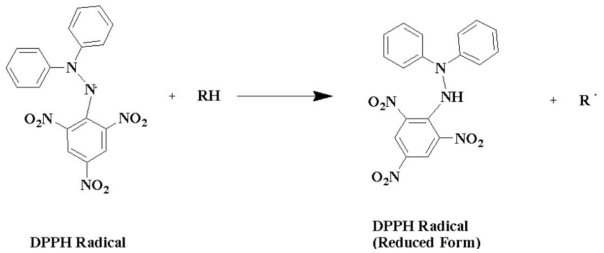


Figure 3: Comparative DPPH radical scavenging activity of MOEE, MOAE, Ascorbic acid and Butylated hydroxy toluene (BHT)

activity of 74.80%, compared to 63.09% for the aqueous extracts of MO Leaves, while ascorbic acid and BHT had 83.69 and 76.86 percent inhibition, respectively. The IC50 values of the two extracts suggested that they had significant DPPH free radical scavenging activity, as shown in (Table 4 and Figure 3). The IC50 value represents the scavenging activity's potency. The IC50 of standard ascorbic acid and BHT were determined to be 122.7 and 1.67.8 µg/mL, respectively. The IC50 of ethanol extract and aqueous extract of *M. Oliefera* leaves were 237.6 and 456.9 µg/mL, respectively, the free radical scavenging activity of an aqueous extract of *M. Oliefera* leaves fraction is the lowest.



Scavenging of Hydrogen Peroxide

Even though hydrogen peroxide would not be a radical, it contributes to oxidative stress. Even lesser amounts of H_2O_2 may be significant in biological systems. Inside the cell,

Table 5: Percentage inhibition of Hydrogen Peroxide scavenging activity by test and standard drugs and their IC50

Hydrogen Peroxide % Scavenging Activity											
Conc. (µg/mL)	*% inhibition*	*IC 50 value*	*Conc. (µg/mL)*	*% inhibition*	*IC 50 value*	*Conc. (µg/ml)*	*% inhibition*	*IC 50 value*	*Conc. (µg/mL)*	*% inhibition*	*IC 50 value*
Ascorbic Acid			*BHT*			*MOAE*			*MOEE*		
5	22.44	11.30 µg/mL	5	19.18	13.67 µg/mL	5	16.13	15.92 (µg/mL)	5	18.16	14.30 (µg/mL)
10	43.75		10	41.45		10	34.99		10	40.33	
15	60.10		15	52.88		15	45.94		15	50.16	

MOAE-*M. oleifera* Aqueous Extract, MOEE-Moringa oleifera Ethanolic Extracts

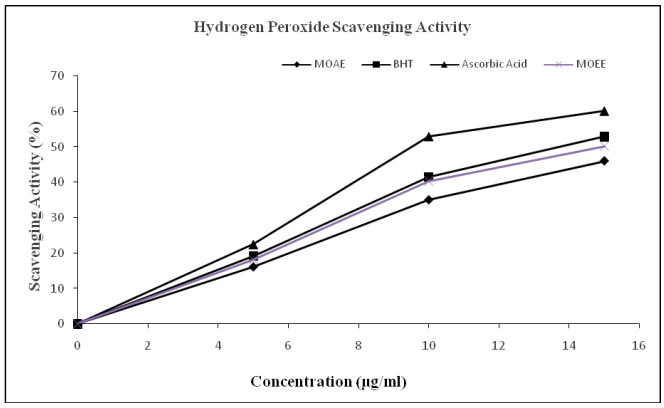


Figure 4: Comparative Hydrogen Peroxide scavenging activity of MOEE, MOAE, Ascorbic acid and Butylated hydroxy toluene (BHT)

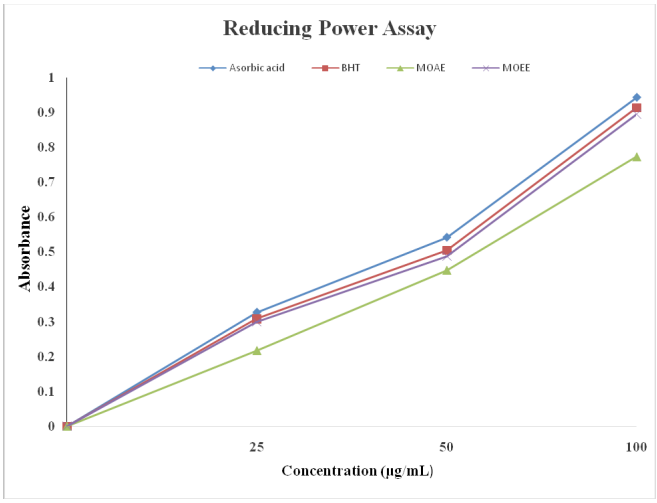


Figure 5: Comparative reducing power capacity of *M. Oliefera* leaf exhact, Ascorbic acid and Butylated hydroxy toluene (BHT)

naturally existing iron complexes are likely to react with H_2O_2 in vivo to produce extremely reactive hydroxyl radicals, which might be the source of many of the cell's harmful effects.[42] Figure 4 shows the scavenging of hydrogen peroxide by various preparations of *M. olefera* leaves (Table 5 and Figure 4). In comparison to aqueous extract, ethanol extract showed excellent activity in depleting H_2O_2, with IC50 values of 14.30 and 15.92 µg/mL, respectively.

Reducing Power

The reducing power was measured by using ferric-to-ferrous reducing activity, which was quantified spectrophotometrically from the production of Perl's Prussian blue color complex.[43] Ascorbic acid and BHT were used to examine the reducing power of various *M. oleifera* leaves extracts as standards (Figure 5). The ethanol extract of *M. oleifera* has the highest reducing power when compared with aqueous extracts of *M. oleifera* leaves. This finding suggests that the ethanol extract of *M. oleifera* leaves may contain polyphenolic chemicals with strong reducing properties. The fact that ethanol extract is the most reducing agent with the maximum phenolic content has been used to justify the claim. The most effective reducing agents were ascorbic acid, BHA, and BHT used as standards. The concept behind this approach is that the absorbance of the reaction mixture increases. An elevation in absorbance suggests that antioxidant activity is increasing. The reducing power of the samples is shown by an increase in the absorbance of the reaction mixture.[44] Reducing power has been attributed to antioxidant activity and may be a useful indicator of antioxidant activity.[45] Compounds having reducing power are electron donors that can reduce oxidized intermediates in lipid peroxidation processes, allowing them to function as primary and secondary antioxidants.[46]

CONCLUSION

The experiments in the present study were based on crude extract and are preliminary. Further research is required to establish a good conclusion on the study's findings. The presence of significant quantities of phenols in *M. oleifera* leaves might be a crucial predictor of antioxidant activity. These findings, together with those from prior research, show that *M. oleifera* lam is an outstanding plant option for improving community health and nutrition, as well as a viable candidate for the development of particular nutraceuticals and bioactive products.

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