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Antioxidant enzyme activity, Phenolics & Flavonoid content in vegetative and reproductive parts of *Moringa Oleifera Lam.*

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ABSTRACT

Plant polyphenols are gaining increasing attention due to their potent antioxidant properties and marked effects in the prevention of various oxidative stress associated diseases. Identification and extraction of phenolic compounds from different plants has become a major area of health and medical research. In the present study, antioxidant activity of methanolic extracts of vegetative and reproductive parts of *Moringa oleifera* Lam. were tested for its free radical scavenging activity by spectrophotometric methods. Total phenolic and total flavonoid content were assayed spectrophotometrically. We observed high amount of total phenolic and flavonoid content in leaves and flowers of *Moringa oleifera* and this may be responsible for increased antioxidant activity in the plant. Antioxidant enzymes like ascorbate and ascorbate peroxidase (APX) were also found to be higher in vegetative and reproductive parts of Moringa *oleifera*. Our study reveals that *M. oleifera* leaves and flowers are potent source of natural antioxidants and the plant is valuable in pharmaceutical industry.

Keywords: Antioxidant enzymes, flavanoids, phenolics, reducing power, scavenging activity, *Moringa oleifera.*

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INTRODUCTION

Antioxidants include natural and synthetic compounds that scavenge free radicals and inhibit oxidation process[1]. Free radicals play important role in pathogenesis of several diseases, such as cancer, rheumatoid arthritis and cardiovascular diseases. Reports are available that show synthetic antioxidants like *Butylated hydroxytoluene* (BHT), Butylated *hydroxyanisole* (BHA) and propyl gallate although used in food and health have harmful effects[1,2]. ,and hence there is a growing interest towards a growing interest towards the study of natural additives from plant source as potential antioxidants. In this respect phenolic compounds have gained significant attention for their antioxidant behaviour and health promoting effects. In plants, anti-oxidative Phenolic compounds are present as covalently bound form. They donate hydrogen to highly reactive radical thereby preventing further radical formation. Phenolics are gaining interest in food industry because they are related to oxidative degradation of lipids and thus improve the nutritional quality of the food. Polyphenolic compounds like flavanoids possess free radical scavenging activity. They also bring about inhibition of hydrolytic & oxidative enzymes and possess anti-inflammatory action[3]. Various herbs and spices have been reported to exhibit antioxidant activity. Some of these common plants include *Ocimum sanctum, Piper cubeba, Allium sativum, Camellia sinensis* etc. Majority of the antioxidant activity in plants is due to the flavones, isoflavones, flavonoids, anthocyanin, coumarin, lignins, catechins and isocatechins[4].

Moringa oleifera is a widely cultivated species belonging to the family Moringaceae and is known for its traditional medicinal properties and industrial uses. Leaves, flowers and tender pods are edible so also leaves act as animal forage, domestic cleaning agent, manure and biopesticides[5]. Seed oil is non sticking, free from rancidity, a good machine lubricant and is used in perfumery, skin and hair care[6]. The plant in general show antioxidant, antimicrobial, antidiabetic, anti-inflammatory and antiulcer properties[7,8,9]. The plant leaves are known for its antihypertensive, hypocholesterolaemic, anti-ulcer and wound healing properties[10]. Hypolipidemic activity in *M.oleifera* fruits are also reported[11]. *M.oleifera* pods and flowers are a rich also a rich source of digestible proteins, calcium, iron, vit-c and carotens[12]. *Moringa oleifera leaves contains* essential amino acids and carotenoids with pharmaceutical properties, supporting the idea of using this plant as a nutritional supplement or constituent in food preparation [9]. Significant antioxidant potential with increase in activity of catalase, glutathione peroxidase (GPX), and glutathione reductase (GR) is reported from *M.oleifera* plant[13] . Traditional practice in Goa is to consume the leaves once a year as potent source of dietary needs. Consumption of

Moringa oleifera leaves is not just a delicacy but has become a traditional cultural practice associated with its medicinal properties and health effects. In view of these multiple uses for *Moringa oleifera* in health care, this work was planned with an objective to study the antioxidant enzymes, phenolics, flavanoid content and the free radical scavenging ability of the enzymes in vegetative (leaves) and reproductive parts (flowers) of *Moringa oleifera* Lam. plant extract.

MATERIALS AND METHOD

Sample Collection

Fresh samples of leaves and flowers of *Moringa oleifera Lam.* belonging to the family Moringaceae, commonly called as mosing in Goa were collected in the month of November, 2010 from our campus. The specimen was authenticated by the Department of Botany, Chowgule College, Margao Goa.

Extraction of the sample

Sun dried plant material(500gms) was homogenized in 1L of methanol overnight. The extraction protocol was slightly modified by grinding the tissue using Remi blender. Extract was then filtered through four layers of muslin cloth and subsequently filtered through Whatman filter paper No.1. The solvent was then evaporated under vacuum using Evator rotary evaporator[14]. The residues were dissolved in few ml of methanol, and stored at 4^{o}c for further use. Study of antioxidant enzyme activity, phenolics, flavonoid, antioxidant enzymes, total protein and sugars content was undertaken.

ASSAY OF ANTIOXIDANT ENZYME ACTIVITY

Antioxidant enzyme activity included DPPH free radical scavenging activity, Reducing power assay, Nitric oxide scavenging activity and hydrogen peroxide scavenging activity.

DPPH free radical scavenging assay

The free-radical scavenging activity of *Moringa oleifera Lam.* (Leaf and flower) extract was measured by decrease in the absorbance of methanolic DPPH solution[15]. A stock solution of DPPH (33 mg in 1 L) was prepared in methanol. 5ml of this stock solution was added to 1 ml of *M.oleifera* plant tissue extract at different concentrations (250–2500 mg/ml). After 30 min of incubation at room temperature, absorbance was measured in a spectrophotometer at 517 nm and was compared with the Gallic acid standard (250–2500 mg/ml). DPPH free radical scavenging activity was expressed as the percentage inhibition calculated using the following formula[16].

% Radical scavenging power = Absorbance [control - (Sample - Blank)]/ Absorbance of control x 100

Reducing power assay

The reducing power of vegetative and reproductive plant extract of *Moringa oleifera* Lam. was determined according to the standard method[17]. Plant extract in the final concentration of 200–1000 µg/ml with 1ml of methanol were mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferrocyanide. The mixture was incubated at 50^{o}C for 20 min. After incubation 2.5 ml of 10% trichloroacetic acid was added to the above incubate. The mixture was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of 1% $FeCl_3$. The absorbance was measured at 700 nm and was compared with the standard BHT (200–1000 µg/ml). Increase in absorbance of the reaction mixture indicated increase in reducing power.

Activity of Nitric oxide scavenging activity

Nitric oxide scavenging activity was measured spectrophotometrically[18]. Sodium nitropruside (5mM) in phosphate buffered saline was mixed with different concentrations of the methanolic extract *of Moringa oleifera Lam* (250–2500 µg/ml) and incubated at 25°C for 30 min. A control without sample extract but with an equivalent amount of methanol was taken. Samples were incubated for 30min. Thereafter, 1.5 ml of the incubated solution was diluted with 1.5 ml of Griess reagent (prepared using 1% sulphanilamide, 2% phosphoric acid and 0.1% N-1- naphthyl ethylenediamine dihydrochloride. The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with N-1-naphthylethylene diamaine dihydrochloride was measured at 546nm and percentage scavenging activity was measured with reference to ascorbate (250–2500 µg/ml) as standard. The capacity to scavenge NO radicals was calculated using the formula.

Scavenging activity (%) = [1 - (A_1 - A_2)/ A_0] x 100 %, where A_0 was the absorbance of the control (the reaction mixture without the extract), A_1 was the absorbance in the presence of the extract and A_2 was the absorbance without Griess reagent.

H_20_2 Scavenging activity

H_20_2 scavenging activity was determined spectrophotometrically at 230nm[19]. Plant material (0.5g) was weighed and homogenized in 0.1mM phosphate buffer pH (7.4) using mortar and pestle. Homogenate was centrifuged at 10,000 r.p.m for 10 minutes. The supernatant was diluted to get the concentration of 250-2500 µg/ml prior to its use in the assay. The reaction mixture containing 0.6ml of H_2O_2 and 3ml of the sample in the final concentration (250-2500 µg/ml) were incubated for 10 minutes and absorbance was measured at 230 nm with Ascorbic acid as standard. A control was maintained for each concentration and the % scavenging activity was

determined using the formula.

% H_2O_2 scavenged = $[(A_0-A_1) / A_0] \times 100$

Where A_0 was the absorbance of the control, and A_1 was the absorbance in the presence of the samples of *M. oleifera* Lam. extract and the standards.

Antioxidant enzyme assay

Sample preparation for enzymatic antioxidant like ascorbate peroxidase (APX) was carried out by standard method[20]. 0.1g of plant tissue (lead and flower) were homogenized using liquid nitrogen in a mortar pestle and was ground using 50mM Tris HCl buffer pH (7.8).The homogenate was centrifuged at 10,000g for 20 min at 4^oC. The supernatant was used as enzyme source.

Activity of Ascorbate peroxidase

APX enzyme assay was carried out spectrophotometrically[21].The reaction mixture in a final volume of 3ml consisting of 50mM phosphate buffer pH(7), 2mM ascorbate ,0.1 mM EDTA, 0.5mM Na-ascorbate, 0.1mM H_20_2 and 100µl of plant extract were mixed by inverting the cuvette and the absorbance was read in a spectrophotometer at 290nm. Readings were taken every 40s interval. The molar extinction coefficient 2.8 $mMol^{-1}$ cm^{-1} was used to calculate ascorbate peroxidase activity.

Non enzymatic antioxidants like total ascorbate

Total ascorbate (ASC+DHA) was assayed using bipyridyl method[22]. Ascorbate reduces F^{3+} to $Fe^{2+}$and F^{3+} forms a red complex with bipyridyl. Dehydroascorbate (DHA, the oxidized form) was measured by reduction of DHA to ascorbate with (10mM) dithiothreitol. Excess DTT was destroyed by N-ethylmaleimide (NEM, 0.5%). The total Ascorbate (ASC+DHA) was studied at 520nm and DHA content was estimated from the difference between assays with and without NEM.

Total phenolic content

Total phenolic content was determined using Folin-Ciocalteu (FC) reagent[23]. The plant extract (0.5 ml) was mixed with 0.75 ml of FC reagent (1:1 diluted with distilled water) and incubated for 5 min at 22°C, and then 0.06% Na_2CO_3 solution was added. After incubation at 22°C for 90 min, the absorbance was measured at 725 nm. The phenolic content was calculated from a gallic acid standard curve.

Total flavonoid content

The total flavonoid content was determined using aluminium chloride ($AlCl_3$) method[24]. The

assay mixture consisting of plant extract (0.5ml), 0.3ml distilled water; 0.03ml of 5% $NaNO_2$ was incubated for 5 min at 25°C. After 5 minutes 0.03 ml of 10% $AlCl_3$ was added and further incubated to another 5 min. The reaction mixture was then treated with 0.2 ml of 1mM NaOH. Finally, the reaction mixture was diluted to 1ml with water and the absorbance was measured at 510 nm. Quercetin was used as standard.

Protein content

Protein content (µg/g F.w.) was studied using BSA as standard [25]. Plant tissue (0.5g) was homogenized in 50 mM phosphate buffer pH (7.8). Extract was centrifuged at low speed and supernatant was used for the assay. An aliquot in known concentration was mixed with 5ml alkaline copper sulphate solution consisting of 2.5% sodium carbonate in 0.1 N sodium hydroxide and 0.5% copper sulphate in 1% potassium sodium tartarate. The contents were mixed and allowed to stand for 10 min at room temperature. Folin ciocalcateau reagent (0.5 ml) diluted 1:1 was added to this incubate, mixed well and was followed by dark incubation at room temperature for 30 min. Absorbance was read at 660nm.

Total Sugars

Total sugars were determined spectrophotometrically[26]. Sugars were extracted twice using 0.1 g of the sample (leaf & flower) in 5 ml 90% hot alcohol. The extract was centrifuged in a Remi R-24 centrifuge for 10 min at 5000 rpm. Supernatant was evaporated to complete dryness & residues were re-dissolved completely in 5 ml of distilled water. For reducing sugars extract in a final volume of 3ml with distil water was mixed with 3ml DNSA (Dinitrosalicylic acid) and warmed in a water bath for 5min. 1ml 40% Rochelle salt was mixed to the incubate and absorbance was read at 510nm.Total sugars were assayed using 5% phenol added to the extract in final volume of 1ml with distilled water. 5ml Conc. H_2S0_4 was added to this mixture and mixed well. Absorbance was read at 490nm. Total sugars (µg/g F.w.) were calculated using glucose as standard.

Statistical Analysis

All experiments were repeated thrice independently with similar results. Data shown are expressed as mean $\pm$ standard deviation (S.D) with readings of 3 samples per tissue. Data analysis was based on ONE WAY analysis of variance (ANOVA). All statistical analysis was performed using Microsoft Excel Version 7.

RESULTS AND DISCUSSION

DPPH radical scavenging activity

Extensive damage to tissues and biomolecules is often caused due to regular generation of free radicals[27]. Many synthetic drugs are known to protect living system against oxidative damage. However, many of these have adverse side effects. An alternative solution to this problem is to consume natural antioxidants from food supplements and traditional medicines[28]. Recently, many natural antioxidants have been isolated from different plant materials [29,30].

Our results with DPPH radical scavenging activity are shown in Figure 1.

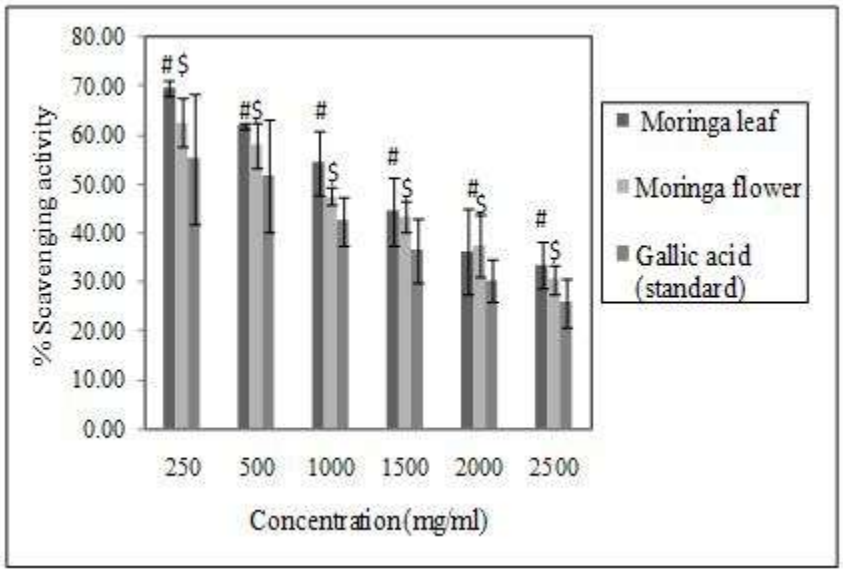

Figure 1: DPPH Percentage (%) scavenging activity in *Moringa oleifera*. The values are mean of 3 experiments ± S.D. Statistical data shows significant difference at $^{\#}P< 0.001$ & $^{\$}P<0.01$.

The percentage (%) scavenging activity was found to be more in the methanolic leaf extract of *Moringa oleifera* than the flower extract. DPPH is a stable nitrogen centred free radical that can be scavenged by oxidants. The change in absorbance of DPPH radical caused by the extract was due to reaction between antioxidant molecule and the extract that resulted in scavenging of the radical by hydrogen donation. This was indicated by the colour change from purple to yellow. The extent of DPPH radical scavenging activity was measured as decrease in intensity of violet colour. When compared to the standard Gallic acid our results with M. *oleifera* plant extract showed significant decline in DPPH radical scavenging activity. The decline may be due to the scavenging ability of Moringa plant extract. We observed maximum DPPH radical scavenging activity in leaf extract (69.63% with 250mg/ml extract) while in 62.63% was observed in the flower extract in comparison with the standard (55.29%). In our study, the radical scavenging activity of methanolic extract of Moringa plant against Hydroxyl ($^{\cdot}OH$) and $O_2^{\cdot -}$ radicals are generated by the Fenton system ($Fe^{2+} + H_2O_2 \rightarrow {}^{\cdot}OH + OH^- + Fe^{3+}$). Therefore, $^{\cdot}OH$ scavenging

activity could be influenced by electron donating and metal chelating abilities of antioxidant compound in Moringa extract. ·Hydroxyl redical is widely accepted as the most potent oxidant and the major damaging species in free radical pathology. It can initiate lipid peroxidation, cause DNA strand breaks, and indiscriminatingly oxidize any organic molecule[31]. Herbal preparations of plant extracts showed better scavenging and reducing power ability[32]. However various researchers have shown relatively low DPPH activity with ginger and rosemary plant extracts[33,34]. Our result revealed that methanolic leaf extract of *Moringa oleifera* Lam. possessed highly significant ($^{\#}P< 0.001$) free radical (·OH) and DPPH scavenging activity compared to the flower extract ($^{\$}P<0.01$) and it increases with increasing concentration.

Reducing power assay

The results of reducing power assay measured as reducing ability of ferric to ferrous in presence of the plant extract are shown in Figure 2. Our results indicate increase in reducing capacity with increasing concentration of plant extract (leaf and flower).The reducing capacity in leaves was found to be higher compared to that of the flower extract at all the concentrations studied. However, when compared to standard BHT the reducing power assay of methanolic leaf and flower extract of *Moringa oleifera* Lam. was found to be lower. Reducing power of leaf and flower extract showed a decline of 69.06% & 81.18% respt.in comparison to the standard BHT at a concentration of 1000µg/ml.

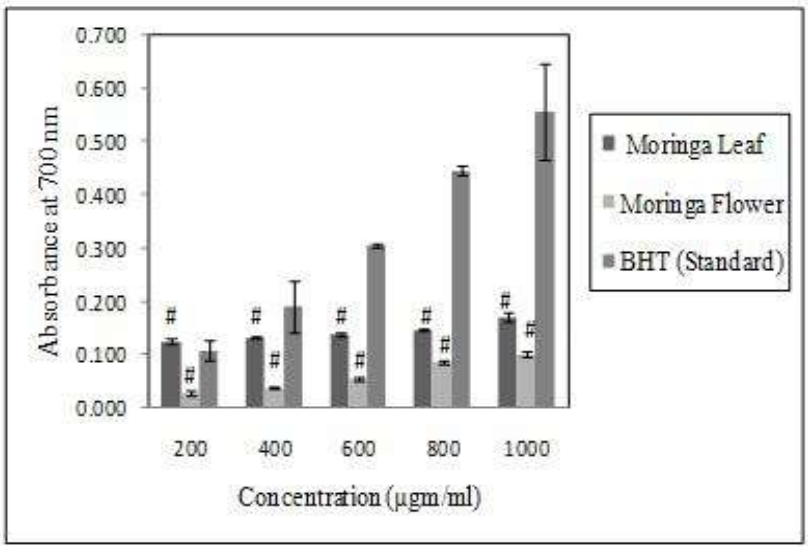

Figure 2: Reducing power assay in *Moringa oleifera*. The values are mean of 3 experiments ± S.D. Statistical data shows significant difference at $^{\#}P< 0.001$.

Nitric oxide (NO)

The results of Nitric oxide radical scavenging activity are shown in Figure 3. The percentage (%)

inhibition of Nitric oxide radical scavenging activity in leaf extract of Moringa was found to be higher than that of the flower extract and showed increase with increasing concentration (250µg/ml-2500µg/ml). However, in comparison to the standard ascorbate, the percentage inhibition of Nitric oxide radical scavenging activity in leaf and flower extract of Moringa showed a decline (40.07% & 36.11% resp.) at 2500µg/ml extract. In our work, the method used to study ·NO scavenging activity was based on the release of ·NO in physiological solution and was determined with Griess reagent by the inhibition of nitrite production. The toxicity of NO increased greatly when it reacts with superoxide radical, forming the highly reactive peroxynitrite anion ($ONOO^-$)[35]. The extract inhibits nitrite formation by directly competing with oxygen in the reaction with Nitric oxide. Our study with methanolic leaf & flower extracts of *Moringa oleifera* Lam. showed significant ($^{\#}P<0.001$) level of % inhibition of Nitric oxide radicals in leaf extract compared to the flower extract. Significant increase in Nitric oxide scavenging activity in *Acalypha indica Linn* root extract is also reported[36]. The increase in % inhibition of nitric oxide radicals is attributed to higher antioxidant activity. Our results with NO activity thus show that the Moringa plant extract has more potent Nitric oxide scavenging activity and both leaf and flower extract proved to be better sources for inhibition of Nitrite formation.

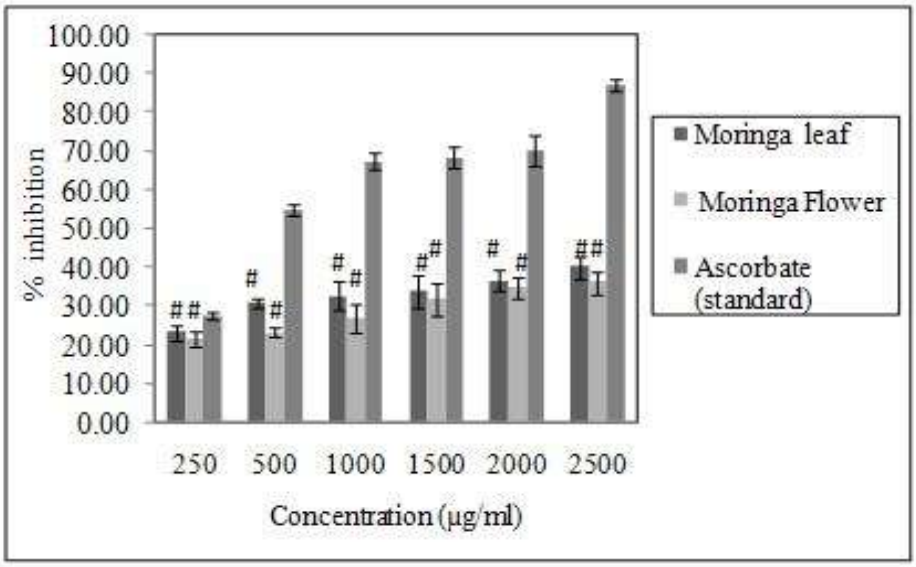

Figure 3: Percentage (%) inhibition of Nitric oxide radical scavenging activity in *Moringa oleifera* . The values are mean of 3 experiments ± S.D. Statistical data shows significant difference at $^{\#}P< 0.001$.

Hydrogen peroxide scavenging activity

Table 1 shows the results of hydrogen peroxide scavenging activity in *M. oleifera* leaf and flower

extract. Our results showed increase in % hydrogen peroxide scavenging activity with increasing concentration of leaf and flower extract (250-2500µg/ml). The scavenging activity of leaf & flower were 55.72% & 52.27% resp. at 2500µg/ml. However, standard ascorbate showed maximum scavenging activity of 60.15%, 54.81% & 43.22% with 250-1000µg/ml concentration respt. in comparison to the leaf and flower extracts. Hydrogen peroxide is a weak oxidizing agent inactivating some enzymes directly by oxidation of essential thiol (-SH) groups. It can cross cell membranes rapidly and once inside the cell, it can probably react with Fe^{2+} or possibly Cu^{2+} ions to form hydroxyl radicals making a way for the origin of many toxic effects[37]. Our study with hydrogen peroxide scavenging activity showed that *M. oleifera* Lam. plant extract (leaf and flower) possessed significantly (#P< 0.001) higher H_2O_2 scavenging activity. Amongst the tissues used leaf showed more activity in comparison to the flower extract. The results indicated that both leaf and flower extracts of Moringa were capable of scavenging H_2O_2 in a concentration dependent manner. Our results are thus in accordance to the earlier report.

Table 1 H_2O_2 percentage (%) scavenging activity in *Moringa oleifera* . The values are mean of 3 experiments ± S.D. Statistical data shows significant difference at $^{\#}P< 0.001$ & $^{*}P<0.05$.

Concentration (µg/ml)	% scavenging activity		
	Ascorbate (Standard)	Moringa leaf	Moringa Flower
250	8.92 ± 0.83	26.84 ± 5.63*	30.66 ± 2.64#
500	16.47 ± 7.79	34.59 ± 6.14*	34.64 ± 1.07#
1000	36.35 ± 15.98	39.55 ± 6.26*	42.23 ± 4.92#
1500	59.74 ± 2.26	47.03 ± 7.80*	46.04 ± 6.05#
2000	78.53 ± 1.11	51.4 ± 8.50*	49.42 ± 6.62#
2500	87.88 ± 0.72	55.72 ± 8.05*	52.27 ± 1.90#

Antioxidant enzyme assay

This was studied as enzymatic antioxidant ascorbate peroxidase (APX) and nonenzymatic antioxidant ascorbate content in vegetative and reproductive parts of *Moringa oleifera Lam.*

Ascorbate & ascorbate peroxidase (APX) enzymes

The results of ascorbate peroxidase and total ascorbate are shown in Table 2 & Figure 4 respt. The ascorbate peroxidase enzyme activity showed a decline over time (0-3min). The leaf tissue of Moringa showed more APX peroxidase activity in comparison to the flower extract. The results of total ascorbate (ASC+DASC) showed that Total ascorbate content in *Moringa oleifera* plant was found to be higher in the flower extract (0.8µmol/g.Fw.) than in the leaf extract (0.43µmol/g.Fw.) APX is important enzyme of the ascorbate-glutathione pathway and is required to scavenge $^{\cdot}OH$ radicals produced mainly in chloroplasts and other cell organelles so that redox state of the cell can be maintained[38]. Ascorbic acid is an important scavenger of

reactive oxygen species (ROS) generated in cell and helps in providing protection to membranes by scavenging $^1O_2$ and $^{\cdot}OH$ radicals. APX utilizes ascorbate as a specific electron donor to reduce H_20_2 to water and thus are important in ascorbate glutathione cycling[39]. In our study APX increase is correlated with increased total ascorbate content in flower and leaf respt. We observed non significant increase in total ascorbate content in leaf and flower extract ($^{ns}P > 0.05$). This may be possibly due to the networking of ascorbate with antioxidant enzymes like Glutathione, Superoxide dismutase (SOD), Catalase and APX. The study however needs thorough understanding of these enzymes at the gene level.

Table 2 Ascorbate peroxidase (APX) activity in *Moringa oleifera* . The values are mean of 3 experiments ± S.D. Statistical data shows significant difference at $^{\#}P< 0.001$

Concentration (ml)	Time in seconds	Moringa Leaf	Moringa Flower
0.1	40	0.35 ± 0.06$^{\#}$	0.34 ± 0.001$^{\#}$
	80	0.09 ± 0.04 $^{\#}$	0.15 ± 0.017 $^{\#}$
	120	0.09 ± 0.03$^{\#}$	0.10± 0.09 $^{\#}$
0.2	40	0.39 ± 0.02 $^{\#}$	0.39 ± 0.002 $^{\#}$
	80	0.15 ± 0.01 $^{\#}$	0.17 ± 0.002 $^{\#}$
	120	0.10 ± 0.01 $^{\#}$	0.11 ± 0.02 $^{\#}$
0.5	40	0.52 ± 0.001$^{\#}$	0.54 ± 0.001$^{\#}$
	80	0.16 ± 0.001 $^{\#}$	0.17 ± 0.001 $^{\#}$
	120	0.13 ± 0.0 02$^{\#}$	0.11 ± 0.001 $^{\#}$
1	40	0.55 ± 0.002 $^{\#}$	0.60± 0.04 $^{\#}$
	80	0.27 ±0.006 $^{\#}$	0.26 ± 0.01 $^{\#}$
	120	0.15 ± 0.009 $^{\#}$	0.25 ± 0.01 $^{\#}$

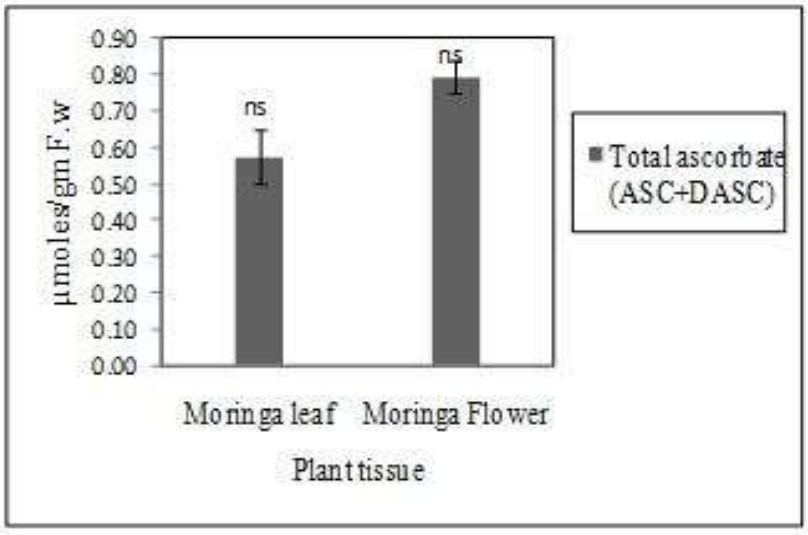

Figure 4: Total Ascorbate (*ASC + DASC*) content in *Moringa oleifera.* The values are mean of 3 experiments ± S.D. Statistical data shows non significant difference at $^{ns}P > 0.05$.

Flavonoid and Phenolics

The results of total Flavanoid and total Phenolic compounds are shown in Table 3. The results of total flavanoid & phenolic content in *M. oleifera* Lam. leaves were 4.53, 0.33mg/ml & 4.59 & 0.406mg/ml in flowers respt. Our results clearly showed that *M. oleifera* flower extract showed 1.32% & 21.21% increase in flavanoid & phenolic content compared to that of the leaf extract.

phenolic & flavanoid compounds are commonly found in the plants and have been extensively exploited because of their multiple biological activities, including antioxidant effects[40]. In Phenolics & Flavanoids at least one hydroxyl ion is substituted with aromatic ring forming chelate complexes with metal ions thus are easily oxidized. They thus serve as important units for donating electrons[40]. The significantly higher phenolic content in leaf and flower extract may have important role in increased antioxidant activity of various antioxidants studied[41].The antioxidant activity of phenolic compounds in earlier reports is shown to be mainly because of their redox properties, allowing them to act as reducing agents, hydrogen donators, or singlet oxygen quenchers[42,43,44].Various investigatory reports on phenolics have shown linear correlation between phenolic content and antioxidant activity in plants[45,46] .

Table 3 Total Flavonoids & Phenolic content in *Moringa oleifera*. The values are mean of 3 experiments ± S.D Statistical data shows significant difference at $^{*}P<0.05$.

Plant tissue	Total Flavonoids (mg/ml)	Total phenols (mg/ml)
Moringa Leaf	4.53 ± 0.03 *	0.33 ± 0.007 *
Moringa Flower	4.59 ± 0.02 *	0.40 ± 0.006 *

Total sugars & Total protein

Results of total sugars & total protein content are shown in Table 4. The results showed 396.34 µg/ml and 110.13µg/ml of total sugars & total protein content in methanolic leaf extract of *Moringa oleifera*. However, the methanolic flower extract of *M. oleifera* Lam. showed 364.61µg/ml & 93.04µg/ml of total sugars and total protein content. The decline in total sugars and proteins in flower extract was 8.02% & 15.5% respt. in comparison to the leaf extract. Our results of protein and sugar content in *M. oleifera* Lam. leaf and flower showed significant increase in total sugar content ($^{\#}P<0.005$) in the leaves compared to the flowers. High amount of crude protein content in extracted and unextracted *M. oleifera* Lam. leaf and twig is also reported[47,48]. The seeds & leaves of *M. oleifera* Lam. are known to possess greater amount of protein content. While, glucose, fructose, raffinose and stachyose are the major carbohydrates reported[47]. In our study, the protein content in leaves was not significantly higher ($^{ns}P>0.05$) to that of the flower. This non significant increase in protein content in leaves could be because of

rapid turnover rate of proteins i.e. synthesis/degradation is higher or it could possibly be due to increased activity of several oxidoreductase type of protein synthesis.

Table 4 Total sugars & Total protein content in *Moringa oleifera*. The values are mean of 3 experiments ± S.D. Statistical data shows significant difference at $^{\#}P< 0.001$ & non significant difference at $^{ns}P >0.05$.

Plant tissue	Total sugars (µg/gm F.w)	Total protein (µg/gm F.w)
Moringa Leaf	$396.43 \pm 1.3^{\#}$	110.13 ± 11.3^{ns}
Moringa Flower	$364.61 \pm 1.0^{\#}$	93.04 ± 2.6^{ns}

CONCLUSIONS

On the basis of the results obtained in the present study, it is concluded that methanolic extract of leaf and flower of *M. oleifera Lam.*exhibit high antioxidant and free radical scavenging activities. The extract also contains large amounts of flavonoids and phenolic compounds with antioxidant potential. Our study indicates that *M. oleifera* Lam. plant is a significant source of natural antioxidant and might be useful in preventing the progress of various oxidative stresses. Leaves & flowers of *M. oleifera* Lam. possess natural antioxidants and hence can be used as potent source to replace synthetic antioxidants in food, health and pharmaceuticals. Influence of *M. oleifera* plant extract on human health and nutrition is highly considerable. However, the components responsible for the anti-oxidative activity in this plant need to be investigated further. There appears to be strong correlation between increase in phenolics & flavanoid content with increased antioxidant enzyme activity as well as in increased synthesis of ascorbate and ascorbate peroxidase enzymes.

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