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# Antioxidant Activity of Different Solvent Extracts of *Moringa oleifera* Leaves under Accelerated Storage of Sunflower Oil

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**Abstract:** The present study was aimed to evaluate the antioxidant activity of different solvent extracts of *Moringa oleifera* leaves by using accelerated aging of sunflower oil. Methanolic and acetone (80 and 100%) extracts of *M. oleifera* leaves were added at the concentration of 0.06% (wt/wt) into the refined, bleached and deodorized sunflower oil. The stabilized sunflower oil samples were subjected to accelerated aging (65°C) for a period of 18 days and analysis was done periodically after every three days. The extent of oxidation was followed by the measurement of AI-, PV-, conjugated dienes-, trienes- and para-anisidine values. The overall order of antioxidant efficacy of the extracts of *M. oleifera* leaves as assessed by various oxidation parameters was followed as; 80% methanolic extract > 100% methanolic extract > 80% acetone extract > 100% acetone extract. The antioxidant activity of *M. oleifera* leaves might be attributed to the presence of high amount of flavonoids, polyphenolics and tocopherol contents. The results of present study revealed that *M. oleifera* leaves might be explored as a viable source of natural antioxidants and nutraceuticals.

**Key words:** *Moringa oleifera*, leaves extracts, lipid oxidation, antioxidant activity, sunflower oil, accelerated aging

## INTRODUCTION

The oxidative reactions limit the shelf life of fresh and processed foodstuff and are a serious concern in the food industry. Also the free radicals leading to the oxidation of biomolecules are implicated in several diseases[1].

Lipid peroxidation is a paradox of aerobic life, affecting both human health and the quality of modern life. Biological systems are lipid rich matrices susceptible to autoxidation unless protected by some mechanisms[2].

Oil industry is one of the most important food industries that have to pay great attention in this regard. Oil that contain relatively high amount of Polyunsaturated Fatty Acids (PUFA) experience stability problems and readily oxidize to give primary hydroxides. The breakdown products of hydroperoxides such as alcohols, aldehydes, ketones and hydrocarbons generally possess offensive off-flavors and make oil rancid and thus lead to economic loses. Strongly oxidized oils could have toxic effects in health and therefore these oils are not suitable for nutritive purposes because of reaction products[3,4]. Now the addition of an antioxidant is popular and applied as a means of increasing the storage period of food products and for improving the stability of lipids and lipid containing foods without loss of sensory and nutritional qualities. It is a generally accepted technological procedure, but these days consumers, assuming those natural compounds are safer, prefer natural antioxidants to the synthetic ones[5].

Current research into free radicals has confirmed that food rich in antioxidants plays an essential role in the prevention of cardiovascular diseases and cancers[6,7].

Plants are a viable source of natural antioxidants. Some plants, especially rosemary and sage, spices and cereals extracts have been found as promising sources of natural antioxidants[8].

Many medicinal plants contain large amount of antioxidants other than vitamin C, vitamin E and carotenoids. The growing consumer preference to the natural products forces the fats and oil industry to seek natural sources for antioxidants rather than investing in synthetic ones. A significant number of plants have been evaluated for their antioxidant activities using different assays[9,10]. Some plants especially rosemary and sage belonging to family *Labiateae* have provided extracts with an effective antioxidant potential, applicable for the protection of oil, fats and salad dressing[11-13]. Leafy green vegetables and beans contain phytochemicals that are chemopreventive. Most of them contain antioxidant substances e.g., indoles, carotenoids, vitamin C and

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phenolics[14,15]. Cereals and legumes containing a wide range of phenolics are also a good source of natural antioxidants[16,17].

A significant number of literature reports are available dealing with the antioxidant potential of plant sources. Lovaas[18] has studied the antioxidative effects of polyamines by measurement of primary and secondary oxidation products of polyunsaturated fatty acids. Choudhary and Kale[19] have investigated the protective efficacy of *Piper betle* leave extracts in a radiation induced lipid peroxidation process. Jasweer *et al.*[20] have applied oleoresin, rosemary and sage extracts as natural antioxidants in palm oil in deep fat frying test. In an antioxidant activity oriented study Vichi *et al.*[21] have evaluated the activity of oregano extracts added to animal fats by means of assessment of the radical scavenging capacity by photochemiluminescence analysis.

Pakistan is an agricultural country and is rich in medicinally important flora. Some literature reports are available which deals with the antioxidant activity of various Pakistani plants[22]. *M. oleifera* Lam. (drumstick tree, horseradish tree) is indigenous to many countries such as Africa, Arabia, Southeast Asia and South America and is often cultivated in hedges and homeyards[23]. The tree is valued mainly for the tender pods, which are esteemed as a vegetable. *M. oleifera Lam,* locally known as Sohanjna is grown and widely cultivated through out the plains of Punjab, Sindh, Baluchistan and North Western Frontier province of Pakistan[24]. A number of medicinal and therapeutic properties have been ascribed to various parts of this multi-purpose plant that included the treatment of ascites, rheumatism and venomous bites and as cardiac and circulatory stimulants[25]. The flowers of *M. oleifera* are considered to possess medicinal values and they have been also reported to contain some flavonoids pigments such as quercetin, kaempherol, isoquercitrin and kaempferitrin[26]. *M. oleifera* leaves can act as a good source of natural antioxidant 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, phenolic and carotenoids[27]. *M. oleifera* seeds are good source of natural antioxidants and the seed oil has excellent keeping quality[28].

However, no efforts have been made to evaluate the antioxidant principles from the abundantly available leaves of *M. oleifera* indigenous to Pakistan. Therefore, the present study was designed in order to investigate the antioxidant activity of *M. oleifera* leaves, by using sunflower-oil as oxidation substrate.

## MATERIALS AND METHODS

Mature leaves of *Moringa oleifera* were collected from the vicinity of Sadiqabad during May-June 2004. The leaves were further identified and authenticated by Dr. Muhammad Ashraf, Professor of Botany, Department of Botany, University of Agriculture, Faisalabad. Refined, Bleached and Deodorized (RBD) Sunflower oil was procured from United Industries Pvt. Limited, Kashmir Road, Faisalabad, Pakistan. All other chemicals and reagents used were of Sigma, Aldrich and E. Merck.

The samples of leaves were ground to pass one mm sieve. Extraction was carried out with methanol and acetone (80 and 100%) by using electrical shaker. After extraction the contents of the flasks were filtered, evaporated to dryness under reduced pressure at 45°C by using a rotary evaporator (EYELA, Rotary Vacuum Evaporator.N.N.Series equipped with an Aspirator and a Digital Water Bath SB-651, Japan) and stored under refrigerator until used for further analysis.

The crude concentrated extracts were separately added into the refined, bleached and deodorized (RBD) sunflower oil at concentration of 600 ppm. The oil samples were stirred for 30 min at 50°C for uniform dispersion. A control (without the addition of any antioxidant extract) sample was also prepared under the same set of analytical conditions. The samples were stored at accelerated conditions (at 65°C for 18 days)[29]. The analysis was carried out periodically after every three days. The oxidative deterioration level was followed by the measurement of antioxidant activity index, peroxide values, conjugated dienes and trienes contents and p-anisidine value.

Determination of Induction Period (IP) and thus calculation of AI was made following the procedure of Metrohm Application Bulletin[30]. An automated Metrohm Rancimat Model 679, capable of operating over a temperature range of 50-200°C was used for the determination of induction period (IP) of stabilized and controlled oil treatments. Analysis was carried out at 120±0.1°C and oxidative stability was measured following the procedure described elsewhere[29]. Briefly, oil (2.5 g) was carefully weighed into each of the six reaction vessels and analyzed simultaneously. IP of the samples were automatically recorded and corresponded to the break point of the plotted curves.

Peroxide value, the titration measure of all peroxides and lipid oxidation products, was calculated following the method of IUPAC[31].

Conjugated dienes and trienes are auto oxidation products, which were determined by measuring the

specific extinctions at 232 and 268 nm. Samples of oil were diluted with iso-octane and spectrum was recorded in the ultraviolet region and absorbance values were noted at 232 and 268 nm by using the method of IUPAC[31].

The determination of P-anisidine value was made following the standard IUPAC method[31]. The oil samples were allowed to react with P-anisidine reagent to produce color compounds and the absorbance values were noted at 350nm using a Hitachi U-2001, model 121-0032 spectrometer. The P-anisidine value was calculated following the above mentioned IUPAC method[31].

SPD: Storage period in days.
SFO-0: Sunflower oil without any extract (control).
SFO-1: Sunflower oil stabilized with 80% Methanolic extract of *M. oleifera* leaves.
SFO-2: Sunflower oil stabilized with 100% Methanolic extract of *M. oleifera* leaves.
SFO-3: Sunflower oil stabilized with 80% Acetone extract *M. oleifera* leaves.
SFO-4: Sunflower oil stabilized with 100% Acetone extract *M. oleifera* leaves.

## RESULTS AND DISCUSSION

AI is an important criterion for the evaluation of effectiveness of antioxidants[30]. It was evident from the results (Table 1) that addition of antioxidant extract has significantly improved the AI values of SFO treatments. The AI value of the control (SFO-0) samples was quite low (1.00) and thus showed the least stability among oil treatments. Eighty percent methanolic extract was found to be the most effective antioxidant extract as noted by the AI (3.28) values of SFO-1 treatment (Table 1).

Table 2 shows the relative increase in Peroxide Value (PV) of SFO treatments stabilized with extracts of *M. oleifera* leaves under accelerated conditions. The control (SFO-0) sample had the highest peroxide value (85.5 m Eq $kg^{-1}$) and thus indicated a higher rate of oxidation. Among different methanolic and acetone extract; 80% methanolic extract was found to be the most effective in retarding the peroxide value as shown by lowest rise in PV (52.1 m Eq $kg^{-1}$) of the SFO-1 treatment. The acetone extracts of *M. oleifera* were also found to retard the rise in PV of SFO treatments and thus exhibited antioxidant activity. However, SFO treatments stabilized with different concentration of acetone extract were found to show high value of PV as compared with those of methanolic extracts. Zia-ur-Rehman *et al.*[32] have reported the antioxidant activity of ginger extract as natural antioxidant to stabilize sunflower oil samples under different temperatures was found to greatly inhibit the rise in PV in a concentration dependent manner. Anwar *et al.*[29] have also described the antioxidant activity of some natural extracts in corn oil (under accelerated aging) using PV as an oxidation parameter and thus an indicator of measurement of antioxidant activity of the extracts.

Table 1: Determination of antioxidant Activity Index (AI) of SFO treatments

| Oil treatments | AI |
|---|---|
| SFO-0 | 1.00±0.05 |
| SFO-1 | 3.28±0.14 |
| SFO-2 | 3.10±0.16 |
| SFO-3 | 2.80±0.14 |
| SFO-4 | 2.68±0.13 |

Values (mean±SD) are average of duplicate samples analyzed individually in triplicate

Table 2: Relative increase in Peroxide values (meq $kg^{-1}$) of SFO treatments stabilized with the extracts of *M. oleifera* leaves

| SPD | SFO-0 | SFO-1 | SFO-2 | SFO-3 | SFO-4 |
|---|---|---|---|---|---|
| 0 | 0.80±0.04 | 0.80±0.04 | 0.80±0.04 | 0.80±0.04 | 0.80±0.04 |
| 3 | 14.3±0.70 | 6.30±0.55 | 9.20±0.55 | 8.80±0.45 | 9.20±0.45 |
| 6 | 27.2±1.60 | 15.5±0.75 | 18.1±0.43 | 18.4±0.89 | 18.9±0.95 |
| 9 | 39.9±1.91 | 23.4±0.60 | 27.2±1.40 | 29.2±1.56 | 29.6±0.89 |
| 12 | 51.3±1.75 | 32.2±1.12 | 36.8±1.90 | 39.0±1.20 | 40.8±2.01 |
| 15 | 69.4±3.40 | 42.1±0.98 | 46.1±1.25 | 49.8±1.18 | 51.6±1.70 |
| 18 | 85.5±2.80 | 52.1±2.60 | 57.4±1.98 | 60.1±3.11 | 63.9±3.07 |

Values (mean±SD) are average of duplicate samples analyzed individually in triplicate

Table 3: Relative increase in conjugated dienes ($^{1\%}\epsilon_{1\ cm\ \lambda\ 232}$) of SFO treatments stabilized with the extracts of *M. oleifera* leaves

| SPD | S-0 | S-1 | S-2 | S-3 | S-4 |
|---|---|---|---|---|---|
| 0 | 1.80±0.10 | 1.80±0.10 | 1.80±0.10 | 1.80±0.10 | 1.80±0.10 |
| 3 | 4.50±0.13 | 2.70±0.12 | 2.70±0.13 | 2.70±0.13 | 2.70±0.14 |
| 6 | 8.00±0.10 | 4.20±0.22 | 4.30±0.09 | 4.10±0.21 | 4.20±0.12 |
| 9 | 13.5±0.68 | 6.00±0.18 | 6.20±0.32 | 6.50±0.16 | 6.70±0.34 |
| 12 | 18.0±0.50 | 8.10±0.41 | 8.40±0.49 | 8.90±0.45 | 9.10±0.46 |
| 15 | 23.5±0.12 | 10.1±0.53 | 10.4±0.51 | 12.0±0.39 | 12.6±0.40 |
| 18 | 29.4±0.75 | 11.9±0.51 | 12.5±0.48 | 15.0±0.74 | 16.2±0.80 |

Values (mean±SD) are average of duplicate samples analyzed individually in triplicate

Table 3 shows the relative increase in the contents of Conjugated Dienes (CD) of SFO treatments under accelerated condition as determined by the measurement of specific extinction at 232 nm. The determination of conjugated dienes measuring specific extinction at 232 nm is a good indicator of oxidative deterioration of auto-oxidized oils[33]. A quite slow followed by a rapid increase in contents of CD during oxidation was noted in all the SFO treatments. The control had highest rise in conjuable oxidation products as compared with those of stabilized oil samples. The SFO treatment with eighty percent methanolic extract of leaves of *M. oleifera* was found to show least contents of conjugated dienes products as compared with those of the other solvent extracts and thus reflected a higher antioxidant activity.

Table 4 shows the relative increase in the contents of Conjugated Trienes (CT) of SFO treatments under accelerated condition as determined by the measurement of specific extinction at 268 nm. The

Table 4: Relative increase in conjugated trienes ($^{1\%}\epsilon_{1\ cm\ \lambda\ 268}$) of SFO treatments stabilized with the extracts of *M. oleifera* leaves

| SPD | SFO-0 | SFO-1 | SFO-2 | SFO-3 | SFO-4 |
|---|---|---|---|---|---|
| 0 | 0.60±0.03 | 0.60±0.03 | 0.60±0.03 | 0.60±0.03 | 0.60±0.03 |
| 3 | 1.40±0.07 | 1.20±0.05 | 1.20±0.06 | 1.20±0.04 | 1.30±0.05 |
| 6 | 2.50±0.12 | 1.90±0.10 | 1.90±0.05 | 1.90±0.08 | 1.90±0.04 |
| 9 | 4.10±0.21 | 2.60±0.08 | 2.80±0.07 | 2.80±0.06 | 2.80±0.15 |
| 12 | 6.10±0.30 | 3.60±0.17 | 3.90±0.20 | 4.10±0.20 | 4.30±0.09 |
| 15 | 8.90±0.40 | 4.70±0.22 | 5.10±0.19 | 5.30±0.17 | 5.50±0.30 |
| 18 | 12.3±0.5 | 5.80±0.20 | 6.80±0.36 | 7.20±0.14 | 7.60±0.27 |

Values (mean±SD) are average of duplicate samples analyzed individually in triplicate

Table 5: Relative increase in para-anisidine value of SFO treatments stabilized with the extracts of *M. oleifera* leaves

| SPD | SFO-0 | SFO-1 | SFO-2 | SFO-3 | SFO-4 |
|---|---|---|---|---|---|
| 0 | 4.00±0.20 | 4.00±0.20 | 4.00±0.20 | 4.00±0.20 | 4.00±0.20 |
| 3 | 10.3±0.40 | 6.30±0.19 | 7.10±0.22 | 7.60±0.17 | 8.10±0.38 |
| 6 | 17.6±0.39 | 9.80±0.50 | 10.6±0.19 | 12.4±0.62 | 13.4±0.29 |
| 9 | 26.2±1.50 | 13.7±0.40 | 14.9±0.60 | 18.2±0.55 | 19.3±0.95 |
| 12 | 34.9±1.25 | 19.9±1.05 | 20.3±1.20 | 24.6±1.21 | 26.6±1.40 |
| 15 | 45.2±2.45 | 24.3±1.40 | 25.6±0.75 | 28.9±1.65 | 32.2±1.23 |
| 18 | 55.1±2.05 | 28.0±1.25 | 33.1±1.89 | 35.1±1.55 | 37.0±1.90 |

Values (mean±SD) are average of duplicate samples analyzed individually in triplicate

contents of conjugated trienes are also a good indicator of the degree of deterioration of oils[34]. A quite slow followed by a rapid increase in contents of conjugated trienes during oxidation was noted in all the sunflower oil treatments. The control had greatest rise in conjuable oxidation products as compared with those of treated oil samples. The SFO treatment with eighty percent methanolic extract of leaves of *M. oleifera* was found to show least contents of conjugated trienes products and thus reflected a higher antioxidant activity. The sunflower samples stabilized with acetone extracts showed higher contents of conjuable oxidation products and thus reflected least antioxidant activity as compared with those of 80 and 100% methanolic extracts. This lowest increase in the values of conjugated dienes and trienes of the sunflower samples stabilized with 80% methanolic extracts of Moringa leaves might be attributed to the presence of antioxidants constituents of leaves.

The control (SFO-0) had the highest para-anisidine value and thus indicated a higher rate of oxidation. The sunflower oil samples stabilized with 80 and 100% methanolic extracts of *M. oleifera* leaves were found to show significantly lower values of para-anisidine as compared with those of acetone extracts and thus reflected a higher antioxidant activity of the former (Table 5).

Literature also revealed a significant number of reports regarding the antioxidant potential of *M. oleifera* leaves. Perumal and Becker[35] reported that in Southern India village people use the fresh leaves of Morga to prepare cow and buffalo ghee from butter fat. It has been found that there is a significant increase in the shelf life of ghee and that Moringa leaves can be a good source of natural antioxidants. Such enhancement of shelf life of ghee may be due to the various types of antioxidant compounds, such as ascorbic acid, carotenoids and phenolic substances which are present in Moringa leaves. Literature revealed that solvent extracts of *M. oleifera* leaves have also exhibited good antioxidant activity in terms of inhibition of peroxidation in linoleic acid system. The presence of relative concentrations of various flavonoids groups and other phenolic substances in various solvent extracts of Moringa leaves might have been involved in the inhibition of peroxidation[35]. Velioglu *et al.*[36] has also reported that the total phenolic contents of fruits and vegetables contained potential antioxidant activities against linoleic acid peroxidation system.

From the results of present study of various oxidation parameters of sunflower oil samples, it is understandable that both the methanolic and acetone extracts of *M. oleifera* has exhibited good antioxidant activity. However, the antioxidant activity of the 80% methanolic extract was found to be significantly higher than acetone extracts, which might be attributed to the high polarity of methanol-water mixture.

The antioxidant activity of different solvent extracts of *M. oleifera* leaves as exhibited in the present analysis might be attributed to the presence of significantly high amount of polyphenolics and other antioxidant substances e.g., anthocyanin, flavonoid, tocopherol and ascorbic acid. The results of present investigation strongly favor the commercial exploitation of *M. oleifera* leaves indigenous to Pakistan for potential antioxidants and nutraceutical applications.

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