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RESEARCH ARTICLE

PRELIMINARY PHYTOCHEMICAL AND INVITRO- ANTIOXIDANT AND ANALYSIS OF *MORINGA OLEIFERA LAM.* LEAF EXTRACT

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ABSTRACT

In the present study, the leaf powder of *Moringa oleifera* Lam. was subjected to preliminary phytochemical and *invitro* antioxidant analysis. The freshly prepared plant leaf extract was subjected to preliminary phytochemical screening, which revealed the presence of alkaloids, tannins, saponins, flavonoids, glycosides, phenolic compounds, terpenoids and steroids. Reducing power, superoxide anion radical, nitric oxide radical and hydroxyl radical scavenging assays were carried out to evaluate the antioxidant potential of the methanolic leaf extract of this plant. The amounts of total phenolic and flavonoid compounds were also determined. The results of the study revealed that the leaf powder of *Moringa oleifera* Lam. can be considered as a potential source of various phytochemicals and natural antioxidants.

INTRODUCTION

Medicinal plants serve as important source of phytochemicals (secondary metabolites) which have protective or disease preventive properties including - antibacterial, anticancer, antifungal, and antioxidant. The naturally occurring antioxidants in them possess the ability to reduce the oxidative damage associated with many diseases, including cancer, cardiovascular disease, cataracts, atherosclerosis, diabetes, arthritis, immune deficiency diseases and aging. The antioxidative effect of the plants is mainly due to their phenolic components, such as flavonoids, phenolic acids, and phenolic diterpenes. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Panovskai, *et al*., 2005). They are found in all parts of plants such as leaves, fruits, seeds, roots and bark (Mathew and Abraham, 2000). Phenolics are of increasing interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food. While, flavonoids are a group of polyphenolic compounds with known properties, which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action. Natural antioxidants especially phenolics and flavonoids from tea, wine, fruits, vegetables and spices are already exploited commercially either as antioxidant additives or as nutritional supplements.

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Also many other plant species have been investigated in the search for novel antioxidants, but generally there is still a demand to find more information concerning the antioxidant potential of plant species as they are safe and also bioactive. In recent years, considerable attention has been directed towards the identification of plants with antioxidant ability (Patel, *et al*., 2010). Hence, in the present study we aim to check the preliminary phytochemical and *invitro* antioxidant potential of the leaf powder of *Moringa oleifera* Lam.

MATERIALS AND METHODS

Collection of plant material

Leaves of *Moringa oleifera* Lam. were collected from Mumbaiand Talegaon – Dabhade (district - Maval, Pune). The plant samples *Moringa oleifera Lam.* (Acc.no.-12778, 12781) was authenticated by the expert taxonomist of St. Xavier's College, Mumbai.

Preliminary Qualitative Phytochemical Screening (Pawaskar, et al., 2017 b, c, d, e, f, g, h)

For preliminary qualitative screening of various phytochemicals about 5g of the *Moringa oleifera Lam.,* leaf powder was extracted separately with 100 ml of methanol and water by continuous shaking with the help of rotary shaker for 8 hours. The extract was filtered, concentrated by evaporation and was used for checking the presence of alkaloids, tannins, saponins, flavonoids, glycosides, phenolic compounds,

terpenoids and steroids using known qualitative assays as followed.

Test for Terpenoids: A volume of 5 ml of the plant extract was mixed with 2 ml of chloroform and concentrated H_2SO_4 was added to form a layer. A reddish brown coloration of the interface was formed to show the presence of terpenoids (Pawaskar, *et al*., 2007; Sivaraj, *et al*., 2011).

Test for Steroids and Phytosterols: 2 ml of acetic anhydride was added to 0.5 ml of the plant extract of each sample with 2 ml of H_2SO_4. The colour change from violet to blue green in the sample indicated the presence of steroids and sterols (Pawaskar, *et al.,* 2007; Sivaraj, *et al*., 2011).

Test for Tannins: 0.5 ml of the plant extract was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added and observed for brownish green or a blue-black coloration (Trease and Evans, 1989). Blue colour indicated the presence of Gallic tannins and green black colour indicated presence of Catecholic tannins (Pawaskar, *et al*., 2007; Sivaraj, *et al*., 2011).

Test for Alkaloids: To 2 ml of plant extract, 1.5 ml of 1% HCl was added. After heating the solution in water bath, 6 drops of Mayors reagents/ Wagner's reagent/ Dragendroff reagent was added. Formation of Orange precipitate indicates the presence of alkaloids (Oguyemi, 1979; Pawaskar, *et al*., 2007; Venkatesan, *et al*., 2009).

Test for Cardiac Glycosides (Keller-Killani Test): To 5 ml of the plant extract was treated with 2 ml of glacial acetic acid containing a drop of ferric chloride solution. Then it was underplayed with 1 ml concentrated sulphuric acid. A brown ring of the interface indicates a deoxy sugar characteristic of cardio glycosides. A violet ring may appear below the ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer (Finar, 1983; Pawaskar, *et al*., 2007; Sivaraj, *et al.*, 2011).

Test for Saponins: 5ml of the plant extract was boiled in 5ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion (Pawaskar, *et al*., 2007; Sivaraj, *et al*., 2011).

Test for Phenols: To 2 ml of the plant extract, 1 ml of 1% ferric chloride solution was added. Blue or green color indicates phenols (Martinez and Valencia, 2003; Pawaskar, *et al*., 2007; Sivaraj, *et al*., 2011).

Test for Flavonoids: A portion of the plant extract was separately heated with 10ml of ethyl acetate in a water bath for 3min. The mixture was filtered and 4ml of each filtrate were shaken with 1ml of dilute ammonia solution. A yellow colour observation indicates the presence of flavonoids (Harborne, 1973; Pawaskar, *et al*., 2007).

Test for Reducing sugars: To 2 ml of crude plant extract, 5 ml of Distilled water was added and filtered. The filtrate was boiled with 3-4 drops of Fehlings solution A and Fehlings B solution in excess (1-2 ml) for 2 minutes. Formation of the orange red precipitate indicated presence of the reducing sugars (Pawaskar, *et al*., 2007; Venkatesan, *et al*., 2009).

Invitro - Antioxidant Study (Pawaskar, et al., 2017 a, f, g, h, i, j)

5g of the dried *Moringa oleifera* Lam. plant leaf powder was weighed and kept for continuous extraction on rotary shaker using 95% methanol (volume - 250ml) for about 18-20 hours. The extract was then concentrated to dryness under reduced pressure and controlled temperature (40–50°C). The residue was weighed and stored in in sealed vials in a freezer until tested. The extract was reconstituted in water to prepare the stock solution of required concentration, which was then diluted and used for the invitro antioxidation assays. The total antioxidant capacity of the plant extract was evaluated by the method of Prieto, *et al,* (1999). The amount of total phenol content of the plant extract was be determined by Folin-Ciocalteau reagent method (McDonald, *et al*., 2001) using gallic acid standard. Total flavonol is determined by colorimetric method using aluminium chloride. The Total Flavonoid Content (Flavones and Flavonols) in the plant sample was expressed as mg of Quercetin equivalents (QE)/g of the plant material. The reducing power of the plant extract was determined according to the method of Oyaizu (1986) and Athukorala, *et al.,* (2006). The scavenging ability for hydroxyl radicals is measured by the method of Kunchandi and Rao (1990). In the nitric oxide radical scavenging assay, the nitric oxide generated from sodium nitroprusside and measured by the Griess reaction. (Green, *et al*., 1982; Marcocci, *et al*., 1994). Measurement of superoxide anion scavenging activity is based on the method described by Robak and Gryglewsky, 1988.

RESULTS AND DISCUSSION

Preliminary Phytochemical Study

Table 1 gives the results of preliminary qualitative screening of various phytochemicals from the leaf extract of *Moringa oleifera* Lam. It showed the presence of terpenoids, steroids & phytosterols, tannins, alkaloids, glycosides, saponins, reducing sugars, phenols and flavonoids. The extraction of various phytochemicals was seen to be more effectively done in polar solvents (ethanol, methanol and water) than the nonpolar solvents. Especially, ethanolic leaf extracts of the *Moringa oleifera Lam.* plant showed presence of most of the tested phytochemicals. Hence, it can be reported that alcoholic extract was the best one for extracting the active principle than others. This may possibly be one of the reasons for highest antibacterial activity shown by the ethanolic leaf extracts of the plants. The results of our preliminary qualitative screening of various phytochemicals from the leaf extracts of *Moringa oleifera* Lam. in different solvents was found to be in accordance with the earlier reports from the similar study done by Josephine *et al* (2010). Josephine *et al* (2010) on phytochemical investigation of the ether, ethanol and water extracts of *Moringa oleifera* Lam. leaves observed that the tannins, steroids and triterpenoids, flavonoids, saponins, anthraquinones, alkaloids and reducing sugars were present in the tested leaf extracts. In our study, the ethanol extract of *Moringa oleifera* Lam. leaves showed presence of terpenoids,

steroids and phytosterols, tannins, alkaloids, glycosides, phenols and flavonoids. Presence of reducing sugars was noted in the methanol and aqueous extracts and the presence of saponins was noted only in the aqueous extract of the plant.

to be in support of the findings of Pari, *et al*., (2007), where by, the methanolic extract of the leaf of *Moringa oleifera* Lam. showed the strong total antioxidant capacity (8.148 ± 0.09 mg of gallic acid equivalents (GAE)/g of the plant material).

Table 1. Results of Preliminary qualitative Screening of various Phytochemicals from the leaf extract of *Moringa oleifera* Lam., in different solvents

Sr. No.	Phytochemicals	PE	CL	EA	AC	ET	ME	WT
1	Terpenoids	-	+	-	+	+	+	-
2	Steroids and Phytosterols	+	-	-	+	+	+	-
3	Tannins	-	+	+	+	+	+	+
4	Alkaloids	+	+	+	-	+	+	+
5	Glycosides	+	+	+	+	+	+	+
6	Saponins	-	-	-	-	-	-	+
7	Phenols	-	-	-	-	+	+	+
8	Flavonoids	-	+	+	-	+	+	+
9	Reducing Sugars	-	-	-	-	-	+	+

(PE-Petroleum ether; CL-Chloroform; EA-Ethyl acetate; AC-Acetone; ET-ethanol; ME-Methanol; WT-Water)

Table 2. Total Antioxidant activity (TAA) expressed as mg of Gallic acid equivalents (GAE)/g of the plant material (leaf powder of *Moringa oleifera Lam.*)

Sr. No.	Plant sample	Total Antioxidant activity (TAA) expressed as mg of Gallic acid equivalents (GAE)/g of the plant material.
1	MOE	8.148 ± 0.09

*All values are expressed as mean ± SD for three determinations

Table 3. Total Phenolic content (TPC) expressed as mg of Gallic acid equivalents (GAE)/g of the plant material (leaf powder of *Moringa oleifera Lam.*)

Sr. No.	Plant sample	Total Phenolic Content (TPC) expressed as mg of Gallic acid equivalents (GAE)/g of the plant material.
1	MOE	9.751 ± 0.08

*All values are expressed as mean ± SD for three determinations

Table 4. Total Flavonoid Content (Flavones and Flavonols) - (TFC) - expressed as mg of Quercetin equivalents (QE) /g of the plant material (leaf powder of *Moringa oleifera Lam.*)

Sr. No.	Plant sample	Total Flavonoid Content (Flavones and Flavonols) - (TFC)- expressed as mg of Quercetin equivalents (QE)/g of the plant material.
1	MOE	27.04 ± 0.02

*All values are expressed as mean ± SD for three determinations

The results of the study have revealed that the *Moringa oleifera Lam.* leaf extract showed considerably high amounts of most of the phytochemicals. Considering wide range of therapeutic uses and the future demand of these phytochemicals, it can be thus concluded that the present study has authenticated the usefulness of the identified plants for medicinal purposes. These species could also be seen as potential sources of useful drugs in future due to their rich contents of phytochemicals.

***In vitro* - Antioxidant Study:** The results of the various invitro antioxidation studies for the methanolic leaf extract of *Moringa oleifera Lam.* are discussed below

Total Antioxidant Activity (TAA)

From the table (2) it is clear that the methanolic leaf extract of *Moringa oleifera* Lam. showed the value of 8.148 0.09 mg of gallic acid equivalents (GAE)/g of the plant material. Pari, *et al*., (2007), had reported that the methanolic leaf extract of *Moringa oleifera* Lam. exhibited the strong antioxidant properties. The value of total antioxidant activity (TAA) of the crude extract of *Moringa oleifera* reported by them was 0.636 ± 0.024 µMOE Trolox/mg. The results of our study were found

Total Phenolic Content (TPC)

From the table (3) it is clear that the methanolic leaf extract of *Moringa oleifera Lam.* showed the value of 9.751 ± 0.08 mg of gallic acid equivalents (GAE)/g of the plant material. Sreelatha & Padma, (2009) had undertaken similar study for the leaf extracts of *Moringa oleifera* Lam., both for the mature and tender leaves. The data obtained by them suggests that the extracts of *Moringa oleifera*, both mature and tender leaves have potent antioxidant activity against free radicals, prevent oxidative damage to major bioMOEecules and afford significant protection against oxidative damage**.** The total phenolic content reported by them was 45.81±0.02 mg gallic acid equivalents (GAE)/g plant material for mature leaf extract and 36.02±0.01 mg gallic acid equivalents (GAE)/g plant material for tender leaf extract. However, in our study, the methanolic extract of the leaf of *Moringa oleifera* Lam. although showed the total phenolic content (9.751 ± 0.08 mg of gallic acid equivalents (GAE)/g of the plant material) but the value differed greatly from the one reported by Sreelatha & Padma, (2009). This might be due to difference in the extraction conditions and also might be due to different environmental conditions.

Table 4. Results of Reducing power assay, showing concentrations of the drug ie. Standard/Plant Extract (leaf powder of *Moringa oleifera* Lam. (mg/ml) and the absorbance (taken at 700 nm)

Tube no.	Concentration of Standard/Plant Extract (mg/ml)	Absorbance of Standard at 700 nm	Absorbance of MOE 700 nm
1.	2	0.42 ± 0.02	0.36 ± 0.01
2.	4	0.53 ± 0.06	0.42 ± 0.04
3.	6	0.62 ± 0.05	0.50 ± 0.03
4.	8	0.70 ± 0.03	0.58 ± 0.05
5.	10	0.75 ± 0.01	0.63 ± 0.02

*All values are expressed as mean ± SD for three determinations

Table 5. Showing concentrations of the drug i.e. Standard/Plant Extract (leaf powder of *Moringa oleifera Lam.* (mg/ml) and the OH scavenged (% activity) shown by the drug i.e. Standard/Plant extract

Tube No.	Concentration of Plant extract (mg/ml)	OH scavenged (% activity) Standard Ascorbic acid	OH scavenged (% activity) MOE
1	10	69.44 ± 0.11	30.55+ 0.25
2	30	73.69 ± 0.10	43.05+ 0.18
3	50	77.77 ± 0.20	48.61+ 0.12
4	70	80.55 ± 0.22	54.17+ 0.14
5	90	83.33 ± 0.09	55.56+ 0.23

*All values are expressed as mean ± SD for three determinations

Table 6. Showing IC50 values (mg/ml of Standard/Plant Extract (leaf powder of *Moringa oleifera* Lam.)

Sr. No.	Plant sample	IC_{50} (mg/ml of plant sample)
1	Standard	7.26 ± 0.10
2	MOE	57.92 ± 0.17

*All values are expressed as mean ± SD for three determinations

Table 7. Showing concentrations of the drug i.e. Standard/Plant Extract (leaf powder of *Moringa oleifera* Lam. (mg/ml) and the Nitric oxide radical scavenged (% activity) shown by the drug i.e. Standard/Plant extract

Tube No.	Concentration of Plant extract (mg/ml)	NO scavenged (% activity) Standard Ascorbic acid	NO scavenged (% activity) MOE
1	10	58.33 ± 0.12	15.00+ 0.21
2	30	63.33 ± 0.10	28.33+ 0.22
3	50	71.66 ± 0.14	48.33+ 0.25
4	70	76.66 ± 0.09	60.00+ 0.19
5	90	78.33 ± 0.08	63.33+ 0.17

*All values are expressed as mean ± SD for three determinations

Table 8. Showing IC50 values (mg/ml of Standard/Plant Extract (leaf powder of *Moringa oleifera* Lam.)

Sr. No.	Plant sample	IC_{50} (mg/ml of plant sample)
1	Standard	9.24 ± 0.10
2	MOE	54.62 ± 0.21

*All values are expressed as mean ± SD for three determinations

Table 9. Showing concentrations of the drug i.e. Standard/Plant Extract (leaf powder of *Moringa oleifera* Lam. (mg/ml) and the SO scavenged (% activity) shown by the drug i.e. Standard Ascorbic acid /Plant extract

Tube No..	Concentration of Plant extract (mg/ml)	SO scavenged (% activity) Standard Ascorbic acid	SO scavenged (% activity) MOE
1	10	72.41 ± 0.10	18.97+ 0.22
2	30	75.86 ± 0.11	29.31+ 0.21
3	50	79.31 ± 0.12	39.66+ 0.23
4	70	82.76 ± 0.17	46.55+ 0.16
5	90	87.93 ± 0.18	51.72+ 0.18

*All values are expressed as mean ± SD for three determinations

Table 10. Showing IC50 values (mg/ml of Standard /Plant Extract (leaf powder of *Moringa oleifera Lam.*)

Sr. No.	Plant sample	IC_{50} (mg/ml of plant extract)
1	Standard	6.6 ± 0.14
2	MOE	67.92 ± 0.19

*All values are expressed as mean ± SD for three determinations

Total Flavonoid Content (TFC)

The total flavonoid content of the methanolic leaf extract of *Moringa oleifera* Lam., as noted by us is 27.04 ± 0.02 mg of quercetin equivalents (QE)/g of the plant material (Table 4). The total flavonoid content reported by Sreelatha & Padma, (2009) in the similar study undertaken by them for the leaf extracts of *Moringa oleifera* Lam. (both for the mature and tender leaves) was 27 ± 0.03 mg quercetin equivalents (QE)/g plant material for mature leaf extract and 15 ± 0.02 quercetin equivalents (QE)/g plant material for tender leaf extract. The results of our study was found to be in accordance with the findings of Sreelatha & Padma, (2009). Our study results indicated that the total flavonoid content of the methanolic leaf extract of *Moringa oleifera* Lam. is 27.04 ± 0.02 mg of quercetin equivalents (QE)/g of the plant material.

Reducing Power

The reducing powers of the methanolic leaf extract of *Moringa oleifera Lam.* is shown in (Table 5 and Figure 1). For the measurements of the reductive ability, we investigated the Fe^{3+} to Fe^{2+} transformation in the presence of the plant extract using the method of Oyaizu (Oyaizu, 1986). The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant (Meir *et al*, 1995).

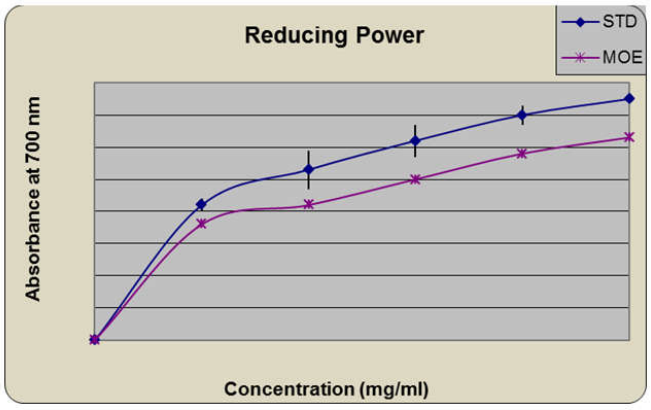

Figure 1. Results of Reducing power assay, showing graph of the concentrations of the drug i.e. Standard/Plant Extract (leaf powder of *Moringa oleifera* Lam. (mg/ml) against the absorbance (taken at 700 nm)

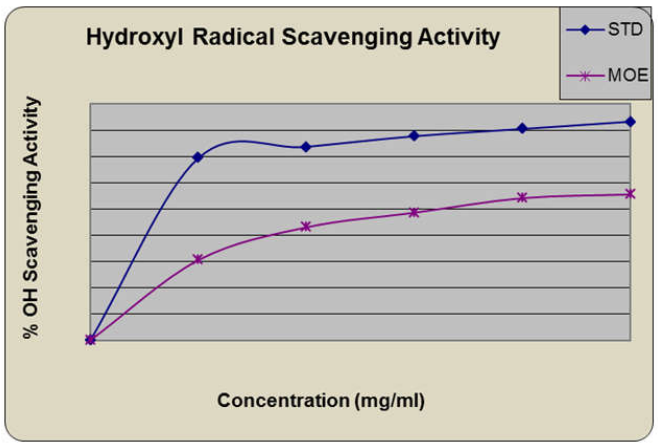

Figure 2. Graph of concentrations of the drug ie. Standard/Plant Extract (leaf powder of *Moringa oleifera Lam.* (mg/ml) and the OH scavenged (% activity) shown by the drug i.e. Standard/Plant Extract

The reducing power increased as the extract concentration increased, indicating some compounds in the tested plant extract, were electron donors and could react with free radicals to convert them into more stable products and to terminate radical chain reactions. The tested plant extract showed significant activity when compared to the standard and the difference was statistically significant ($p < 0.01$). Pari, *et al*., (2007) in their reports, indicated that the leaf extract of *Moringa oleifera* Lam. offered the stronger antiradical properties and reducing power and Koruthu, *et al*., (2011) also indicated ethanol to be a better solvent than methanol in extraction of reducing compounds from the leaf extract of *Moringa oleifera* Lam. and the reducing power of the extract was found to be lower than that of ascorbic acid, chosen as the standard. However, Reddy and Urooj, (2010) in their study reported that - among the four extracts of the *Moringa oleifera* Lam. leaves tested by them, acetone extract showed significantly high reducing capacity (with 0.465 O.D at 700 nm), followed by methanol extract (with 0.415 O.D at 700 nm), and water extract (with 0.411 O.D at 700 nm) but none of the extracts could reach the ascorbic acid as reported by them. All the above mentioned results were found to be in support of the results of our study which indicated that the leaf extract of *Moringa oleifera* Lam. exhibits stronger reducing power.

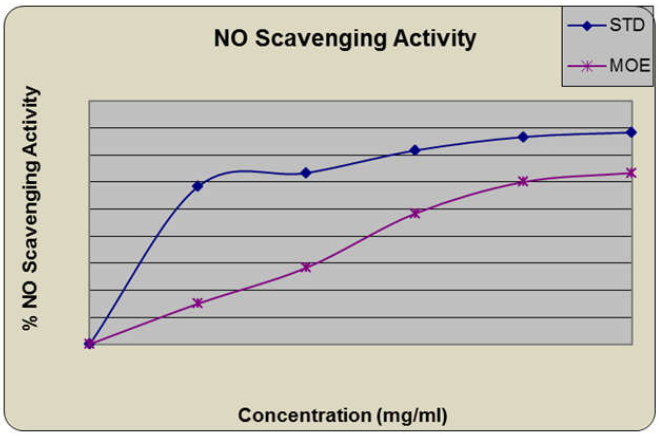

Figure 3. Graph of concentrations of the drug ie. Standard/Plant Extract (leaf powder of *Moringa oleifera Lam.* (in mg/ml) and the NO scavenged (% activity) shown by the drug ie. Standard/Plant extract

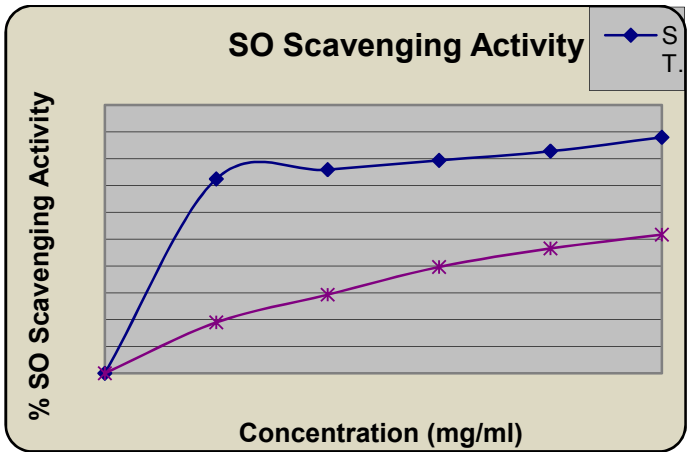

Figure 4. Graph of concentrations of the drug i.e. Standard/Plant Extract (leaf powder of *Moringa oleifera* Lam. (mg/ml) against the SO scavenged (% activity) shown by the drug i.e. Standard /Plant extract

Hydroxyl radical scavenging Assay (OH - scavenging)

Moringa oleifera Lam., showed (55.56 ± 0.23%) inhibition (Table 6). The maximum scavenging capacity on hydroxyl radicals exhibited by the ascorbic acid, used as a standard, was (83.33 ± 0.09 %) at 90 mg/ml concentration. The reduction ability is in a dose dependent manner, increasing with increasing concentrations (Figure 2). The IC_{50} value of methanolic leaf extracts of *Moringa oleifera* Lam. was found to be (57.92 ± 0.17) among the plant extracts under study and was very close to the IC_{50} value of the ascorbic acid standard (7.26 ± 0.10 mg/ml) suggesting very high scavenging potential on hydroxyl radicals. (Table 7). We tested the scavenging activities of methanolic leaf extracts of *Moringa oleifera* Lam. in the concentration range from 10-90 mg/ml (Table 6 and Figure 2). In the range tested, we found that the maximum scavenging activity (% inhibition) on hydroxyl radicals - was seen when the concentrations of methanolic leaf extracts was about 90 mg/ml. At the highest concentration tested (90 mg/ml) *Moringa oleifera* Lam. showed (55.56 ± 0.23 %) inhibition. The maximum scavenging capacity on hydroxyl radicals exhibited by the ascorbic acid, used as a standard, was (83.33 ± 0.09 %) at 90 mg/ml concentration. The reduction ability is in a dose dependent manner, increasing with increasing concentrations. The IC_{50} value of methanolic leaf extracts of *Moringa oleifera* Lam. was found to be (57.92 ± 0.17 mg/ml) suggesting very low scavenging potential on hydroxyl radicals. No previous report on the hydroxyl radicals scavenging activity, however, was found for the leaf extract of *Moringa oleifera* Lam. Hence our study can be considered as the first to report the same.

Nitric oxide radical scavenging Assay (NO - scavenging)

The results of the study are mentioned in Table 8 and the IC_{50} value (mg/ml of Plant sample) is indicated in Table 9. Figure 3, illustrates the percentage inhibition of nitric oxide generation by the above mentioned plant and the ascorbic acid as a standard used. In the present study, the crude methanolic extract of the leaves of *Moringa oleifera* Lam. was checked for its inhibitory effect on nitric oxide production. Our study showed that the plant leaf extract under study effectively prevented the formation of peroxynitrate and can be used to prevent the adverse effect of metabolities of NO. In the tested concentration range from 10-90 mg/ml. (Figure 3), we found that the maximum scavenging activity (% inhibition) on nitric oxide radicals - was seen when the concentrations of methanolic leaf extract of the plant was about 90 mg/ml. At the highest concentration tested (90 mg/ml), *Moringa oleifera Lam.* showed (63.33 ± 0.17%) inhibition. The maximum scavenging capacity on nitric oxide radicals exhibited by the ascorbic acid, used as a standard, was (78.33 ± 0.08 %) at 90 mg/ml concentration. The reduction ability in a dose dependent manner; increasing with increasing concentrations. The concentration of *Moringa oleifera Lam.* needed for 50% inhibition of nitric oxide radical (IC_{50} value) was found to be 54.62 ± 0.21 mg/ml; whereas (9.24 ± 0.10 mg/ml) was needed for ascorbic acid used as standard. Sreelatha & Padma, (2009) reported that the mature leaf extract of *Moringa oleifera* Lam. (12 µg concentration) to be better scavenger than the tender leaf extract and the reported IC_{50} value for the matured leaf extract of *Moringa oleifera* Lam. was 56.77±0.45µg/ml. However, according to our results, the methanolic leaf extract of *Moringa oleifera* Lam. showed (63.33 ± 0.17 %) as the maximum scavenging activity of nitric oxide at the highest concentration tested (90 mg/ml) with the IC_{50} value (54.62 ± 0.21 mg/ml).

Superoxide anion radical scavenging (SO) Activity

The results of the study are mentioned in Table 10 and the IC_{50} values (mg/ml of Plant extract) is indicated in Table 11. Figure 4, illustrates the percentage inhibition of superoxide anion radicals by the methanolic leaf extract of *Moringa oleifera Lam.* and the ascorbic acid as a standard used. In the present study, the superoxide anion radicals are derived in PMS□NADH□NBT system, superoxide radical reduces NBT to blue coloured formazan that is measured at 560 nm (Khanam *et al*., 2004) and the decrease in absorbance at 560 nm indicates the consumption of superoxide anion in the reaction mixture, thereby exhibiting a dose dependent increase in superoxide scavenging activity (Bora and Sharma, 2010).
We tested the superoxide anion radicals scavenging activities methanolic leaf extract of *Moringa oleifera* Lam. in the concentration range from 10-90 mg/ml (Figure 4). In the range tested, we found that the maximum scavenging activity (% inhibition) on superoxide anion radicals - was seen when the concentrations of methanolic leaf extract of the plant was about 90 mg/ml. At the highest concentration tested (90 mg/ml), *Moringa oleifera* Lam. showed (51.72 ± 0.18%) inhibition. The maximum scavenging capacity on superoxide anion radicals exhibited by the ascorbic acid, used as a standard, was (87.93 ± 0.18 %) at 90 mg/ml concentration. The reduction ability in a dose dependent manner; increasing with increasing concentrations.

The concentration of *Moringa oleifera* Lam. needed for 50% inhibition of superoxide anion radical (IC_{50} value) was found to be (67.92 ± 0.19 mg/ml); whereas (6.6 ± 0.14 mg/ml) was needed for ascorbic acid used as standard. Sreelatha & Padma, (2009) reported that the mature leaf extract of *Moringa oleifera* Lam. (12 µg concentration) to be better superoxide anion radicals scavenger than the tender leaf extract and the reported IC_{50} value for the matured leaf extract of *Moringa oleifera* Lam. was 12.71±0.15 µg/ml. However, according to our results, the methanolic leaf extract of *Moringa oleifera* Lam. showed (51.72 ± 0.18 %) the maximum superoxide anion radicals scavenging activity at the highest concentration tested (90 mg/ml) with the IC_{50} value (67.92 ± 0.19 mg/ml). The results obtained in the present study indicated that the *Moringa oleifera Lam.,* showed comparatively good scavenging activity i.e. inhibition of hydroxyl radical, nitric oxide and superoxide anion scavenging and reducing power activities and contain a noticeable amount of total phenols and flavonoid content when compared with the respective standards. Thus it can be concluded that, the leaf powder of *Moringa oleifera Lam.* can be used as easily accessible source of natural antioxidant and as a possible food supplement or in pharmaceutical industry. However, further work should be performed on the isolation and identification of the antioxidant components in the leaf powder of *Moringa oleifera Lam.* as the components responsible for the antioxidant activity of this plant leaf powder are currently unclear.

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