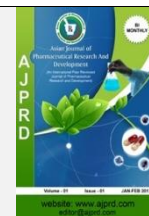


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Research Article

GC-MS Profiling and Antioxidant Activities of Ethanol Extract of Fresh Seeds of Miracle Tree-*Moringa Oleifera* lam.Arumugam. P*, Saraswathi.K¹, Sivaraj.C²¹Department of Biotechnology, Karpaga Vinayaga College of Engineering and Technology, Madhuranthagam, Kancheepuram-603 308²Phytochemistry and Natural Products Laboratory, ARMATS Biotek Training and Research Institute, Guindy, Chennai.

ABSTRACT

Objectives: *Moringa oleifera* Lam. or munga is one of the most important plant widely cultivated in India. It belongs to family Moringaceae. The plant is also known as Horse - radish tree, Drumstick tree. Every part of this plant contains a valuable medicinal feature. It contains rich source of the vitamin A, vitamin C and milk protein. Current research studies were carried out for evaluating the antioxidant activities and GC-MS analysis of ethanol extract of fresh seeds of *Moringa oleifera*.

Methods: Antioxidant activities such as DPPH[•] radical, Superoxide (O₂^{•-}) radical, ABTS^{•+} radical cation, phosphomolybdenum reduction and Fe³⁺ reduction were carried out for the ethanol extract of fresh seeds of *Moringa oleifera*. Identification of the active compounds present in the ethanol extract of fresh seeds of *Moringa oleifera* were detected by GC-MS profiling.

Results: The maximum DPPH[•] radical and Superoxide (O₂^{•-}) radical scavenging activities were 79.28±0.43% and 63.8±0.26% at 120 µg/mL concentration and the IC₅₀ values were 20.12 µg/mL and 85.91 µg/mL concentrations respectively. The maximum ABTS^{•+} radical cation scavenging activity was 83.26±0.37% at 12 µg/mL concentration and the IC₅₀ value was 6.10 µg/mL concentration respectively. The maximum Mo⁶⁺ reduction and Fe³⁺ reduction were 86.83±0.25% and 61.86±0.42% at 120 µg/mL concentration and the RC₅₀ values were 18.20 µg/mL and 46.20 µg/mL concentrations respectively. Flavone, 7-Chloro-2,3-dihydro-3-methyl-5-phenyl-1H-benzodiazepin-2-one, Kaempferol, Pyrimidine, 5-ethyl-2-[4-(4-ethylcyclohexyl)phenyl]-, 8-Carboxy-1-methyl-1,4,5,6,7,8-hexahydropyrrolo[2,3-b]azepin-4-one-3-carboxylic acid were the phytochemical compounds revealed from the ethanol extract of fresh seeds of *Moringa oleifera* by GC-MS profiling.

Conclusion: The selected antioxidant methods and the GC-MS analysis proves *Moringa oleifera* as a potent antioxidant agent and thereby scavenges the free radicals existing in the environment.

Keywords: Antioxidant, Superoxide (O₂^{•-}) radical, ABTS^{•+} radical cation, Fe³⁺ reduction, GC-MS analysis.

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INTRODUCTION

Certain foods are essential for maintaining good nutrition and health in humans. It is well recognized that foods are the main sources of nutrients used to meet our nutritional needs. However, foods, particularly those of plant origin, contain a wide range of non-nutrient phytochemicals that are elaborated by plants for their own defense and for other biological functions. When man ingests these plant foods to meet his nutritional needs, he also ingests a wide variety of these non-nutrient phytochemicals. Many plants and herbs are considered to have medicinal value, as described in the ancient Indian

medical system (Ayurveda) and also in folk medicine. They have been in use as part of home remedies for several common ailments^{1,2}. These phytochemicals present in commonly consumed plant foods are normally non-toxic and have the potential for preventing chronic diseases. Foods that have disease-preventing potential are designated 'functional foods'. Functional foods are foods that provide health benefits beyond basic nutrition. 'The appeal of functional foods lies in their potential to lower the incidence of diet related diseases. It is interesting to note that several nutrients like vitamin E (tocopherols), provitamin A (β-carotene), ascorbic acid and selenium also

have disease-preventing and healthpromoting potentials, just like phytochemicals.

The plant kingdom represents a rich storehouse of organic compounds, many of which have been used for medicinal purposes and could serve as lead for the development of novel agents having good efficacy in various pathological disorders in the coming years³. *Moringa oleifera* is the most widely cultivated species of a monogeneric family, Moringaceae that is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. *Moringa* leaves have been reported to be a rich source of β -carotene, protein, vitamin C, calcium and potassium and act as a good source of natural antioxidants; and thus enhance the shelf-life of fat containing foods due to the presence of various types of antioxidant compounds such as ascorbic acid, flavonoids, phenolics and carotenoids. Almost all the parts of this plant: root, bark, gum, leaf, fruit [pods], flowers, seed and seed oil have been used for various ailments in the indigenous medicine of South Asia, including the treatment of inflammation and infectious diseases along with cardiovascular, gastrointestinal, hematological and hepato-renal disorders. The seeds of *Moringa* are considered to be antipyretic, acrid, bitter¹.

Taxonomic Classification of *Moringa oleifera*

Kingdom: Plantae

Sub kingdom: Tracheobionta

Super Division: Spermatophyta

Division: Magnoliophyta

Class: Magnoliopsida

Sub class: Dilleniidae

Order: Capparales

Family: Moringaceae

Genus: *Moringa*

Species: *oleifera*

Botanical name: *Moringa oleifera* Lam.

Moringa oleifera is a small or middle-sized tree, ranges in height from 5 to 10 m. It is found wild and cultivated throughout the plains, especially in hedges and in house yards, thrives best under the tropical insular climate, and is plentiful near the sandy beds of rivers and streams⁴ (Figure

1). *Moringa oleifera* is rich in compounds containing the simple sugar, rhamnose called glucosinolates and isothiocyanates^{5,6}. The mucilage from the pods designated as drumstick polysaccharide, the investigation of which revealed the presence of galactose, dextrose, xylose and sodium, potassium, magnesium, calcium salts of glucuronic acid⁷. *Moringa* seeds are very effective for high turbidity water and show similar coagulation effects to alum. The coagulation effectiveness of *Moringa oleifera* varies depending on the initial turbidity and it has been reported that *Moringa oleifera* could reduce turbidity by between 92% and 99%⁸. *Moringa* seeds also have softening properties in addition to being a pH correct ant [alkalinity reduction], as well as exhibiting a natural buffering capacity, which could handle moderately high to high alkaline surface and ground waters. The *Moringa* seeds can also be used as an antiseptic in the treatment of drinking water⁹. It is believed that the seed is an organic natural polymer¹⁰.

The leaves and seeds of *Moringa oleifera* Lam. may protect against some effects of the arsenic toxicity which is especially important and contamination of ground water by arsenic has also become a cause of global public health concern. *Moringa oleifera* seeds have even been found to work better for water purification function^{11,12}. **Seeds:** Seeds are round 1cm in diameter with brownish semi – permeable seed hull with 3 papery wings hulls of seed are brown to black but can be white if kernels are of low viability. Viable seed germinate within 2 weeks, each tree can produce around 15,000 to 25,000 seeds/year. Average weight is 0.3 gm/seed¹³. Crushed seeds are a viable replacement of synthetic coagulants. Reactive oxygen species (ROS) have an important role in the aetiology of several noncommunicable diseases. Oxidants and free radicals such as singlet molecular oxygen ($-O_2$), superoxide ($-O$), hydroxyl (OH) peroxide (O-O-H) and lipid peroxides (LOO) are known to cause tissue damage. The total antioxidant potential of a food or diet can be determined by its capacity to prevent lipid peroxidation in an in vitro system. Green leafy vegetables are good sources of antioxidants, contributed by their content of carotenoids, flavonoids and tocopherols. However, the potency of antioxidants present in foods in vivo will depend not only on their levels in the foods but also on their bioavailability, that is, the extent to which the active forms of antioxidants are released from the food and absorbed through the gut^{14,15,16}.



Figure 1: Habitat of *Moringa oleifera*

MATERIALS AND METHODS

Collection and Extraction process of *Moringa oleifera*

The fresh seeds of *Moringa oleifera* were collected from the market at Maduvinkarai, Chennai, Tamil Nadu, India. The fresh seeds of *Moringa oleifera* were separated from the pulp and cut into fine pieces aseptically and soaked in ethanol for 72 hours. The glassy green coloured supernatant was filtered, condensation was carried out in a medium sized sterile petriplate at room temperature naturally which yields gummy green extract^{17,18}.

In vitro antioxidant activities

DPPH[•] radical scavenging activity

The radical scavenging activity of ethanol extract of fresh seeds of *Moringa oleifera* was carried out by the reduction DPPH[•] free radical method¹⁹. One mL of ethanol extract of fresh seeds of *Moringa oleifera* with various concentrations (20-120 µg/mL) was mixed with 1 mL of 0.1 mM DPPH solution in methanol. The mixture was then allowed to stand for 30 min incubation in dark. One mL of methanol mixed with 1 mL of DPPH solution was used as the control. The decrease in absorbance was measured at 517 nm using UV-Vis spectrophotometer. Ascorbic acid was used as the standard reference. The percentage of inhibition was calculated as:

$$\% \text{ of DPPH}^{\bullet} \text{ radical inhibition} = \left[\frac{\text{Control} - \text{Sample}}{\text{Control}} \right] \times 100$$

Superoxide (O₂^{•-}) radical scavenging activity

Superoxide (O₂^{•-}) radical scavenging activity was carried out by the method²⁰ and the reaction mixture contains different concentrations (20-120 µg/mL) of ethanol extract of fresh seeds of *Moringa oleifera* with 50 mM of phosphate buffer (pH-7.4), 200 µL of 1.5 mM of riboflavin, 200 µL 12 mM of EDTA and 100 µL 50 mM of NBT, added in that sequence. The reaction was started by illuminating the reaction mixture for 15 min in UV lamp. After illumination, the absorbance was measured at 590 nm using UV-Vis spectrophotometer. Ascorbic acid was used as the standard reference. The percentage of inhibition was calculated as:

$$\% \text{ of Superoxide (O}_2^{\bullet-}) \text{ radical inhibition} = \left[\frac{\text{Control} - \text{Sample}}{\text{Control}} \right] \times 100$$

ABTS^{•+} (2,2-azino bis (3-ethylbenzo thiazoline-6-sulfonic acid) radical cation scavenging activity

The ethanol extract of fresh seeds of *Moringa oleifera* from the stock solution was taken in various concentrations and this assay was performed according to the method²¹. The stock solutions included 7.4 mM ABTS solution and 2.6 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 hours at room temperature in the dark. Fresh ABTS solution was prepared for each experiment. The ethanol extract of fresh seeds of *Moringa oleifera* in varying concentrations (2-12 µg/mL) were allowed to react with 500 µL of the ABTS

solution for 15 minutes in dark condition and the absorbance was measured at 734 nm using UV-Vis spectrophotometer. Ascorbic acid was used as the standard reference. The percentage of inhibition was calculated as:

$$\% \text{ of ABTS}^{\bullet+} \text{ radical cation inhibition} = \left[\frac{\text{Control} - \text{Sample}}{\text{Control}} \right] \times 100$$

Phosphomolybdenum reduction activity

The antioxidant capacity of the ethanol extract of fresh seeds of *Moringa oleifera* was assessed as described⁽²²⁾. The ethanol extract of fresh seeds of *Moringa oleifera* with varying concentrations ranging (20-120 µg/mL) was combined with reagent solution containing ammonium molybdate (4 mM), sodium phosphate (28 mM) and sulphuric acid (600 mM). The reaction mixture was incubated in water bath at 95°C for 90 min. The absorbance of the coloured complex was measured at 695 nm using UV-Vis spectrophotometer. Ascorbic acid was used as the standard reference. The percentage of reduction was calculated as:

$$\% \text{ of Phosphomolybdenum reduction} = \left[\frac{\text{Sample} - \text{Control}}{\text{Sample}} \right] \times 100$$

Ferric (Fe³⁺) reducing power activity

The reducing power of ethanol extract of fresh seeds of *Moringa oleifera* was determined by slightly modified method²³. One mL of ethanol extract of fresh seeds of *Moringa oleifera* of different concentrations (20-120 µg/mL) was mixed with phosphate buffer (1 mL, 0.2 M, pH -6.6) and potassium ferricyanide [K₃Fe (CN)₆] (1 mL, 1 % w/v). The mixtures were then incubated at 50°C for 20 min in water bath. 500 µL of trichloroacetic acid (10 % w/v) was added to each mixture, followed by 100 µL of freshly prepared Ferric chloride (0.01%, w/v) was added and the absorbance was measured at 700 nm using UV-Vis spectrophotometer. Ascorbic acid was used as the standard reference. The percentage of reduction was calculated as:

$$\% \text{ of Fe}^{3+} \text{ reduction} = \left[\frac{\text{Sample} - \text{Control}}{\text{Sample}} \right] \times 100$$

Statistical analysis

All the experiments were conducted in triplicates and data given in tables were average of three replicates. All data were reported as the mean ± standard deviation of three replicates.

Gas Chromatography–Mass Spectrometry (GC–MS) Profiling

In GC-MS analysis, the ethanol extract of fresh seeds of *Moringa oleifera* was injected into a HP-5 column (30 m X 0.25 mm i.d with 0.25 µm film thickness), Agilent technologies 6890 N JEOL GC Mate II GC-MS model. Following conditions were used: Helium as carrier gas, flow rate of 1 mL/min; and the injector was operated at 200°C and column oven temperature was programmed as

50-250°C at a rate of 10°C/min injection mode. Following MS conditions were used: ionization voltage of 70 eV; ion source temperature of 250°C; interface temperature of 250°C; and mass range of 50-600 mass units²⁴.

Identification of components

The database of National Institute Standard and Technology (NIST) having more than 62,000 patterns was used for the interpretation on mass spectrum of GC-MS. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library.

RESULTS AND DISCUSSION

DPPH[•] radical and Superoxide (O₂⁻)-radical scavenging activities of ethanol extract of fresh seeds of *Moringa oleifera*

Evaluation of antioxidant activity by DPPH method is the best screening option for herbal based drugs. DPPH[•] (1,1-Diphenyl-2-picrylhydrazyl) is a stable nitrogen centered free radical which has an unpaired valence electron at one atom of nitrogen bridge. The ability of ethanol extract of fresh seeds of *Moringa oleifera* to scavenge free radicals formed was assessed using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH)²⁵. The ethanol extract of fresh seeds of *Moringa oleifera* demonstrated high capacity for scavenging free radicals by reducing the stable DPPH (1,1-diphenyl-2- picrylhydrazyl) radical to the yellow coloured 1,1-diphenyl-2-picrylhydrazine and the reducing capacity

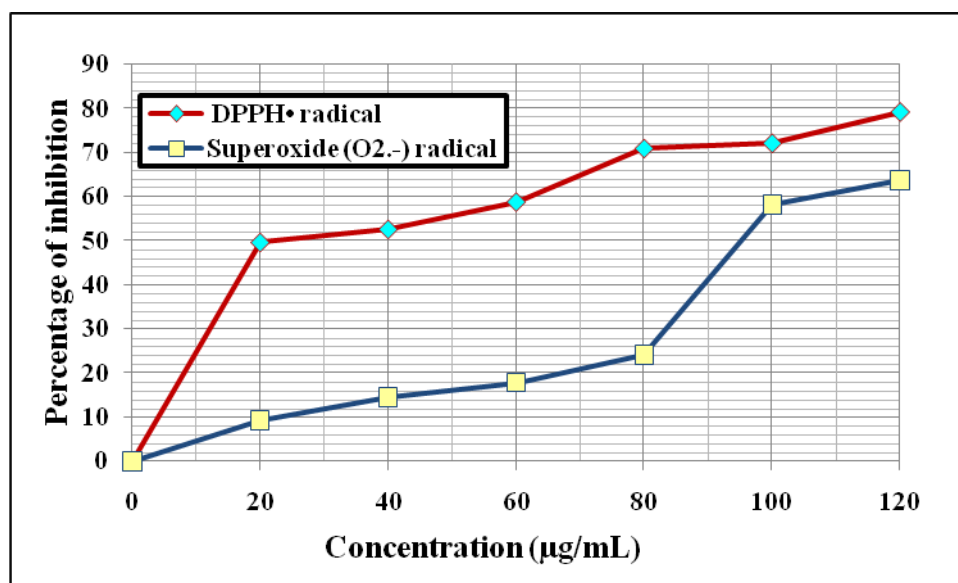
increased with increasing concentration of the extract. The maximum DPPH[•] radicals scavenging activity of ethanol extract of fresh seeds of *Moringa oleifera* was 79.28±0.43% at 120 µg/mL concentration (Table 1). The IC₅₀ value for the ethanol extract of fresh seeds of *Moringa oleifera* was found to be 20.12 µg/mL concentration respectively (Graph 1) and was compared with standard (Ascorbic acid, IC₅₀ = 12.93µg/mL concentration).

Superoxide anion is also very harmful to cellular components and their effects can be magnified because it produces other kinds of free radicals and oxidizing agents. Flavonoids are effective antioxidants, mainly because they scavenge superoxide anions. Superoxide anions derived from dissolved oxygen by the riboflavin-light-NBT system will reduce NBT in this system. In this method, superoxide anion reduces the yellow dye (NBT²⁺) to blue formazan, which is measured at 590 nm using UV-Vis spectrophotometer. Antioxidants are able to inhibit the blue NBT formation and the decrease of absorbance with antioxidants indicates the consumption of superoxide anion in the reaction mixture²⁶. The maximum superoxide (O₂⁻)-radical scavenging activity of ethanol extract of fresh seeds of *Moringa oleifera* was 63.8±0.26% at 120 µg/mL concentration (Table 1 and Graph 1) and the IC₅₀ value for the ethanol extract of fresh seeds of *Moringa oleifera* was found to be 85.91 µg/mL concentration respectively. It was compared with the standard of ascorbic acid (IC₅₀ = 15.41 µg/mL concentration).

Table 1: DPPH[•] radical and Superoxide (O₂⁻)-radical scavenging activities of ethanol extract of fresh seeds of *Moringa oleifera*

S.No	Concentration (µg/mL)	Percentage of inhibition*	
		DPPH [•] radical	Superoxide (O ₂ ⁻)-radical
1	20	49.7±0.15	9.32±0.34
2	40	52.66±0.24	14.55±0.10
3	60	58.87±0.19	17.91±0.21
4	80	71±0.36	24.25±0.17
5	100	72.18±0.11	58.2±0.32
6	120	79.28±0.43	63.8±0.26

(*Average value of 3 replicates)



Graph 1: DPPH[•] radical and Superoxide (O₂⁻)-radical scavenging activities of ethanol extract of fresh seeds of *Moringa oleifera*

ABTS^{•+} radical cation scavenging activity of ethanol extract of fresh seeds of *Moringa oleifera*

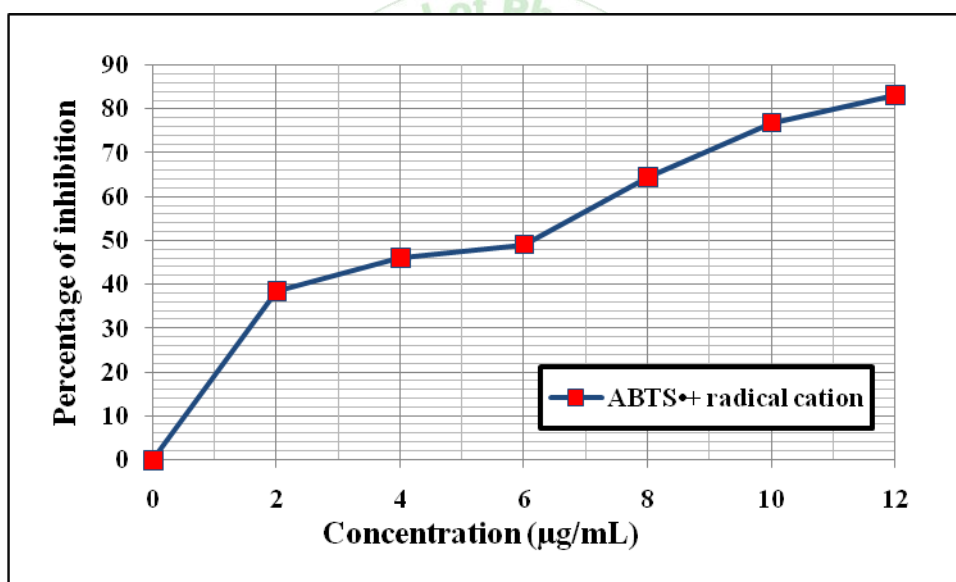
ABTS^{•+} is a blue chromophore produced by the reaction between ABTS and potassium persulfate and ABTS^{•+} radical cation gets reduced in the presence of ethanol extract of fresh seeds of *Moringa oleifera* and the remaining radical cation concentration was then quantified at 734 nm. It can be prepared using K₂S₂O₈ as an oxidant. The blue-green colour of ABTS solution is formed by the loss of an electron by the nitrogen atom of ABTS (2, 2-

azinobis (3ethylbenzothiazolin-6-sulfonic acid)). The decolourization of the solution takes place in the presence of hydrogen donating antioxidant (nitrogen atom quenches the hydrogen atom)²⁷. The maximum ABTS^{•+} radical cation scavenging activity of ethanol extract of fresh seeds of *Moringa oleifera* was 83.26±0.37% at 12 µg/mL concentration (Table 2 and Graph 2) and the IC₅₀ value for the ethanol extract of fresh seeds of *Moringa oleifera* was found to be as 6.10µg/mL concentration respectively, which was compared with standard ascorbic acid (IC₅₀ = 5.87 µg/mL concentration).

Table 2: ABTS^{•+} radical cation scavenging activity of ethanol extract of fresh seeds of *Moringa oleifera*

S.No	Concentration (µg/mL)	Percentage of inhibition*
		ABTS ^{•+} radical cation
1	2	38.59±0.45
2	4	46.08±0.12
3	6	49.17±0.19
4	8	64.53±0.33
5	10	76.94±0.25
6	12	83.26±0.37

(*Average value of 3 replicates)



Graph: 2 ABTS^{•+} radical cation scavenging activity of ethanol extract of fresh seeds of *Moringa oleifera*

Phosphomolybdenum reduction and Ferric (Fe³⁺) reducing power activities of ethanol extract of fresh seeds of *Moringa oleifera*

The total antioxidant activity of ethanol extract of fresh seeds of *Moringa oleifera* was measured spectrophotometrically by phosphomolybdenum reduction method, which is based on the reduction of Mo (VI) to Mo (V) by the formation of green phosphate/Mo (V) complex at acidic pH, with a maximum absorption at 695 nm²⁸. The maximum phosphomolybdenum reduction of ethanol extract of fresh seeds of *Moringa oleifera* was 86.83±0.25% at 120µg/mL concentration with the RC₅₀ value of 18.20 µg/mL concentration respectively

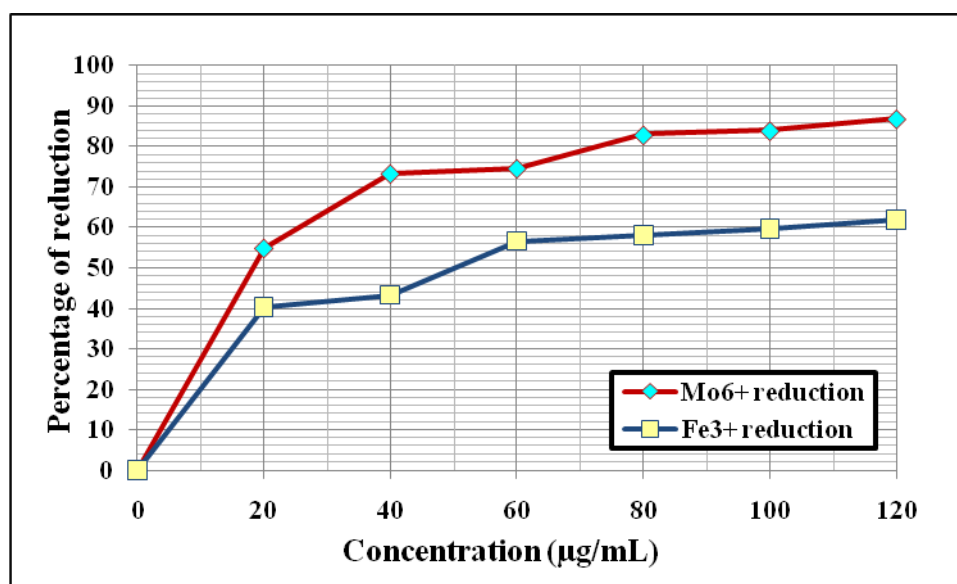
(Table 3 and Graph 3). It was compared with the standard ascorbic acid (RC₅₀ = 10.18 µg/mL).

The reducing power of Fe³⁺ to Fe²⁺ by ethanol extract of fresh seeds of *Moringa oleifera* was studied and showed reduction ability in a dose dependent manner. The maximum reduction of ethanol extract of fresh seeds of *Moringa oleifera* was 61.86±0.42% at 120 µg/mL concentration (Table 3 and Graph 3). Fe (III) reduction is often used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action²⁹. The RC₅₀ value for the ethanol extract of fresh seeds of *Moringa oleifera* as found to be 46.20µg/mL concentration respectively and was compared with the standard (20.46µg/mL concentration) Ascorbic acid.

Table 3: Phosphomolybdenum reduction and Ferric (Fe^{3+}) reducing power activities of ethanol extract of fresh seeds of *Moringa oleifera*

S.No	Concentration ($\mu\text{g/mL}$)	Percentage of reduction*	
		Mo^{6+} reduction	Fe^{3+} reduction
1	20	54.92 \pm 0.21	40.18 \pm 0.37
2	40	73.33 \pm 0.13	43.29 \pm 0.31
3	60	74.6 \pm 0.16	56.55 \pm 0.28
4	80	82.88 \pm 0.32	57.94 \pm 0.13
5	100	83.91 \pm 0.44	59.56 \pm 0.10
6	120	86.83 \pm 0.25	61.86 \pm 0.42

(*Average value of 3 replicates)


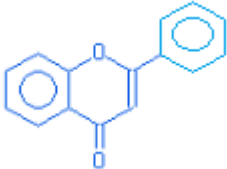
**Graph: 3** Phosphomolybdenum reduction and Ferric (Fe^{3+}) reducing power activities of ethanol extract of fresh seeds of *Moringa oleifera*

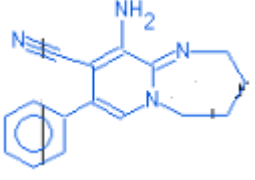
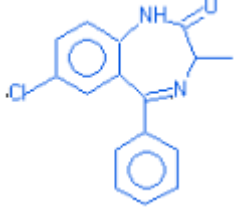
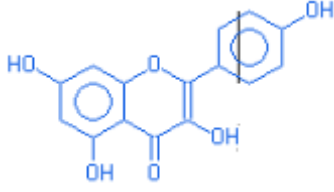
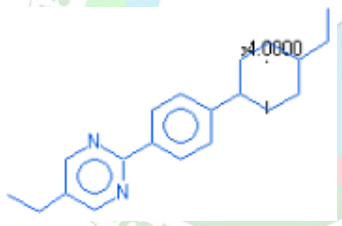
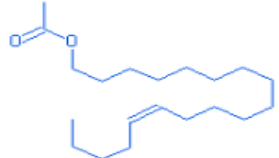
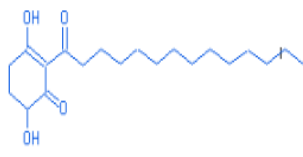
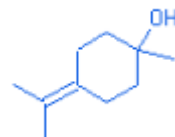
Gas Chromatography–Mass Spectrometry (GC–MS) Profiling

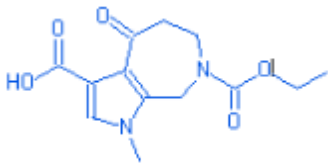
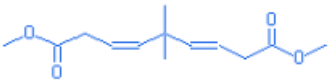
GC-MS is an analytical technique used for many applications which has very high sensitivity and specificity. In recent years GC-MS studies have been increasingly applied for the analysis of medicinal plants as this technique has proved to be a valuable method for the analysis of nonpolar components and volatile essential oil, fatty acids, lipids and alkaloids. It also plays a fundamental

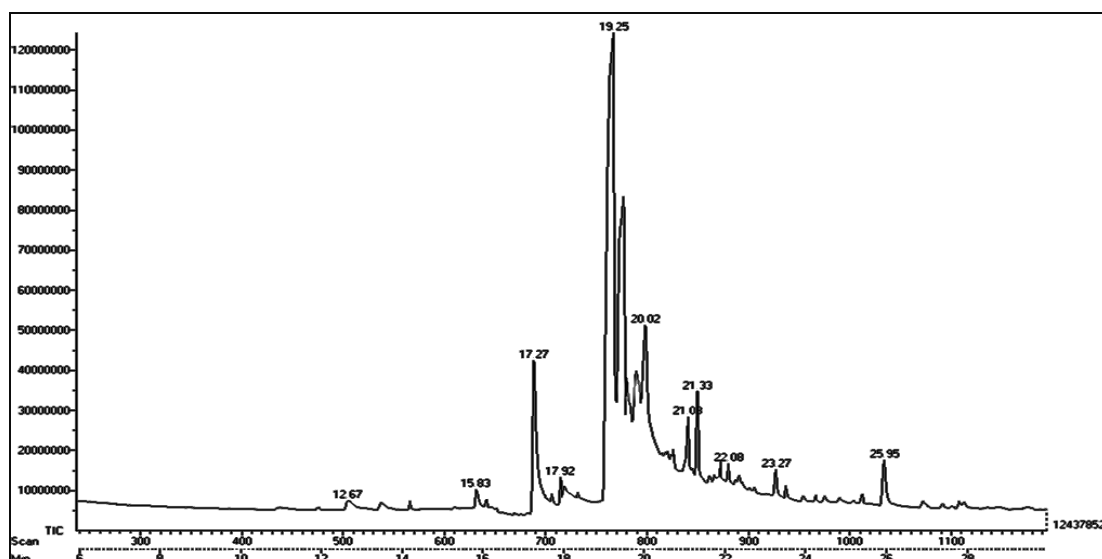
role as an analytical technique for quality control and standardization of phytochemical molecules^{30,31,32}. The GC-MS analysis of ethanol extract of fresh seeds of *Moringa oleifera* (Table 4 and Graph 4) revealed the presence of different bioactive compounds (phytochemical constituents) that could contribute the antioxidant effect and other biological properties of *Moringa oleifera* seeds (Table 5). The active principles with their Retention time (RT), Molecular formula and Molecular weight (MW) were recorded.

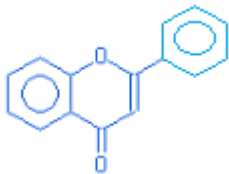
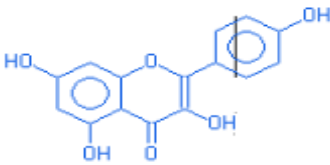
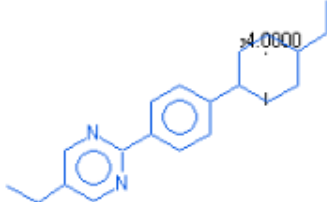
Table 4: GC–MS Profiling of ethanol extract of fresh seeds of *Moringa oleifera*

S. No	COMPOUND NAME	COMPOUND STRUCTURE	RT	MOLECULAR WEIGHT (g/mol)	MOLECULAR FORMULA
1	Nonane		12.67	127.72	C_9H_{20}
2	Flavone		17.27	222	$\text{C}_{15}\text{H}_{10}\text{O}_2$

3	2,3,4,5-Tetrahydro-8-phenyl-9-cyano-10-aminopyrido[1,2-a]diazepin		19.25	264	$C_{16}H_{16}N_4$
4	7-Chloro-2,3-dihydro-3-methyl-5-phenyl-1H-benzodiazepin-2-one		20.02	284	$C_{16}H_{13}ClN_2O$
5	Kaempferol		21.08	286	$C_{15}H_{10}O_6$
6	Pyrimidine,5-ethyl-2-[4-(4-ethylcyclohexyl)phenyl]-		21.33	294.44	$C_{20}H_{26}N_2$
7	Z-13-Octadecen-1-yl acetate		23.27	310.35	$C_{20}H_{38}O_2$
8	2-Cyclohexen-3,6-diol-1-one, 2-tetradecanoyl-		25.95	338.32	$C_{20}H_{34}O_4$
9	Cyclohexanol,1-methyl-4-(1-methylethylidene)-		15.83	153.72	$C_{10}H_{18}O$

10	8-Carboxy-1-methyl-1,4,5,6,7,8-hexahydropyrrolo[2,3-b]azepin-4-one-3-carboxylic acid		22.08	280.21	C ₁₅ H ₂₁ N ₃ O ₂
11	3,6-Nonadienedioic acid,5,5-dimethyl-,dimethyl ester		17.92	240.50	C ₁₂ H ₁₄ O ₄

Graph: 4 GC-MS Chromatogram of ethanol extract of fresh seeds of *Moringa oleifera*Table: 5 Pharmacological properties of ethanol extract of fresh seeds of *Moringa oleifera*

S.No	Compound Name	Pharmacological activity ⁽³³⁻³⁵⁾
1	Flavone 	Production of Reactive Oxygen Species (ROS) can be reduced by flavonoids. Relevance of plant defense mode of action is highly possible by flavonoids. Formation of oxygen radicals can be prevented by flavonoids thereby inhibiting the enzyme activity.
2	Kaempferol 	Oxidative Stress, Cardiovascular activity, Anticancer, Antidiabetic, Anti-inflammatory, Anti-aging, Antiallergic, Antiplatelet aggregation, Bone disorders and Anti-obesity activities.
3	Pyrimidine,5-ethyl-2-[4-(4-ethylcyclohexyl)phenyl]- 	Antiviral, Antitumour, Antimicrobial, Anti-inflammatory, Antidiabetic, Antihistaminic, Analgesic, Antipyretic, Antihypertensive activities.

CONCLUSION

Reactive oxygen species (ROS) or oxidants formed in our body due to exogenous and endogenous factors are found to be responsible for many diseases. Day to day research is revealing the potential of phytochemical antioxidants as health benefactors. This is due to their ability to neutralize the free radicals or reactive oxygen species or oxidants responsible for the onset of cell damage. Antioxidants are often added to foods to prevent the radical chain reactions of oxidation, and they act by inhibiting the initiation and propagation step leading to the termination of the reaction and delay the oxidation process. Due to safety concerns of synthetic compounds, food industries have focused on finding natural antioxidants to replace synthetic compounds. In addition, there is growing trend in consumer preferences for natural antioxidants, all of which has given more impetus to explore natural sources of antioxidants. The results of the present study in concern with the ethanol extract of fresh seeds of *Moringa oleifera* provides promising guidelines proving to be the richest source of antioxidant properties. Hence, the pharmacological mechanism of *Moringa oleifera* considering at molecular level shall be evaluated for converting into an active drug for treating several diseases.

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