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Full Length Article

Antioxidant Potential and Biochemical Analysis of *Moringa oleifera* Leaves

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Abstract

Moringa oleifera is extremely valuable plant which has been widely grown in the tropical and subtropical areas. Four accessions {originated from Faisalabad (M-Fsd), Multan (M-Mln), India (PKM1) and China (M-China)} of *M. oleifera* were analyzed for total phenolics, superoxide dismutase, peroxidase, catalase, protease, esterase, proteins, ascorbate peroxidase, total antioxidant capacity, malondialdehyde and total oxidant status (TOS) during October(autumn), December (winter), January (winter), February (early spring) and March (late spring). Significant variations were detected in antioxidant activity of leaf extracts among the accessions. Comparison of antioxidant activities during different months revealed that during March (late spring) strongest antioxidant activity was detected in *M. oleifera* leaves. Genotype "M-China" had the maximum antioxidant activity as compared to other accessions. In conclusion, present findings provided evidence that under agro climatic conditions of Pakistan, seasons and accessions significantly influenced the antioxidant and biochemical profiles of this plant. *Moringa* leaves have highest levels of enzymatic antioxidants i.e. POD, CAT and SOD during March and therefore, should be utilized in this month to get maximum health related benefits. In general, accessions M-China and M-Mln have relatively better antioxidant and biochemical profiles as compared to other locally grown accessions.

Keywords: Antioxidants; APX ; CAT; POD; Protein profiling; SDS PAGE; SOD; TAC

Introduction

"Moringaceae" is a single genus family of the class Magnoliopsida with thirteen renowned species and *Moringa oleifera* is the most commonly known and consumed species (Sengupta and Gupta, 1970; Morton, 1991; Abiodun *et al.*, 2012; Owolabi; 2013; Basra *et al.*, 2015; Daba, 2016). It is generally acclimatized to tropics as well as subtropics therefore it is referred to as by various names like drumstick tree, ben tree or horse reddish tree (Paliwal and Sharma, 2011). It is native to South Asia primarily in Himalaya's foothills (Bangladesh and PKM1) (Luqman *et al.*, 2011). It has been cultivated and naturalized in other countries like Pakistan, Srilanka, Afghanistan, Bangladesh, philippines (Daba, 2016) East and West Africa all the way through West Indies, from Mexico to Peru, Brazil and Paraguay (Fahey, 2005; Meena *et al.*, 2010; Paliwal and Sharma, 2011; Koul and Chase, 2015). *M. oleifera* and *M. concanensis* are the two species that are reported in Pakistan. The earlier specie is widely grown in irrigated areas of the country as well as in Sindh province and the later specie is not common and restricted to merely a secluded area of Tharparkar (Anwar *et al.*, 2005).

A wide range of therapeutic and medicinal characteristics has been attributed to different parts of this versatile tree due to the presence of antioxidants, vitamins like riboflavin , nicotinic acid, ascorbic acid, Vitamin-A, vitamin B1-thiamine and vitamin B-choline, oils and fatty acid (Dillard and German, 2000; Charoensin, 2014). Approximately every part of *M. oleifera* that is, fruit, root, seed, gum, bark flowers, leaf, and seed oil have been employed for various disorders in the indigenous South Asian medicine, involving the management of inflammation and infectious diseases (Caceres *et al.*, 1991; Anwar *et al.*, 2007; Kumar *et al.*, 2009; Upadhyay *et al.*, 2015). Flowers, leaves, seed, fruit, bark, roots and immature pods work as circulatory and cardiac stimulants (Makonnen *et al.*, 1997; Verma *et al.*, 2012) and have antitumor, anti-inflammatory, antimicrobial, antiprogestational, antiaging, antiepileptic, antiulcer and antipyretic activity (Pal *et al.*, 1995; Paliwal and Sharma, 2011; Ganatra *et al.*, 2012; Koul and Chase, 2015). These different parts of tree have also been utilized for nutritional purposes as well (Ashfaq *et al.*, 2012). *M. oleifera* leaves and flowers have also been stated to possess flavonoid pigments for example, rhamnetin, kaempferol, isoquercitrin and kaempferitrin (Bukar *et al.*, 2010; Dehshahri *et al.*, 2012) in addition to vitamins C and A, phenolics and carotenoids (Sankhyan *et al.*, 2013). Moringa is known for its antioxidants and is getting popularity as human and animal food supplement. In this view the present project was designed with objective to test/confirm antioxidant potential and biochemical constituents of

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Moringa under local climatic conditions of Pakistan using four accessions from different origins. In parallel, effect of local climatic conditions during different months on antioxidant potential and biochemical constituents of *M. oleifera* was also assessed to find out most suitable time and accession for leaf harvest with maximum benefits.

Materials and Methods

Fresh young compound leaves (from middle branches) of four different accessions of *M. oleifera* tree (Voucher specimen number: No. 66250 KUH; local name: Sohanjna) with origin from Faisalabad (M-Fsd), Multan (M-Mln), India (PKM1) and China (M-China) were collected from plants grown at Department of Crop Physiology, University of Agriculture, Faisalabad, Pakistan. Leaf samples were collected during October (autumn), December (winter) (2013), January (winter), February (early spring) and March (late spring) (2014). Local weather parameters of specified months are given in Table 1.

Evaluation of Antioxidant Enzyme Activities

Extraction of leaf samples: Fresh compound leaves (0.2 g) were extracted in 1.5 mL (50 mM) potassium phosphate buffer (pH 7.4). Samples were centrifuged at 14462 xg for 10 min at 4°C. The supernatant was separated and used for the determination of different enzymatic and non-enzymatic activities.

Total Antioxidant Capacity (TAC)

The reduction of 2, 2-azino-bis (3-ethylbenzothiazoline-6-sulfonate) radical cation ($ABTS^{\bullet+}$ that is blue-green in colour) by antioxidants to its original colourless ABTS form is the basis of the ABTS assay. The $ABTS^{\bullet+}$is decolorized by antioxidants according to their antioxidant content (Nenadis *et al.*, 2007). The assay mixture contained reagent R1 (mixture of sodium acetate buffer solution and glacial acetic acid, pH5.8), sample extract and reagent R2 (mixture of sodium phosphate buffer solution, glacial acetic acid, hydrogen peroxide and ABTs). The contents of the tubes were mixed and allowed to stand for 6 min. Absorbance was measured at 660 nm. The ascorbic acid was used to develop a calibration curve. The TAC values were expressed as milli molar ascorbic acid equivalent L^{-1}.

Total Phenolic Content (TPC)

A micro colorimetric method as described by (Ainsworth and Gillespie, 2007) was applied with some modifications for total phenolics content, in which Folin-Ciocalteau (F-C) reagent was used. A 0.5 g leaf sample was homogenized in 500 µL ice cold 95% methanol using an ice cold mortar and pestle. The samples were then incubated at room temperature for 48 h in the dark. The samples were then subjected to centrifugation at 14462xg for 5 min at room temperature. The supernatant was removed and used for TPC estimation. A 100 µL of supernatant was mixed with 100 µL of 10% (v/v) F-C reagent, vortex thoroughly and then 800 µL of 700 mM Na_2CO_3 was added. Samples were then incubated at room temperature for 1 h. Blank corrected absorbance of samples was measured at 765 nm. A standard curve was prepared using different concentration of gallic acid and a linear regression equation was calculated. Phenolic content (GAE per fresh weight) of samples was determined using linear regression equation. The results were expressed as gallic acid equivalents (GAE) per gram fresh weight.

Superoxide Dismutase (SOD) Activity

For the estimation of SOD activity, leaves were homogenized in a medium composed of 50 mM potassium phosphate buffer (pH 7.0), 0.1 mM EDTA, and 1 mM dithiothreitol (DTT) as described by (Dixit *et al.*, 2001). The activity of SOD was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) following the method of (Giannopolitis and Ries, 1977). One unit of SOD activity was defined as the amount of enzyme that caused 50% inhibition of photochemical reduction of NBT.

Catalase (CAT) Activity

For the estimation of catalase activity, leaves were homogenized in a medium composed of 50 mM potassium phosphate buffer (pH 7.0) and 1 mM dithiothreitol (DTT). Catalase (CAT) was estimated using the method described by (Beers and Sizer, 1952). For measurement of CAT activity, the assay solution contained 50 mM phosphate buffer (pH 7.0), 59 mM H_2O_2, and 0.1 mL enzyme extract. The decrease in absorbance of the reaction solution at 240 nm was recorded after every 20 sec. An absorbance change of 0.01 min^{-1} was defined as 1 U of CAT activity. Enzyme activity was expressed on a fresh weight (F. wt.) basis.

Esterases Activity

The α-esterases and β-esterases were determined according to the method of (Van Asperen, 1962) using α–naphthyl acetate and β–naphthyl acetate as substrates, respectively. The reaction mixture consisted of substrate solution (30 m M α or β–naphthyl acetate, 1% acetone and 0.04 M phosphate buffer (pH 7) and enzyme extract. The mixture was incubated for exactly 15 min at 27°C in dark and then 1 mL of staining solution (1% Fast blue BB and 5% SDS mixed in ratio of 2:5) was added and incubated for 20 min at 27°C in dark. Amount of α- and β-naphthol produced was measured by recording the absorbance at 590 nm. Using standard curve, enzyme activity was α or β naphthol produced in µM min^{-1} per g F. wt.

Peroxidase (POD) Activity

For the estimation of POD, leaves were homogenised in a medium composed of 50 mM potassium phosphate buffer (pH 7.0), 0.1M EDTA and 1 mM DTT. Activity of peroxidase (POD) was measured using the method of

(Chance and Maehly, 1955) with some modification. For measurement of POD activity, the assay solution contained distilled water (545 µL), 200 mM phosphate buffer (pH 7.0), 200 mM guaiacol, 400 mM H_2O_2 and 15 µL enzyme extract. The reaction was initiated after adding the enzyme extract. The increase in absorbance of the reaction solution at 470 nm was recorded after every 20 s. One unit of POD activity was defined as an absorbance change of 0.01 min^{-1}. Enzyme activity was expressed on F. wt basis.

Total Oxidant Status (TOS)

Total oxidant status was determined by using (Erel, 2005) formulated method which is based on the oxidation of ferrous ion to ferric ion by oxidants present in the sample in an acidic medium and the measurement of ferric ion by xylenol orange (Harma *et al.*, 2005). The assay mixture contained reagent R_1, reagent R_2 and sample extract. After 5 min. the absorption was measured at 560 nm by using spectrophotometer. A standard curve was prepared using hydrogen peroxide. The results were expressed in µmol H_2O_2 equivalent per L.

Malondialdehyde (MDA) Content

The level of lipid peroxidation in the leaf tissue was measured in terms of malondialdehyde (MDA, a product of lipid peroxidation) content determined by the thiobarbituric acid (TBA) reaction using method of (Heath and Packer, 1968) with minor modifications as described by (Rajinder, *et al.*, 1981). A 0.25 g leaf sample was homogenized in 0.1% TCA. The homogenate was centrifuged at 14462xg for 5 min. To 1 ml aliquot of the supernatant 20% TCA containing 0.05% TBA were added. The mixture was heated at 95°C for 30 min and then quickly cooled in an ice-bath. After centrifuging at 14462xg for 10 min, the absorbance of the supernatant at 532 nm was read and the value for the non-specific absorption at 600 nm was subtracted. The MDA content was calculated by using extinction coefficient of 155 mM^{-1} cm^{-1}.

Ascorbate Peroxidase (APX) Activity

For the estimation of APX activity, leaves were homogenized in a medium composed of 50 mM potassium phosphate buffer (pH 7.0). APX activity was measured using the method of (Dixit Pandey *et al.*, 2001). Assay buffer was prepared by mixing 200 mM potassium phosphate buffer (pH 7.0), 10 mM ascorbic acid and 0.5 M EDTA. For measurement of APX, the activity assay solution contained assay buffer made up of 10 mM ascorbic acid, 0.5 M EDTA and 200mM potassium phosphate buffer, H_2O_2 (1 mL) and supernatant 50 µL. The oxidation rate of ascorbic acid was estimated by following the decrease in absorbance at 290 nm after every 30 sec (Chen and Asada, 1989).

Protease Activity

For estimation of protease activity, samples were extracted in 50 mM potassium phosphate buffer pH 7.8. Protease activity was dictated by the casein digestion assay described by (Drapeau, 1974). By this method one unit is that amount of enzyme, which releases acid soluble fragments equivalent to 0.001 A280 per minute at 37°C and pH 7.8. Enzyme activity was expressed on Fresh weight basis.

Protein Content

For protein estimation, leaves were homogenized in a medium composed of 50 mM potassium phosphate buffer (pH 7.0). Estimation of quantitative protein was executed by method of (Bradford, 1976). For protein estimation in samples, 5 µL of supernatant and 0.1N NaCl were mixed with 1.0 mL of Bradford dye and the mixture was allowed to stand for 5 min. to form a protein dye complex. Absorbance was calculated at 595 nm by using spectrophotometer. Enzyme activity was expressed on Fresh weight basis.

Statistical Analysis

All the data was reported as mean ± SD. Descriptive statistics was applied to analyze and organize the resulting data. Data were analyzed using two-way ANOVA with three replications. Significance of data was tested by analysis of variance and Tukey (HSD) Test at $p<0.05$ and where applicable at $p<0.01$ using XL-STAT software.

Results

Total Antioxidant Capacity (TAC)

Significant variation was observed among the accessions during different months (Fig. 1). In general, highest activity was detected in accession M-Mln (17.5 µM/g f.wt.) during December (winter) while the lowest TAC activity was observed in accession M-Fsd (4.8 g f.wt.) during January (winter). TAC activity was detected to be statistically same in accession PKM1 during October (autumn) (6.4 µM/g), December (winter) (5.7 µM/g), February (early spring) (6.1 µM/g) and March (late spring) (6.7 µM/g) while this level was statistically higher as well as different during January (winter) (14.9 µM/g). TAC activity was also compared among different tested accessions of *M. oleifera* during different months. During October (autumn), comparatively higher TAC activity was detected in accession M-China (16.4 µM/g) and this level was also statistically higher as detected in accession M-Fsd (14.9 µM/g) and M-Mln (14.3 µM/g). Least TAC activity was observed in accession PKM1 (6.4 µM/g). During December (winter), TAC activity was detected to be statistically same and higher in accession M-Fsd (17.4 µM/g) and M-Mln (17.5 µM/g) respectively than accession M-China and PKM1 while least activity was observed in accession PKM1 (5.7 µM/g). During January (winter), TAC activity remained same in

Table 1: Local weather parameters for different months

Date, Year	Month	Temperature			R.H.	Rainfall	Wind Speed
		max.	min.	avg.			
24, 2013	October (autumn)	31.1	18.2	24.7	59	00.0	02.6
24, 2013	December (winter)	19.2	04.9	12.1	57	00.0	03.7
24, 2014	January (winter)	20.2	06.0	13.1	71	00.0	04.4
24, 2014	February(early spring)	23.1	10.7	16.9	54	00.0	04.5
24, 2014	March (late spring)	20.6	15.9	18.3	84	16.0	05.3

accession M-China (15.1 µM/g) and PKM1 (14.9 µM/g) respectively. During February (early spring), highest TAC activity was detected in accession M-China (15.1 µM/g) and this level was also significantly higher as compared to other accessions. During March (late spring), highest TAC activity was observed in accession M-China (16.0 µM/g), while this level was detected to be statistically same in accessions PKM1 (6.7 µM/g) and M-Mln (6.7 µM/g) respectively. Least TAC activity was observed in accession M-Fsd (4.962 µM/g).

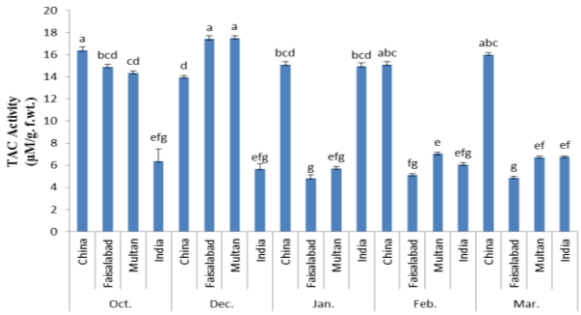

Fig. 1: Comparison of total antioxidant capacity of leaves from different accessions of *Moringa oleifera* during different months

Total Phenolic Content (TPC)

There was no significant variation observed among the accessions of *M. oleifera* during different months (Fig. 2). In general, highest TPC was detected during March (late spring) in the genotype M-China (31811 µM/g) while lowest TPC was observed in genotype PKM1 (2296.7 µM/g) during October (autumn). TPC was also compared among different tested accessions of *M. oleifera* during different months. During October (autumn), comparatively higher TPC was detected in accession M-Fsd (2881.1 µM/g) but the level was statistically same to the accession M-China (25400 µM/g) and M-Mln (27255.6 µM/g). However, the least TPC was observed in accession M-China (25400.0 µM/g) which was also statistically lower than accession M-Fsd. During December (winter), TPC was observed to be statistically same among the accessions M-Mln (24700.0 µM/g), PKM1 (25677.8 µM/g) and M-Fsd (26622.2 µM/g). However, as compared to October (autumn), TPC increased significantly in accession M-China (28244.4 µM/g). During January (winter), TPC was observed to be same in accessions M-China (27111.1 µM/g), M-Mln (26077.8 µM/g) and PKM1 (28522.2 µM/g) and M-Fsd (28522.2 µM/g). Though as compared to December (winter), TPC increased significantly in genotype M-China (27111.1 µM/g). During February (early spring) and March (late spring), TPC was detected to be statistically same among all accessions. However, the level of this content increased significantly in accession M-China (29411.1 µM/g) as compared to January (winter). During March (late spring), as compared to all previously mentioned months, TPC was detected to be increased appreciably among the accession M-China (31811.1), M-Mln (31711.1 µM/g) and PKM1 (31422.2 µM/g).

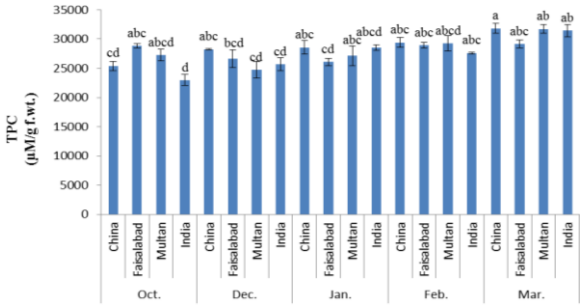

Fig. 2: Comparison of total phenolic content of leaves from different accessions of *Moringa oleifera* during different months

Accessions: Faisalabad (M-Fsd), Multan (M-Mln), India (PKM1) and China (M-china)

Superoxide Dismutase (SOD) Activity

SOD activity was highly significant and variable during different months October (autumn), December (winter), January (winter), February (early spring) and March (late spring) in the accessions M-Fsd, M-China, M-Mln and PKM1 (Fig. 3). In general, highest SOD activity was detected in the accession M-Mln (204.0 units/g) during March (late spring). Lowest activity was observed in the accession M-Fsd (67.4 units/g) during January (winter). SOD activity was also compared among different tested accessions of *M. oleifera* during different months. During October (autumn), substantial SOD activity was detected in accession M-Fsd (182.7 units/g) followed by M-China (169.0 units/g), M-Mln (117.3 units/g) and PKM1 (107.3 units/g). However, SOD activity was detected to be statistically same in accession M-Mln and PKM1 and also in M-China and M-Fsd. During December (winter), SOD activity was observed to be same in all accessions. However, as compared to October (autumn) the SOD activity increased in accession M-Mln (137.1 units/g) during December (winter). During January (winter), SOD activity was highest in accession M-Mln (173.8 units/g) followed by

PKM1 (165.5 units/g), M-China (112.0 units/g) and M-Fsd (67.4 units/g). SOD activity was observed to be same in accession PKM1 and M-Mln. Though, as compared to December (winter) SOD activity was increased in accession M-Mln. During February (early spring), SOD activity was detected to be same in three accession M-Fsd (113.5 units/g), M-Mln (120.9 units/g) and PKM1 (127.3 units/g). However, as compared to January (winter) the level of this activity decreased in accession M-Mln. During March (late spring), Significant SOD activity was observed in all accessions with maximum SOD activity in accession M-Mln (204.0 units/g) as compared to February (early spring). However, an overall increase was seen during March (late spring) followed by October (autumn), January (winter), December (winter) and February (early spring).

Catalase (CAT) Activity

Significant variation was observed among the accessions during all months (Fig. 4). Generally, highest CAT activity was detected in accession M-China (2709.0 units/g) during March (late spring). Lowest CAT activity was observed in the accession PKM1 (310.0 units/g) during February (early spring). CAT activity was also compared among different tested accessions of *M. oleifera* during different months. During October (autumn), comparatively higher CAT activity was observed in genotype M-China (2540.0 units/g) and it was statistically different as detected between accessions M-Fsd (2210.0 units/g) and PKM1 (2330.0 units/g), whereas lowest CAT activity was observed in the case of accession M-Mln (460.0 units/g). During December (winter), maximum CAT activity was observed in the accession M-Fsd (2630.0 units/g) and the level was also statistically higher as compared to other three accessions where CAT activity was detected to be same between the accession M-Mln (340.0 units/g), M-China (500.0 units/g) and PKM1 (880.0 units/g). During January (winter), CAT activity was highest in the accession PKM1 (1321.0 units/g) followed by M-China (930.0 units/g) and M-Mln (710.0 units/g) but least CAT activity was detected in accession M-Fsd (370.0 units/g). CAT activity was detected to be same between accession M-China and PKM1 as well as between M-Fsd and M-Mln. During March (late spring), relatively higher CAT activity was observed in accession M-China (2709.0 units/g) and this level was also statistically higher as compared to accession M-Fsd (720.0 units/g), PKM1 (1640.0 units/g) and M-Mln (1910.0 units/g) whereas level of CAT activity was detected to be same in accessions M-Mln and PKM1.

Esterase Activity

Esterase activity varied among all accessions during the months of October(autumn), December (winter), January (winter), February (early spring) and March (late spring) (Fig. 5). In general, the highest esterase activity was detected in accession M-Fsd (308.7 µM/min/g) during

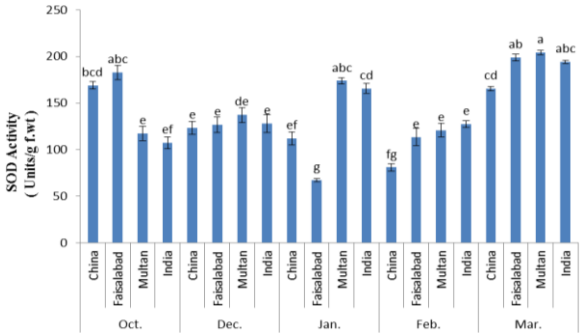

Fig. 3: Comparison of superoxide dismutase of leaves from different accessions of *Moringa oleifera* during different months

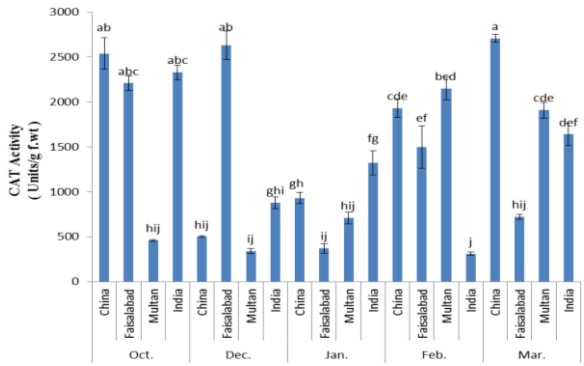

Fig. 4: Comparison of catalase activity of leaves from different accessions of *Moringa oleifera* during different months

Accessions: Faisalabad (M-Fsd), Multan (M-Mln), India (PKM1) and China (M-china)

December (winter) while lowest esterase activity was detected in accession M-Mln (31.9 µM/min/g) during February (early spring). An overall increase in esterase activity was observed during December (winter) followed by October (autumn), March (late spring), January (winter) and February (early spring). Esterase activity was also compared among different tested accessions of *M. oleifera* during different months. During October (autumn), esterase activity was observed to be same among the accessions M-China (235.2 µM/min/g), M-Fsd (267.2 µM/min/g) and PKM1 (213.3 µM/min/g). Least activity was detected in accession M-Mln (138.6 µM/min/g). During December (winter), relatively higher activity was detected in the accession M-Fsd (308.7 µM/min/g) while the level of activity was observed to be statistically same when compared to accession M-China (66.9 µM/min/g) and PKM1 (64.9 µM/min/g). During January (winter), M-China (81.8 µM/min/g), M-Fsd (61.0 µM/min/g), M-Mln (124.5 µM/min/g) and PKM1 (64.4 µM/min/g) as well as during February (early spring), M-China (34.0 µM/min/g), M-Fsd (79.0 µM/min/g), M-Mln (31.9 µM/min/g) and PKM1 (31.9 µM/min/g) esterase activity was detected to be statistically same among the

accessions. During March (late spring), relatively higher esterase activity was detected in accession M-Fsd (215.0 µM/min/g) and this was also statistically higher when compared to accession M-China (187.3 µM/min/g) and statistically same to the accession PKM1 (165.5 µM/min/g) and M-Mln (184.4 µM/min/g) respectively.

Peroxidase (POD) Activity

POD activity varied to a great extent among the four accessions of *M. oleifera* during different months (Fig. 6). Generally, highest POD activity was detected in the accession M-Mln (76733.3 units/g) during March (late spring) while lowest POD activity was observed in same accession (4183.3 during February (early spring). POD activity was also compared among different tested accessions of *M. oleifera* during different months. During October (autumn), maximum POD activity was detected in the accession M-Fsd (24150.0 units/g) as compared to other accessions i.e. M-Mln (8566.7 units/g), M-China (9100 units/g) and PKM1 (5083.3 units/g). The least POD activity was detected in accession PKM1 and this level was statistically lower as compared to accession M-Fsd while same when compared with accessions M-China and M-Mln. During December (winter), POD activity was detected to be same in all four accessions i.e. M-China (23133.3 units/g), M-Fsd (24566.7 units/g), PKM1 (24600 units/g). However, as compared to October (autumn), POD activity increased significantly during December (winter) in accession PKM1 (24600.0 units/g). During January (winter), comparatively higher POD activity was observed in accession PKM1 (27516.7 units/g) and it was significantly higher as detected in accessions M-China (10083.3 units/g) and M-Mln (15316.7 units/g). Though, as compared to December (winter), POD activity increased significantly during January (winter) in accession PKM1. During February (early spring), again relatively higher POD activity was detected in accession PKM1 (45383.3 units/g) while least POD activity was observed in accession M-Mln (4183.3 units/g), which was also found to be statistically same to accession M-China (19583.3 units/g). However, as compared to January (winter), POD activity increased during February (early spring) in accession PKM1. During March (late spring), POD activity was observed to be statistically same in the accession M-Mln (76733.3 units/g), M-Fsd (71500.0 units/g) and PKM1 (76016.7 units/g) but relatively lower POD activity was detected in accession M-China (48300.0 units/g).

Total Oxidants Status (TOS)

There was no significant variation among the accessions during different months except during December (winter) and February (early spring) (Fig. 7). Generally, highest TOS was detected in accession M-China (221.7 µM/g) during December (winter). Lowest TOS activity was detected in the accession PKM1 (69.4 µM/g) during March (late

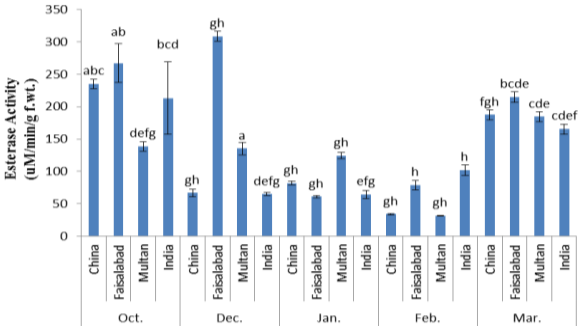

Fig. 5: Comparison of esterase activity of leaves from different accessions of *Moringa oleifera* during different months

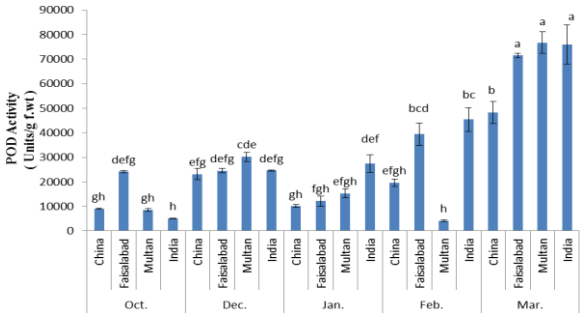

Fig. 6: Comparison of peroxidase activity of leaves from different accessions of *Moringa oleifera* during different months

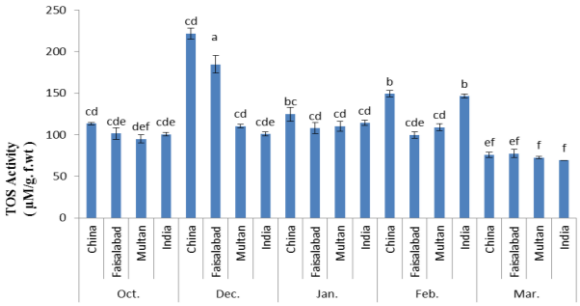

Fig. 7: Comparison of total oxidant status of leaves from different accessions of *Moringa oleifera* during different months

Accessions: Faisalabad (M-Fsd), Multan (M-Mln), India (PKM1) and China (M-china)

spring). TOS activity was also compared among different tested accessions of *M. oleifera* during different months. During October (autumn) and March (late spring), non-significant difference was observed in TOS activity among accessions. During December (winter), TOS activity was observed to be comparatively and statistically higher in genotype M-China (221.7 µM/g) as compared to accession M-Fsd (184.8 µM/g) while same when compared with accessions M-Mln (110.4 µM/g) and PKM1 (100.9 µM/g) respectively. During February (early spring), TOS activity was observed to be statistically same in M-China (149.3

µM/g) and PKM1 (146.5 µM/g) as well as in M-Fsd (100.3 µM/g) and M-Mln (109.0 µM/g), respectively. TOS remained statistically same during January (winter), M-China (124.9 µM/g), M-Fsd (107.9 µM/g), M-Mln (110.4 µM/g), PKM1 (114.1 µM/g) and March (late spring). M-China (76.0 µM/g), M-Fsd (77.5 µM/g), M-Mln (72.6 µM/g) and PKM1 (69.4 µM/g) respectively. An overall highest TOS was observed during December (winter) followed by February (early spring), January (winter), October (autumn) and March (late spring).

Malondialdehyde (MDA) Content

MDA content varied to a great extent among the four accessions of *M. oleifera* during all months (Fig. 8). Generally, maximum MDA content was detected in accession M-China (159.2 µM/g) during October (autumn). Minimum MDA content was noticed in accession M-Mln (26.6 µM/g) during February (early spring). MDA content was also compared among different tested accessions of *M. oleifera* during different months. During October (autumn), comparatively higher MDA content was observed in the accession M-China (159.2 µM/g).This level was also statistically same to accession M-Fsd (136.8 µM/g) and M-Mln while statistically lower in accession PKM1 (80.0 µM/g). During December (winter), MDA content in accession M-Fsd was comparatively higher while statistically same between accessions M-Mln (115.9 µM/g) and M-Fsd (124.1 µM/g) as well as between PKM1 (60.4 µM/g) and M-China (43.1 µM/g). During January (winter), MDA content was detected to be comparatively higher in accession M-Fsd (60.9 µM/g) while statistically same to genotype M-China (32.0 µM/g), M-Mln (47.5 µM/g) and PKM1 (35.9 µM/g). During February (early spring), MDA content was maximum in accession M-China (79.0 µM/g) and this content was statistically same while minimum in accession PKM1 (50.1 µM/g) and M-Fsd (48.8 µM/g) respectively. Lowest MDA content was detected in accession M-Mln (26.6 µM/g). During March (late spring), relatively higher MDA content was observed in accession M-Fsd (95.7 µM/g) and it was also statistically higher as compared to accessions M-China (38.7), M-Mln (35.6 µM/g) and PKM1 (48.3 µM/g), while level of this content was statistically same to accessions M-China, M-Mln and PKM1.

Ascorbate Peroxidase (APX) Activity

APX activity varied extensively among the four accessions of *M. oleifera* during different months (Fig. 9). In general, APX activity was found to be highest in the accession M-China (630.0 µM/g) during March (late spring) whereas lowest APX activity was detected in the same accession (32.0 µM/g) during January (winter). APX activity was also compared among different tested accessions of *M. oleifera* during different months. During October(autumn), maximum APX activity was detected in accession M-China (1680.0 units/g) this level was statistically same to the

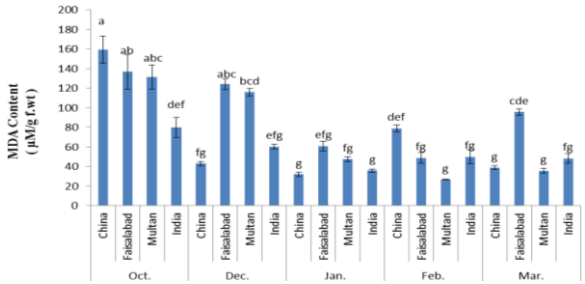

Fig. 8: Comparison of malondialdehyde content of leaves from different accessions of *Moringa oleifera* during different months

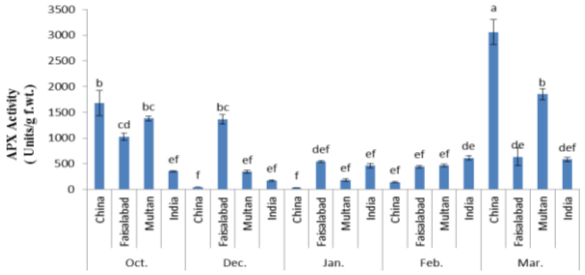

Fig. 9: Comparison of ascorbate peroxidase activity of leaves from different accessions of *Moringa oleifera* during different months

accession M-Mln (1380.0 units/g), while different when compared with accession M-Fsd (1020.0 units/g) and PKM1 (350.0 units/g). During December (winter), APX activity was detected to be highest in the accession M-Fsd (1360.0 units/g) while the least APX activity was observed in the accession M-China (43.1 units/g) and this level was statistically lower as compared to accession M-Fsd, while same when compared with the accession M-Mln (340.0 units/g) and PKM1 (170.0 units/g). During January (winter), APX activity was detected to be same in all four accessions M-China (32.0 units/g), M-Fsd (170.0 units/g), M-Mln (32.0 units/g) and PKM1 (540.0 units/g). During February (early spring), APX activity was again observed to be statistically same among the accessions. During March (late spring), comparatively higher APX activity was observed in accession M-China (630.0 units/g) and it was statistically higher as detected in accession M-Mln (1850.0 units/g) while APX activity was statistically same to accession M-Fsd (630.0 units/g) and PKM1 (580.0 units/g) when compared with accession M-China (630.0 units/g).

Protease Activity

Protease activity significantly altered among four accessions during different months (Fig. 10). Highest protease activity was observed in accession M-Fsd (10053.3 units/g) during February (early spring). Lowest protease activity was detected in the accession PKM1 (2633.3 units/g) during March (late spring). During October (autumn), comparatively higher protease activity was detected in accession M-Fsd (8253.3 units/g), which was

also statistically higher as compared to M-China (5430.0 units/g) and PKM1 (5363.3 units/g) while statistically same to the accession M-Mln (6920.3 units/g). During December (winter), relatively higher protease activity was detected in accession M-Mln (8470.0 units/g), which was statistically same to the accession M-Fsd (7416.7 units/g), while also statistically higher in comparison with accession PKM1 (4640.0 units/g) and M-China (5213.3 units/g). During January (winter), non-significant difference was detected in Protease activity among four accessions i.e., M-China (6746.7 units/g), M-Fsd (6886.7 units/g), M-Mln (7076.7 units/g) and PKM1 (7736.7 units/g). During February (early spring), comparatively higher protease activity was detected in accession M-Fsd (10053.3 units/g), which was also significantly higher than M-Mln (8456.7 units/g), M-China (7196.5 units/g) and PKM1 (5880.0 units/g). During March (late spring), maximum protease activity was observed in accession M-Fsd (5873.3 units/g) and least activity was detected in accession PKM1 (2633.3 units/g) which was also statistically lower as compared to M-Fsd (5873.3 units/g) while same when compared with M-China (3472.3 units/g) and M-Mln (3146.7 units/g).

Protein Content

A significant variation was observed in protein content among the accessions of *M. oleifera* in different months (Fig. 11). The maximum protein content was observed in accession PKM1 (392.2 mg/g) during February (early spring). Lowest protein content was observed in the accession M-Mln (31.6 mg/g) during October (autumn). Protein content was also compared among different tested accessions of *M. oleifera* during different months. In case of October (autumn), Maximum protein content was detected in accession PKM1 (168.9 mg/g) which was also statistically same to the accession M-China (145.1 mg/g). Least protein content was observed in M-Mln (31.6 mg/g) and this level was statistically lower as compared to accession M-Fsd (71.1 mg/g). During December (winter), highest protein content was observed in the accession M-Fsd (188.2 mg/g) which was also statistically higher as compared to other accessions while protein content was observed to be statistically same in three accessions M-China (58.1 mg/g), M-Mln (79.6 mg/g) and PKM1 respectively. During January (winter), maximum protein content was detected in accession M-Fsd (352.6 mg/g) which was also statistically same to the accession M-China (351.7 mg/g) while different from accession M-Mln (41.1 mg/g) and PKM1 (240.9 mg/g). Lowest protein content was observed in accession M-Mln which was observed to be statistically lower as compared to accession M-Fsd (352.6 mg/g). During February (early spring), comparatively higher protein content was detected in accession PKM1 (392.2 mg/g) and this content was also statistically different when compared to M-China (203.8 mg/g), M-Fsd (82.2 mg/g) and M-Mln (61.6 mg/g) while protein content was also observed

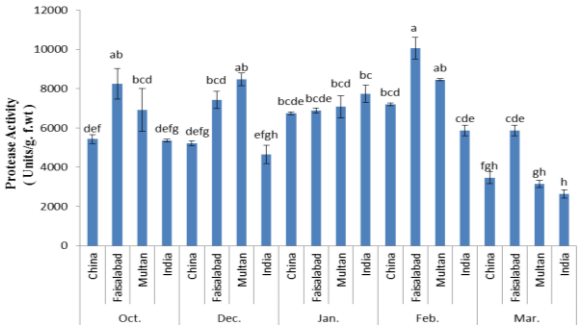

Fig. 10: Comparison of protease activity of leaves from different accessions of *Moringa oleifera* during different months

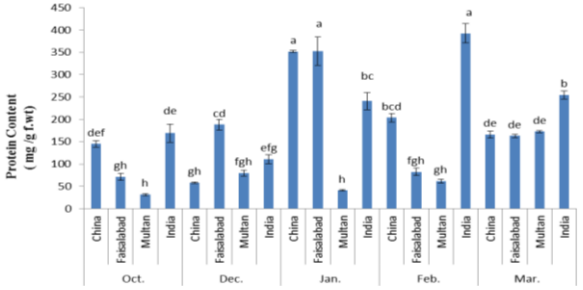

Fig. 11: Comparison of protein content of leaves from different accessions of *Moringa oleifera* during different months

Accessions: Faisalabad (M-Fsd), Multan (M-Mln), India (PKM1) and China (M-china)

to be statistically same between the accessions M-Mln and M-Fsd respectively. During March (late spring), highest protein content was detected in accession PKM1 (254.2 mg/g) and this was also statistically different when compared to accessions M-China (165.6 mg/g), M-Fsd (162.9 mg/g) and M-Mln (172.4 mg/g), respectively while protein content remained statistically same among the accessions M-China, M-Fsd and M-Mln. On the whole, net increase in protein content was noted during February (early spring) followed by January (winter).

Discussion

Moringa oleifera plant is used as a source of food with remarkable properties in humans as well as animals (Ganguly *et al.*, 2010). Therefore, knowledge about biochemical constituents of leaves can help to plan strategies focused on proper application of this resource. Moringa accessions showed variation in antioxidants within themselves and seasons. Comparison of different parameters in individual accessions during different months revealed that accession M-China depicted the maximum Malondialdehdye (MDA) content during October (autumn) among all other months (i.e. December (winter), January (winter), February (early spring) and March (late spring). The MDA is the end product of lipid peroxidation and is

considered to be an indicator of oxidative degradation which brings alteration in characteristics of membrane i.e. membrane bound enzyme activity loss (Sharma *et al.*, 2012). It also represents the extent of damage to the plant cells (Zhang, 2013). The maximum level of MDA during October is logical as trees shed their leaves during autumn.

The Moringa leaves are tremendous source of phenolic compounds which have been stated as a significant group of secondary metabolites in medicinal plant and these phenols contribute directly to antioxidant activity. Hence, it has been vastly utilized as anti-urolithiatic, anti-ulcer, anti-diabetes, antipyretic, anti-inflammatory, anti-hypoglycemia anticonvulsant, malnutrition and hepatoprotective, anti-hypertension, anti-malaria, anti-cancer, and anti-microbial activities (Abdulaziz *et al.*, 2015). Leaf extract of *M. oleifera* can be used for the butter stabilization at refrigeration (Nadeem *et al.*, 2013). In present study, during March (late spring), accession M-China depicted the maximum TPC (31811 µM/g). Literature revealed remarkable variation in total phenolic content when effect of location and seasons on TPC was checked in *M. oleifera* leaves. Mardaan sample were observed to have highest TPC (12.79 g/100 g) then Balakot (11.94 g), Chakwal (10.54 g/100 g), Jamshoro (8.99 g/100 g) and Nawabshah (8.82 g/100 g) respectively. In general TPC was higher in December (winter) (11.17 g/100 g) for all the locations investigated (Iqbal and Bhanger, 2006). This might be because of the reason that growth of Moringa leaves occurs in June while leaves got old during December (winter) to March (late spring). In newly opened leaves phenolic content has been observed to be lowest which increases steadily with the leaves maturity or increase in age (Iqbal and Bhanger, 2006). However, for the samples from Mardaan,TPC was hiher in Mrach (Iqbal and Bhanger, 2006) and same observation was recored in prest study.

Catalase is the major enzymatic hydrogen peroxide scavenger which is involved in the conversion of H_2O_2 into water and oxygen as well as in scavenging hydrogen peroxide produced in photorespiratory oxidation, fatty acids beta oxidation and in mitochondrial electron transport (Quan *et al*., 2008). In present study, it was observed that accession M-China depicted maximum CAT (2709.0 units/g) activity and accession M-Mln showed maximum SOD (204.0 Units/g) and CAT (76733.3 units/g) activities during March (late spring). Since Moringa is a rich source of antioxidants the antioxidant property of its leaves have been found to be a contributing factor for improved memory by alteration of brain monoamines as well as hyperthyroidism regulation in rat models by the increase of superoxide dismutase and catalase activity (Tahiliani and Kar, 2000; Ganguly *et al.*, 2010). *M. oleifera* leaves has been found helpful in improving memory by decreasing the lipid peroxidation activity in rat models and can provide protection against Alzheimer's disease (Ganguly *et al.*, 2010). Previously, lyophilized *M. oleifera* aqueous alcoholic extract was evaluated for studying the cardioprotective effect in myocardial infarction model induced with isoproterenol and the results showed considerable biochemical enzymes modulation such as catalase, glutathione peroxidase, superoxide dismutase (Nandave *et al*., 2009). POD helps to scavenge reactive oxygen species which can cause oxidative injury to the cell (Vicuna, 2005). Based on present findings supported by previous literature, it can be recommended that Moringa leaves should be harvested during March to get maximum levels of enzymatic antioxidants i.e. POD, CAT and SOD and related health benefits.

ABTS is frequently used by the food industry and also agricultural researchers to measure the antioxidant capacities of foods. ABTS assay is used to measure the relative ability of antioxidant to scavenge the ABTS with compared with Trolox standard (Fitriana *et al.*, 2016). During December (winter), accession M-Fsd depicted the highest esterase activity (308.7 µM/min/g) while accession M-Mln depicted the maximum TAC activity (17.5 µM/g) whereas accession M-China depicted the maximum TOS activity (221.7 µM/g). Previously, Antioxidant activities of *M. oleifera* extracts with various solvents were measured and it was found to be highest in methanol extracts when measured by ABTS method (Fitriana *et al.*, 2016).This finding provided scientific evidence for use of *M. oleifera* leaves as one of nutrition food to prevent diseases in Indonesia and the fact that *M. oleifera* leaves can be used as antioxidant source (Fitriana *et al*., 2016). Present finding further supported this aspect related to *M. oleifera* reported previously.

APX is an important constituent of the glutathione ascorbate cycle (Noctor and Foyer, 1998) and is involved in the detoxification of hydrogen peroxide into water by the means of ascorbate acting as a specific donor of electron. APX also helps in the maintenance of non cyanogenic level of hydrogen peroxide in plant cell (Dabrowska *et al.*, 2007) as hydrogen peroxide is a strong oxidizing agent and is detrimental for plant cell if present in high amount. Reason behind higher APX activity during the month of March (late spring) in accession M-China (3060.0 units/g. wt.) and M-Mln (1850.0 units/g. wt.) and during October (autumn) in accessions M-China (1680.0 units/g. wt.), M-Fsd (1020.0 units/g. wt.) and M-Mln (1380.0 units/g. wt.) might be the temperature difference which is relatively high during these months as compared to December (winter), January (winter) and February (early spring) which makes these accessions tolerant to abiotic stress whereas all other accessions with low APX activity are sensitive to abiotic stress. Highest protease activity (10053.3 units/g) was depicted by accession M-Fsd during February (early spring). Proteases are involved in the proteins modification after translation, in enzymes maturation and its activity as well (Schaller, 2004). Overall increase has been observed in protease activity, showing the sensitivity of these accessions to abiotic stresses and more proteolytic enzyme production (Hameed *et al.*, 2012).

Conclusion

Present work has showed that though *M. oleifera* is a potent antioxidant source but seasons have significant impact on the antioxidant potential of different accessions of *M. oleifera* leaves, as March (late spring) showed strongest antioxidant activity followed by December (winter), February (early spring), October(autumn), and January (winter). It is recommended that in agro climatic conditions of Pakistan, *Moringa* leaves should be harvested during March to get maximum levels of enzymatic antioxidants i.e., POD, CAT and SOD and related health benefits. In general, accessions M-China and M-Mln have relatively higher levels of antioxidants as compared to other tested accessions and should be given preference to get maximum benefits of this plant.

References

Abdulaziz, R.A., Z.D. Dhiya and S.J. Muhammad, 2015. DPPH antioxidant activity, total phenolic and total flavonoid content of different part of Drumstic tree (*Moringa oleifera* Lam.). *J. Chem. Pharm. Res.,* 7: 1423–1428

Abiodun, O., J. Adegbite and A. Omolola, 2012. Chemical and physicochemical properties of Moringa flours and oil. *Global J. Sci. Front. Res.*, 12: 224–227

Ainsworth, E.A. and K.M. Gillespie, 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin Ciocalteu reagent. *Nat Protoc*., 2: 875–877

Anwar, F., M. Ashraf and B.M. Iqbal, 2005. Interprovenance variation in the composition of *Moringa oleifera* oilseeds from Pakistan. *J. Amer. Soc.*, 82: 45–51

Anwar, F., S. Latif, M. Ashraf and A.H. Gilani, 2007. *Moringa oleifera*: a food plant with multiple medicinal uses. *Phytother Res.*, 21: 17–25

Ashfaq, M., S.M. Basra and U. Ashfaq, 2012. Moringa: A miracle plant for agro-forestry. *J. Agric. Fores. Soc. Sci.*, 8: 115–122

Basra, S.M.A., W. Nouman, H.U. Rehman, M. Usman and Z.H. Nazli, 2015. Biomass production and nutritional composition of *Moringa oleifera* under different cutting frequencies and planting spacings. *Int. J. Agric. Biol*., 17: 1055–1060

Beers, R.F. and I.W. Sizer, 1952. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *J. Biol. Chem.*, 195: 133–140

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem.*, 72: 248–254

Bukar, A., A. Uba and T. Oyeyi, 2010. Antimicrobial profile of *Moringa oleifera* Lam. extracts against some food borne microorganisms. *J. Pure Appl. Sci.*, 3: 43–48

Caceres, A., O. Cabrera, O. Morales, P. Mollinedo and P. Mendia, 1991. Pharmacological properties of *Moringa oleifera*. 1: Preliminary screening for antimicrobial activity. *J. Ethnopharmacol*., 33: 213–216

Chance, B. and A. Maehly. 1955. Assay of catalases and peroxidases. *Methods Enzymol.*, 2: 764–775

Charoensin, S., 2014. Antioxidant and anticancer activities of *Moringa oleifera* leaves. *J. Med. Plants Res.*, 8: 318–325

Chen, G.X. and K. Asada, 1989. Ascorbate peroxidase in tea leaves: Occurrence of two isozymes and the differences in their enzymatic and molecular properties. *Plant Cell Physiol.*, 30: 987–998

Daba, M., 2016. Miracle Tree: A Review on Multi-purposes of *Moringa oleifera* and Its Implication for Climate Change Mitigation. *J. Earth Sci. Clim.*, 7: 1–5

Dehshahri, S., M. Wink, S. Afsharypuor, G. Asghari and A. Mohagheghzadeh, 2012. Antioxidant activity of methanolic leaf extract of *Moringa peregrina* (Forssk.) Fiori. *Res. Pharma. Sci.*, 7: 111

Dillard, C.J. and J.B. German, 2000. Phytochemicals: nutraceuticals and human health. *J. Sci. Food Agric.* 80: 1744–1756

Dixit, V., V. Pandey and R. Shyam, 2001. Differential antioxidative responses to cadmium in roots and leaves of pea (*Pisum sativum* L. cv. Azad). *J. Exp. Bot.*, 52: 1101–1109

Drapeau, G., 1974. *Protease from Staphylococcus Aureus*. Academic Press, New York, USA

Erel, O., 2005. A new automated colorimetric method for measuring total oxidant status. *Clin. Biochem.*, 38: 1103–1111

Fahey, J.W., 2005. *Moringa oleifera*: A review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Part 1. *Phytochemistry*, 47: 123–157

Fitriana, W.D., T. Ersam, K. Shimizu and S. Fatmawati, 2016. Antioxidant Activity of Moringa oleifera Extracts. *Indones. J. Chem.*, 16: 297–301

Ganatra, T., U.H. Ghoshi, P.N. Bhalodia and T.R. Desai, 2012. A panoramic view on pharmacognostic, pharmacological, nutritional, therapeutic and prophylactic values of *Moringa oleifera* Lam. *Int. Res. J. Pharm.*, 3: 6

Ganguly, R., R. Hazra, K. Ray and D. Guha, 2010. Effect of *Moringa oleifera* in experimental model of Alzheimer's disease: Role of antioxidants. *Ann. Neurosci.*, 12: 33–36

Giannopolitis, C.N. and S.K. Ries, 1977. Superoxide dismutases I. Occurrence in higher plants. *Plant Physiol.*, 59: 309–314

Harma, M., M. Harma and O. Erel. 2005. Oxidative stress in women with preeclampsia. *Amer. J. Obstet. Gynecol*., 192: 656–657

Heath, R.L. and L. Packer, 1968. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.*, 125: 189–198

Iqbal, S. and M. Bhanger, 2006. Effect of season and production location on antioxidant activity of *Moringa oleifera* leaves grown in Pakistan. *J. Food Comp. Anal*., 19: 544–551

Koul, B. and N. Chase, 2015. *Moringa oleifera* Lam.: Panacea to several maladies. *J. Chem. Pharm. Res.*, 7: 687–707

Kumar, A., M. Chalamaiah, R. Kumar and K. Babu, 2009. Preliminary studies on biotransformation of drumstick (*Moringa oleifera*) and watermelon (*Citrullus lanatus*) seed oils using baker's yeast. *Asian J. Biol. Sci.*, 2: 118–123

Luqman, S., S. Srivastava, R. Kumar, A.K. Maurya and D. Chanda, 2011. Experimental assessment of *Moringa oleifera* leaf and fruit for its antistress, antioxidant and scavenging potential using *in vitro* and *in vivo* assays. *J. Evid Based Complementary Altern. Med.*, 2012: 1–12

Makonnen, E., A. Hunde and G. Damecha, 1997. Hypoglycaemic effect of Moringa stenopetala aqueous extract in rabbits. *Phytother. Res.*, 11: 147–148

Meena, A., A. Sachan, R. Kaur, B. Pal and B. Singh, 2010. *Moringa oleifera*: A review. *J. Pharm. Res.*, 3: 840–842

Morton, J.F., 1991. The horseradish tree, *Moringa pterygosperma* (Moringaceae). A boon to arid lands. *Econ. Bot.*, 45: 318–333

Nadeem, M., M. Abdullah, I. Hussain, S. Inayat, A. Javid and Y. Zahoor, 2013. Antioxidant potential of *Moringa oleifera* leaf extract for the stabilisation of butter at refrigeration temperature. *J. Food Sci.*, 31: 332–339

Nenadis, N., O. Lazaridou and M.Z. Tsimidou, 2007. Use of reference compounds in antioxidant activity assessment. *J. Agric. Food Chem.*, 55: 5452–5460

Owolabi, J., O. Ghazal, M. Dare, J. Olanrewaju, A. Caxton-Martins and F. William, 2013. Prophylactic and regenerative effects of alcoholic extract of *Moringa oleifera* on rat lung tissue following lead-induced damage. *Eur. J. Anat.*, 17: 115–122

Pal, S.K., P.K. Mukherjee and B. Saha, 1995. Studies on the antiulcer activity of *Moringa oleifera* leaf extract on gastric ulcer models in rats. *Phytother. Res.*, 9: 463–465

Paliwal, R. and V. Sharma, 2011. Pracheta. A review on horse radish tree (*Moringa oleifera*): A multipurpose tree with high economic and commercial importance. *Asian J. Biotech.*, 3: 317–328

Quan, L.J., B. Zhang, W.W. Shi and H.Y. Li, 2008. Hydrogen peroxide in plants: a versatile molecule of the reactive oxygen species network. *J. Integ. Plant Biol.*, 50: 2–18

Rajinder, P.P. Dhindsa and T.A. Thorpe, 1981. Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. *J. Exp. Bot.*, 32: 93–101

Sankhyan, N., A. Sharma, C.A. Seth, A. Chauhan and S. Kulshrestha, 2013. Determination and comparison of vitamin C content from *Moringa oleifera* by different methods. *Int. J. Agric. Sci. Res.*, 3: 67–70

Sengupta, A. and M. Gupta, 1970. Studies on the seed fat composition of Moringaceae family. *Fette. Seifen. Anstr*., 72: 6–10

Tahiliani, P. and A. Kar, 2000. Role of *Moringa oleifera* leaf extract in the regulation of thyroid hormone status in adult male and female rats. *Pharmacol. Res.*, 41: 319–323

Upadhyay, P., M.K. Yadav, S. Mishra and P. Sharma, 2015. *Moringa oleifera*: A review of the medical evidence for its nutritional and pharmacological properties. *Int. J. Res. Pharm. Sci.*, 5: 12–16

Van Asperen, K., 1962. A study of housefly esterases by means of a sensitive colorimetric method. *J. Insect Physiol.*, 8: 401–416

Verma, V.K., N. Singh, P. Saxena and R. Singh, 2012. Anti-ulcer and antioxidant activity of *Moringa oleifera* (Lam) leaves against aspirin and ethanol induced gastric ulcer in rats. *Int. Res. J. Pharm.*, 2: 46–57

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