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Nutritional Scrutiny, Chemical Profiling and Antioxidant Potentials of Crude Extracts of *Moringa oleifera* Lam. (Moringaceae) from Kasur, Pakistan

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ABSTRACT

Moringa oleifera L. is a miraculous plant due to the presence of essential nutrients, phyto-constituents and natural antioxidants. This study evaluated the aqueous and methanol extracts of *M. oleifera* leaves for various nutritional parameters, phyto-constituents and antioxidant activities. Results indicated varying contents of moisture (78.0±2.2, 7.3±0.7%), ash (0.8±0.1, 1.50±0.8%), fat (0.9 ±0.2, 1.8±0.9%), crude fiber (2.1±0.3, 20.5±1.7%), crude protein (7.1±0.8, 22.7±1.9%) carbohydrates (11.1±1.3, 46.2±2.1%) and energy kcal/100g (81.0±2.5, 292±4.2). Proteins, carbohydrates, hydroxyl-anthraquinone, tannins, alkaloids, saponins, flavonoids, terpenoids and saponins were present in *Moringa* leaves powder except phytosterol and fixed oil. In the methanol extract of *Moringa* fresh leaves, TPC recorded was 76.7±2.5 mg GAE/g and TFC was 24.6±0.40 mg QE/g while in the dried leaves powder, TPC and TFC were 86.2±1.8 mg GAE/g and 29.8±0.4 mg QE/g which were higher than the aqueous extracts. Antioxidant activity of *Moringa* dried leaves methanol extract with DPPH displayed maximum percentage inhibition (92.5±3.2%) than aqueous extract (65.2±2.5%) and BHT (57.6±2.1%) at 100µg/ml. Same tendency was observed in the reducing power assay for *Moringa* dried leaves powder methanol extract Conclusively, *M. oleifera* possess a wealth of nutrients and bioactive compounds with potential antioxidant activity for extraordinary applications in the food and pharmaceutical industries.

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INTRODUCTION

Since the ancient time, plants are regarded as a significant source of medicine. According to the World Health Organization, up to 80% population of the world is still dependent on herbal preparations as medicine to treat different diseases[1]. The *Moringa oleifera* Lam. is a famine resistant plant from the Moringaceae family[2]. It is a plant of tropical forests whose all parts including the gum, seed, fruit, flowers, leaves, bark and roots are rich in proteins, vitamins and minerals like calcium, phosphorous, potassium, folic β-carotene, iron and acid. The fresh leaves or dried leaves powders of *Moringa* are employed for the development of food products to have improved nutritional quality and therapeutic effects[3]. In the ancient times, leaves of this plant were given to animals as feed[4] and were used in human diet for better health. As its popularity rises, its various parts like roots, pods and seeds were found to be nutritious and medicinally significant. That's why; this plant is taken as a food ingredient, nutraceutical and medicine due to the presence of essential phytochemicals. It has been said that the phytochemicals in different parts of a particular plant makes it very significant and versatile medicinally[5-7]. Numerous phytochemicals have been extracted and reported from *Moringa,* including phenolics, flavonoids, tannins, alkaloids, saponins and glucosides etc. Polyphenolic compounds like flavonoids and phenolic acids are abundantly present in the dried leaves of *Moringa*[9,10].

Previously, phytochemicals like carbonic acid, 2-Isopropoxyethyl propionate (16.87%), 1,3-dioxolan-2-one, 4,5-dimethyl- (6.16%), 4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- (8.98%), 1,3-dihydroxyacetone dimer (3.85%), 2-hydroxy-2-methyl- (3.14%), butyl 2-pentyl ester (20.64%), alpha-d-glucose (3.44%), azetidin-2-one 3,3-dimethyl-4-(1-aminoethyl)- (4.67%), tetra acetyl-d-xylonic nitrile (5.03%) and butanedioic acid, were documented in *M. oleifera* leaves aqueous extracts. Compounds like 2-ethyl-2-(hydroxymethyl)- (21.19%), 1,3-propanediol, 2-methyl-, octyl ester (15.02%), propionic acid, n-ethyl-n-nitroso- (5.21%), ethanamine, 9,12,15-octadecatrienoic acid,(Z,Z,Z)- (5.00%), 4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- (4.18%), benzeneacetonitrile, monomethyl malonate (2.56%), n-hexadecanoic acid (2.57%), 3-oeoxy-d-mannoic lactone (3.29%) were also recognized in the methanol extracts of *M. oleifera* leaves[11].

The phytochemicals from *Moringa* leaves and seeds have been reported with therapeutic potencies[12,13] against cardiovascular diseases[14], hypolipidemic disorders[15],

with biological activities including antispasmodic and diuretic[16], antiobesity[17], antiulcer[18], antihypertensive[19,20], antitumor and apoptotic[21,22], hepatoprotective[23,24], antidiabetic[25-27], antimicrobial[28], wound healing[29], analgesic[30], antipyretic[31], antiasthmatic[32], anti-inflammatory[33,34], antiurolithiatic[35] and antioxidant[36,37] activities. *Moringa* plant have also protective effects against immune disorders[38] and neurodegenerative diseases including Parkinson's[39] and Alzheimer's[40].

As there is emergent interest in the assessment of nutritional and therapeutic efficacies of natural compounds from plants origin with their utilization in the development of products and drugs in the food and pharmaceutical industries, this study further extents the nutritional parameters, phytochemical profiles and antioxidant potentials of aqueous and methanol extracts of *M. oleifera* fresh leaves and dried leaves powder from the Kasur district of Pakistan.

METHODOLOGY

Moringa sample preparation

Fresh leaves of *M. oleifera* were acquired from the Kasur district of Pakistan and the collected specimens were identified by experts from the Government College University, Lahore, Pakistan. Primarily, the leaves were cleaned up with distilled water and placed in aluminum trays at 27°C for 5 days to shade dry. The dried leaf specimen was then grinded (Grinding mill, Germany) and the obtained powder was sieved with a 2 mm pore size siever and stored at -4°C for further experimentation[41].

Nutritonal evaluation

For moisture analysis, *Moringa* fresh leaves and dried leaves powder were placed in oven at 105 to 110°C to a constant weight. Nutritional parameters like, crude lipid, crude protein, crude fiber and total ash content were estimated by adapting AOAC standard methods[42]. Estimation of total carbohydrates was done using the method described in literature[43,44]. The values of energy in Kcal/100 g were estimated by multiplying obtained values of carbohydrates, lipids and proteins by factors of 4 and 9 and the sum obtained was presented in kilocalories[45].

Solvent extraction and phytochemical analysis

A 1% (w/v) stock concentration of extract was obtained from fresh and dried leaves powder of *Moringa* using methanol and water as extraction solvents. Following standard procedures given by Harborne[46] and Kokate[47], the *Moringa* extracts were tested with positive and negative controls for qualitative testing of phytochemicals like amino acids, alkaloids, triterpenoids, tannins, phytosterols, anthroquinone, flavonoids, cardiac glycosides, carbohydrates, glycosides, saponins, fixed oils/fats and proteins.

Estimation of TPC and TFC

The total phenolic content (TPC) of *Moringa* fresh leaves and dried leaves powder were determined quantitatively by folin-ciocalteau reagent method at 760 nm[48] with some modifications[49] and the obtained results were presented in mg GAE/100g. For the total flavonoid content (TFC) estimation in *M. oleifera* fresh leaves and dried leaves powder, the aluminium chloride colorimetric method was used[50] with minor modifications[51]. Quercetin was used as a standard compound for flavonoids quantification and the obtained values were presented as mg QE/100g.

Antioxidant potentials of *Moringa*

The free radical scavenging potency of *M. oleifera* fresh leaves and dried leaves powder was assessed using DPPH method[52] with some modifications[53,54] where the antioxidants minimize the free radicals absorbing light at 517 nm. The ability of aqueous and methanol extracts of *Moringa* fresh leaves and dried leaves powder to reduce iron (III) to iron (II) (total reducing power) was evaluated following Oyaizu[55] with slight modification[53,54] and compared to a strong reducing agent BHT. The absorbance (700 nm) of the samples was plotted against each concentration taken.

Statistical analysis

Data was analyzed statistically and standard deviation (SD±) was estimated in the Microsoft excel program. Differences at $p < 0.05$ were considered significant[56].

RESULTS and DISCUSSION

Nutritional evaluation of *M. oleifera*

Nutritional analysis of *M. oleifera* fresh leaves and dried leaves powder performed using the proximate analysis is critical in determining the nutritional quality of *Moringa*. The results of nutritional parameters assessed in *M. oleifera* leaves are given in Table 1 where the fresh leaves and dried leaves powder exhibited varying contents of moisture (78.0 ± 2.2, 7.3 ± 0.7%), ash (0.9 ± 0.1, 6.50 ± 0.8%), fat (0.8 ±0.2, 1.8 ± 0.9%), crude fiber (2.1 ± 0.3, 20.5 ± 1.7%), crude protein (7.1 ± 0.8, 22.7 ± 1.9%), carbohydrates (11.1 ± 1.3, 46.2± 2.1%). The total energy estimated in *Moringa* fresh leaves and dried leaves powder were 81.0 ± 2.5, 272 ± 3.2 Kcal/100g.

These results regarding the moisture and ash contents recorded in *Moringa* dried leaf powder (7.3%, 6.5%) are in line with the data reported in previous studies on *Moringa* where mean contents of moisture recorded were between

5 -10% and ash between 6-11%[57,58]. In other investigations, elevated levels of moisture and slightly low ash contents were reported in *Moringa* on the basis of dryness of the original sample[59,60]. The ash of *Moringa* may possess inorganic minerals that are necessary for growth and development. It has been found that *Moringa* possess more Ca and iron as compared to spinach, that's why; the leaves powder of *Moringa* could be utilized as a substitute for iron medications for the treatment for anemia. It has been also found that *Moringa* leaves have around 25.5–31.03 mg of zinc/kg[61].

The mean protein contents of fresh leaves and dried leaves powder of *Moringa* were 7.1±0.8 and 22.7 ±1.9% respectively. These results regarding the protein contents are in accordance with the data reported by Valdez-Solana *et al.*[62] and Fejér *et al.*[63]. However, Moyo *et al.*[64], Castillo-López *et al.*[65] and Fokwen *et al.*[66] reported slightly lower (27.2%) protein contents in *Moringa*.

Other nutritional parameters assessed in fresh leaves and dried leaves powder of *Moringa* include fat (0.9±0.2, 1.8±0.9%), crude fiber (2.1±0.3, 20.5±1.7%), crude protein (7.1±0.8, 22.7±1.9%) and carbohydrates (11.1±1.3, 46.2±2.1%). These outcomes are consistent with the results of earlier inquiries of Yameogo *et al.*[67], Witt[68] and Isitua *et al.*[69]. The nutrient variation in *Moringa* could be due to the seasonal fluctuations such as climate, location, geography and some other environmental factors[70]. The physicochemical analysis confirmed that *M. oleifera* is a miraculous plant and its leaves are a high quality food source making itself a candidate plant to be utilized directly in human diet or in the improvement of balanced diets in animal nutrition[71]. In spite of this difference, proximate analysis displayed that the leaves of *M. oleifera* remained good sources of fats, fiber, carbohydrate and proteins which are the primary sources of energy and are used in food and medicinal products like bread, biscuits, drinks, cookies, soups and medicinally coated capsules.

Table 1. Results of nutritional parameters of *M. oleifera* fresh leaves and dried leaves powder

Parameters	Percentage values	
	***Moringa* leaves (Fresh)**	***Moringa* dried leaves (Powder)**
Moisture	78.0±2.2	7.3±0.7
Ash	0.9±0.1	6.5±0.8
Fat	0.8±0.1	1.8±0.9
Crude fiber	2.1±0.3	20.5±1.7
Crude protein	7.1±0.8	22.7±1.9
Carbohydrates	11.1±1.3	41.2±2.1
Energy (Kcal/100g)	81±2.5	272±3.2

Values are the mean (± SD) of three readings presented as (%) g/100 g

Phytochemical analysis of *M. oleifera*

Basic phytochemical analysis for confirming the presence of major phytocompounds is critical because many drugs' active principles are secondary metabolites present in plants. Findings showed that the aqueous and methanol extracts of *Moringa* leaves possess many phytochemical groups including tannins, alkaloids, saponins, flavonoids, protein, terpenoids, hydroxyl anthraquinone and carbohydrates, but no phytosterol or fixed oil was found in both the extracts (Table 2). These outcomes are in accordance with the results of Vergara-Jimenez *et al.*[72], Ayoade *et al.*[73], and Sudha *et al.*[74]. The utilization of methanol and water as extraction solvents indicated the presence of assorted active principles with solvents selective solubility with different polarities used in succession, inferring the solvents significance as a promising factor[75]. Furthermore, the evidence points to the significance of a particular test as a key factor in endorsing phytochemicals presence. Since *M. oleifera* leaves are rich in amino acids and carbohydrates, it is proposed that these nutrients are very beneficial and may be utilized as a growth promoters and nutritional supplements[76,77]. Phytochemical data of *M. oleifera* may be used to produce lead compounds in the quest for innovative herbal medications[78,79].

Table 2. Phytochemical profile of aqueous and methanol extracts of *M. oleifera* fresh leaves

Phytochemicals	Tests/ Experiments	Blank	Control	Aqueous extract	Methanol extract
Tannin	Ferric chloride	-	+++	+	++
Alkaloids	Dragendorff's test	-	++	+	++
Saponins	Foam test	-	++	-	+
Triterpenoids	Salkowski	-	+++	++	+++
Phytosterols	Liebermann-Burchard	-	+++	-	-
Cardiac glycoside	Keller killani	-	+++	-	+
Flavonoids	Lead acetate	-	+++	++	+++
Hydroxyanthraquinone	Potassium hydroxide	-	+++	++	+
Amino acid	Millon's test	-	++	+	++
Fixed oils and fats	Copper sulphate	-	++	-	-
Carbohydrates	Molisch's test	-	+++	++	+++
Proteins	Biuret test	-	++	+	++

++++ (Very high), +++ (High), ++ (Moderate), + (Low), - (Nil), Positive controls and blank (water)

Total phenolic content (TPC) in *M. oleifera*

Phenolic compounds are much prevalent in plants with potential antioxidant activities as they generate hydrogen ions that form stable intermediate radicals[80]. This study assessed the TPC with folin-ciocalteu method where the *Moringa* fresh leaves aqueous extract displayed lower (60.2±1.3 mg GAE/g) and the methanol extract showed higher TPC content (76.5±1.7 mg GAE/g). While, the aqueous extract of dried leaves powder contains 68.3±1.5 mg GAE/g and methanol extract has TPC of 86.2±1.8 mg GAE/g (Figure 1, Table 3). The TPC reported here are faintly lower as compared to the TPC content (9535.3±57.74 mg/100g) reported by Ilyas *et al.*[81] whereas; slightly higher values of TPC were described in *Moringa* leaves by Abdulkadir *et al.*[82]. The variations in TPC might be associated with the difference in polyphenolics extraction methods or solvents polarity and also the plants geographical distribution.

Previous data shows that the *Moringa* leaves are rich in valued compounds like vitamin, protein, calcium, iron and antioxidants including ascorbic acid, carotenoids, phenols and flavonoids[83]. Due to the polyphenols and other antioxidants, many researchers have claimed that the methanol extract of *M. oleifera* leaves exhibit a strong antioxidant action[84]. Many studies have also proposed that the *Moringa* leaves possess anti-diabetic, anti-inflammatory, anti-epileptic, anti-hypertensive, and antitumor activities and these are associated with the phenolic compounds present in the plant. Further, the phenolic compounds have strong antioxidant activities against tissue impairments instigated by the free radicals[85,86].

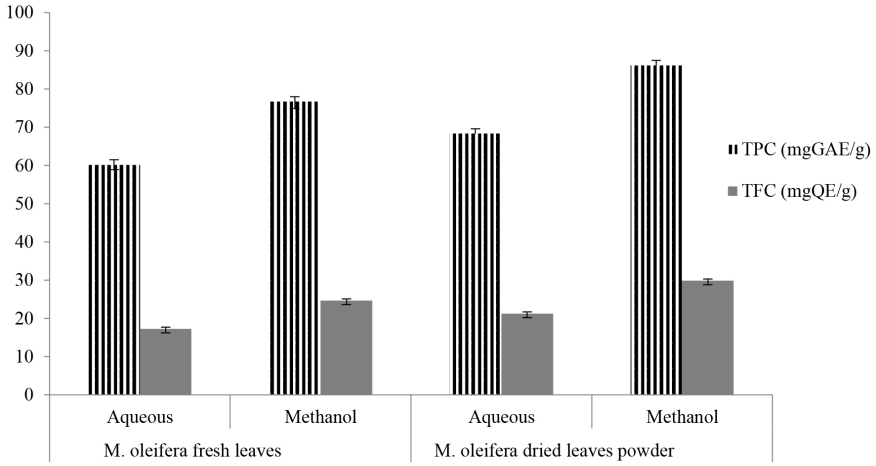

Figure 1. Total phenolic and flavonoid contents in methanol and aqueous extracts of *M. oleifera* fresh leaves and dried leaves powder

Table 3. Total phenolic and flavonoid contents in *M. oleifera* fresh leaves and dried leaves powder

Extracts	*M. oleifera* fresh leaves		*M. oleifera* dried leaves powder	
	TPC (mgGAE/g)	TFC (mgQE/g)	TPC (mgGAE/g)	TFC (mgQE/g)
Aqueous (H_2O)	60.2±1.3	17.2±0.2	68.3±1.5	21.2±0.3
Methanol (MeOH)	76.7±1.7	24.6±0.5	86.2±1.8	29.8±0.4

Data are represented as mean (± SD) of three readings

Total flavonoid content (TFC) in *M. oleifera*

It was found that the methanol extract of *Moringa* dried leaves powder has greater concentration of flavonoids (29.6±0.4 mg QE/g) than its aqueous extract (21.2±0.3 mg QE/g) (Figure 1, Table 3). Flavonoids were confirmed in both the aqueous and methanol extracts of *Moringa* fresh leaves and dried leaves powder. According to Lin *et al.*[87], flavonoids are polyphenolic compounds mostly found in dried leaves of *Moringa*. They are secondary metabolites which are most prevalent phytochemical group. According to Masood *et al.*[88], plant's antioxidant capacity is correlated with its level of TPC and TFC. Flavonoids have positive effects on the human body and provides protection against various diseases[89]. The leaves of *M. oleifera* contain variety of flavonoids; however the most prevalent flavonoids with significant pharmacological action are quercetin, apigenin, kaempferol and isorhamnetin[90]. Flavonoids also possess potential anti-microbial, anti-inflammatory, antioxidant, anti-allergic potentials and other significant biological activities[91,92].

Antioxidant activity of *M. oleifera* extracts

The free radical scavenging potentials of plant extracts improves with the increase in extract concentration. This pattern of radical scavenging was observed in the present inquiry where the percentage inhibitions recorded in methanol extract of *Moringa* dried leaves powder were 35.5, 50.2, 65.9, 78.1, and 92.5% at 20, 40, 60, 80, and 100 µg/ml concentrations , while the percentage inhibitions of the aqueous extract at same concentrations were 25.4, 34.1, 45.6, 55.3, and 65.2 respectively. Similarly, the percentage inhibitions of the standard synthetic antioxidant BHT at same concentrations recorded were 21.5, 30.2, 40.7, 48.3, and 57.6% (Figure 2). The aqueous and methanol extracts of *Moringa* dried leaves powder showed the similar patterns of DPPH scavenging activities which are higher than BHT.

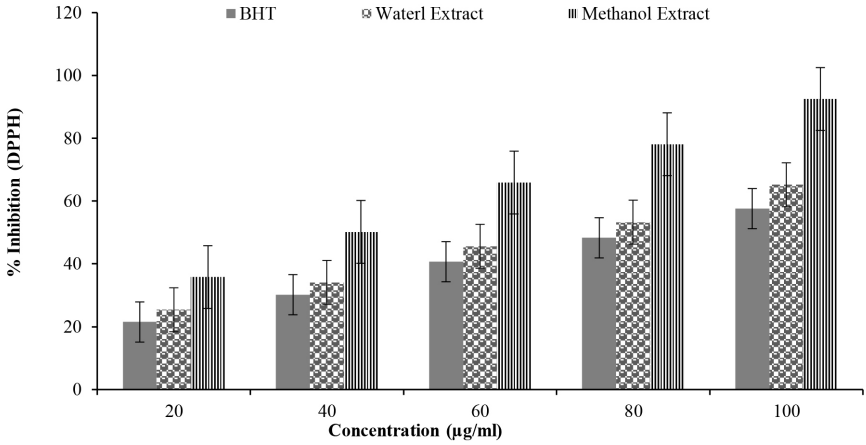

Figure 2. Percentage inhibition (DPPH) of aqueous and methanol extracts of *M. oleifera* dried leaves powder and BHT

The phytochemicals bear antioxidant properties due to the ability to prevent production free radicals, or scavenging free radicals in the body or chelating/reducing the content of transition metal[93,94]. An essential antioxidant method is considered to be the inhibition of the chain start phase by scavenging different reactive species like the free radicals[53,54,95,96].

ROS (reactive oxygen species) are continuously generated in animals due to some environmental factors encountered in daily life[97]. In such conditions, antioxidants are generated by the body cells to maintain the body's equilibrium with free radicals. Oxidative stress describes any imbalance brought on by a variety of diseases in the regular physiological system of the body. According to Karim *et al.*[98] at the harsh level, the oxidative stage transforms cellular damage into different chronic diseases. It has been found that the antioxidants have a good effect on these types of chronic diseases by preventing the initiation of any damage[99-101].

As per the findings of this study, the methanol extract of *Moringa* dried leaves powder displayed higher percentage of DPPH inhibition than the aqueous extract and BHT which are in accordance with previous investigations of Kumar *et al.*[102], Almaghrabi *et al.*[103] and Landazuri *et al.*[104].

The increased DPPH scavenging activity of methanol extracts of *Moringa* reported here might be associated with the presence of total phenolic and flavonoid contents. By providing hydrogen atoms to DPPH, these hydroxyl phenolic compounds can remove it from the environment. In order to evaluate the

antioxidant potentials of herbal extracts, the DPPH scavenging method is now frequently used[105-107] which is highly accurate, sensitive, and quick, depends on the transformation of unstable purple DPPH molecules into yellowish DPPH molecules in the presence of antioxidants[108,109].

The reducing power of *Moringa* dried leaves powder extract was assessed and compared with standard reference BHT on ferric to ferrous reduction in the presence of Fe (II) - stabilising ligand (Figure 3). The reducing power might be attributed to the hydrogen donating capacity, which is commonly related with the presence of reductants[110]. The extracts can convert $[Fe(CN)_6]_3$- to $[Fe(CN)_6]_4$-, which then interacts with Fe^{3+} to form $Fe_4[Fe(CN)_6]_3$ which is a Prussian blue coloured complex[111]. Our findings revealed that these extracts have a degree of hydrogen donation ability which varies with concentration.

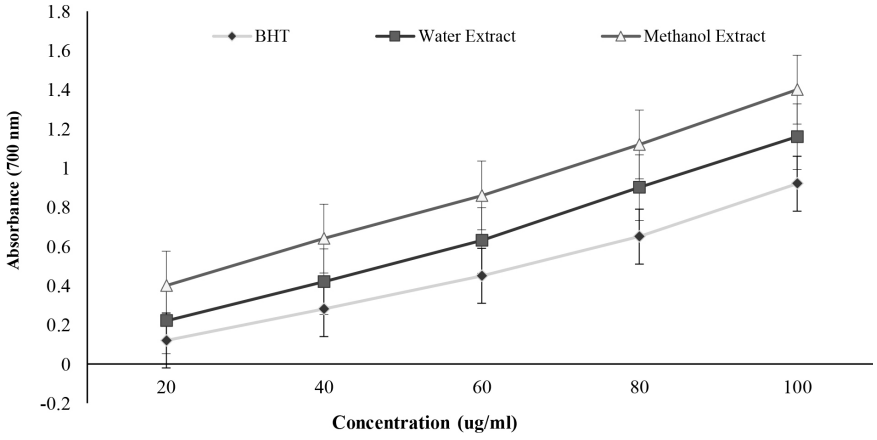

Figure 3. Reducing power of aqueous and methanol extracts of *M. oleifera* dried leaves powder and BHT

The reducing power increased with concentration and these capabilities were superior to those of standard synthetic antioxidant BHT. *Moringa* dried leaves powder extracted with methanol was found to be the most powerful reducing agent, followed by the aqueous extract and BHT. These findings form a clear relationship between the reduction efficacy and antioxidant capability of *Moringa* extracts with high phenolic content as the phenols with a greater number of hydrolysable groups (OH groups) connected to the ring are potent reducing agents (proton donors), resulting in the termination of free radical chain reactions[112].

Considering its myriad benefits, *Moringa* really does seem to be a Marvel plant. As such, this plant must be adapted as a high-quality, inexpensive memento from nature. In addition to its remarkable health benefits, this study found that the crude methanol and aqueous extracts of *M. oleifera* leaves possess essential nutrients, phytochemicals and natural antioxidants that could be significant for industrial and medicinal purposes. To have improved nutrition, the extracts of *Moringa* dried leaves powder should be used for better effects. To fully investigate and utilize the wonders of the *Moringa* tree, more robust research and product development strategies are required. The moment has come to investigate its route for food usage, standardize and commercialize technology for producing value-added and highly nutritious products.

CONFLICT OF INTEREST STATEMENT

Nothing to declare.

AUTHOR CONTRIBUTIONS

Design: Muhammad Khalid SAEED

Acquisition of data: Muhammad Khalid SAEED

Analysis of data: Muhammad Khalid SAEED

Drafting of the manuscript: Muhammad Khalid SAEED and Adil HUSSAIN

Critical revision of the manuscript: Muhammad Khalid SAEED and Adil HUSSAIN

Statistically analysis: Muhammad Khalid SAEED

Technical and Financial Support: Muhammad Khalid SAEED, Naseem ZAHRA (these authors contributed equally)

Supervision: Muhammad Khalid SAEED, Asma SAEED and Quratulain SYED (these authors contributed equally)

Other (Specify): NA

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