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Chemical and oxidative properties of *Moringa oleifera* dried-ground parts extracted with different solvents

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Abstract
High concentration of antioxidants and low concentrations of anti-nutritional chemicals are found in various parts of Moringa species. The current study investigated the chemical composition, total energy, minerals and oxidative characteristics of several solvent extracts of dried-ground Moringa oleifera (MO) seeds, bark, and leaves. The MO parts' chemical composition, mineral content, and total energy were all measured. The antioxidant activity (DPPH, ABTS, and FRAP tests), total phenolics, and total flavonoids of different solvent extracts were also assessed. Seeds had significantly ($p \leq 0.05$) higher quantities of dry matter (94.49%), protein (22.67%), fat (34.29%), and total energy (565.97 Kcal/mol) compared to other parts. The bark contained much more carbohydrates (65.52%) than the other parts, but the leaves had a high ash level (6.62%). The leaves contained significantly greater quantities ($p \leq 0.05$) of Ca, Na, Mg, K, S, Mn, Fe, and Zn, whereas the seeds are high in phosphorus. The solvents' effectiveness in extracting total phenolics and flavonoids from the three parts varied significantly, with leaves having higher levels than the other parts. Furthermore, the leaves exhibited higher antioxidant activity than the other parts. In addition, antioxidant activity was significantly correlated with total phenolics and flavonoids. Except for the seeds, extracts generated using aqueous organic solvents produced greater total phenolics and flavonoids, as well as better antioxidant activity, with leaves outperforming other sections in terms of antioxidant characteristics.

Keywords: Moringa, leaves, seeds, bark, phenolics, flavonoids, minerals

Introduction

Plants include a large number of physiologically active phytochemicals. Due to safety concerns, numerous of these plant secondary metabolites are useful sources of natural antioxidants that are preferable to synthetic ones (van Wyk and Prinsloo, 2020) [41]. The biologically active secondary metabolites have been shown to scavenge free radicals via a range of biological pathways, lowering the risk and progression of diseases like cancer, cardiovascular disease, and neurological disorders (Karthikeyan *et al*, 2022) [23]. Moringa is a wholesome and nutritious food in the arid tropics, making it a desirable component in the development of sustainable communities (Olson *et al*, 2016) [30]. When used as a protein supplement, it increases milk production in goats and ewes that are accustomed to hot conditions (Babiker *et al*, 2017) [3]. A nutritional study found that the high vitamin A concentration of dry Moringa leaf powder compares favorably to milk powder in terms of calcium and protein content. The leaves of the plant also contain strong antioxidants, including isothiocyanates, which are known for their antibacterial, hypotensive, hypoglycemic, and anticancer properties (Olson *et al*, 2016) [30]. Moringa oleifera can be cultivated in tropical and subtropical locations in all types of soils; it is a drought-tolerant plant that can withstand dry seasons of up to 6 months (Su and Chen 2020) [38]. M. oleifera's nutrient profile is primarily determined by soil composition, climatic features, cultivation circumstances, processing, and storage quality (van Wyk and Prinsloo, 2020) [41]. Moringa leaves are high in minerals such as calcium, iron, potassium, and multivitamins, which are necessary for animal performance and milk production, as well as a good source of protein (El-Badawi *et al*, 2023) [18]. Moringa oleifera leaf meal is high in protein and can be used as a supplement to increase milk production (kholif *et al*. 2019). Oluwaniyi *et al*. (2020) [24, 31] discovered that Moringa oleifera leaves and stem bark have significant amounts of crude fiber, moisture, and ash, but the seeds contain comparatively modest amounts of the same parameters but high levels of protein and crude fat. Furthermore, they found that the leaves had the most macroelements (Na, K, Ca, Mg, and Fe), whereas the seeds contained the most microelements (Cu, Zn, Cd, Pb, and Ni). The leaves also have the highest antioxidant content and activity compared to the other parts. Many factors influence the extraction of bioactive compounds from plant material, including solvents, methods, and extraction timeframes; for large-scale manufacture, the ideal extraction process must be both successful and efficient (Jha and Sit, 2022). Al-Dabbas (2017) [1, 22] used a model system to show that distinct polar extracts from M. peregrine's leaves and seeds have different antioxidant capabilities. These activities are mostly related to the content of flavonoids and phenolic chemicals. He also noted that M. peregrina can inhibit undesired oxidation processes and may be an effective antioxidant source. It is likely that distinct bioactive compounds with different polarities and chemical characteristics will not dissolve in the same solvent (Jha and Sit, 2022) [22]. The most effective solvents for extracting polyphenols from a plant matrix include aqueous solutions including ethanol, methanol, acetone, and ethyl acetate. Polar solvents are often employed in this application (Wu *et al*, 2022). Aqueous methanol is more effective at extracting phenolic compounds from flaxseed (Wu *et al*, 2022). MO has the potential to become a substantial phytochemical source in underdeveloped countries where starvation and famine are serious problems. Thus, the goal of this research is to investigate the chemical composition and oxidative properties of *Moringa oleifera* parts extracted with different solvents.

Materials and Methods

Materials

Moringa oleifera tree parts were obtained from an agricultural farm in Khartoum, Sudan, during the 2021-2022 season. The leaves, bark, and seeds were carefully picked and thoroughly cleaned of debris and dirt. The pieces were air-dried, ground to pass through a 0.1 mm mesh, and stored at 4°C until use. The chemicals and reagents utilized in this study were of laboratory grade.

Chemical composition and total energy determination

The MO parts were dried to a constant weight at 105°C to determine their moisture content. Crude protein, fat, and ash were assessed using the AOAC (2006) standard techniques. Carbohydrate (nitrogen-free extract) was calculated using difference. The energy was calculated using the Atwater factors, as described by Osborne and Voogt (1978) [32]. One gram of carbohydrates provides 4 kcal, one gram of protein provides 4 kcal, and one gram of fat supplies 9 kcal.

Minerals analysis

According to Amaglo *et al*. (2010) [6], the material was dissolved in a 1:1 solution of HNO3 and HCl. After 72 hours of standing at 24°C, the mixture was filtered through a Millipore vacuum filter (0.45µm). The filtrate was diluted by 1:4 with ultrapure water. The analysis was conducted using ionic liquid chromatography. An electrolytic self-regenerating system, a conductivity detector, and a quaternary gradient pump were installed in the HPLC. The elution solvent was methane-sulfonic acid (25 mmol/L) at a flow rate of 1.0 ml per minute. The standard dilution for the calibrations was created using analytical purity commercial solutions, which were then dried at 140 °C for four hours before being re-suspended in 0.01 mol/L of each HNO_3: HCl (1:1). Mineral concentrations were given in mg per 100 gm of dry plant matter.

Extract preparation

For six hours at ambient temperature in an orbital shaker (Gallenkamp, UK), about 20 gm of each part was extracted using a variety of solvents, including water, absolute methanol, aqueous methanol (50:50 v/v), absolute ethanol, aqueous ethanol (50:50 v/v), absolute acetone, and acetone. Extracts and residues were separated using Whatman No. 1 filter paper. The precipitates were extracted again with the same fresh solvent before being mixed. The mixed extracts were concentrated and solvent-free at 45 °C under decreased pressure with a rotary evaporator (EYELA, SB-651, Rikakikai Co. Ltd. Tokyo, Japan).

Determination of total phenolics (TPC)

The TPC was evaluated using the Folin-Ciocalteu method, which was described by Chaovanalikit and Wrolstad (2004) [15]. Exactly 200 µl of the extract was mixed with roughly 400 µl of Folin-Ciocalteu reagent. Methanol was used in the control experiment. The solution was well mixed after being diluted with deionized water to a total volume of 4.6 ml. Following ten minutes of standing at room temperature, precisely one milliliter of a 20% Na2CO3 solution was added, immediately mixed, and left to incubate for two hours. A spectrophotometer (PD-UV, Apel, Saitama, Japan) was used to detect the absorbance at 765 nm. The TPC of the samples was expressed in mg GAE/100-gram dry weight, and the standard used was 1 mg/ml of Gallic acid. The values were given as means ± SD, and each measurement was carried out three times.

Determination of total flavonoid content (TFC)

The TFC of the extract was determined using the Kim *et al*. (2003) [25] technique. Exactly 300 µL of 5% $NaNO_2$ solution, 300 µL of 10% aluminum chloride, and 1 mL of methanolic extract were combined and incubated for five minutes at 25 ºC. Then 2 ml of sodium hydroxide (1 mol/L) was added. The solution was thoroughly vortexed after adding H2O to reach a volume of 10 mL. Using a spectrophotometer (PD-303UV spectrophotometer, Apel, Saitama, Japan) the absorbance was measured at 510 nm. A calibration curve was made using different doses of catechin (R^2 = 0.974). TFC was measured in milligrams of catechin equivalents (CE) per gram of sample (DW).

Antioxidant activity determination

2, 2-diphenyl-1-picrylhydrazyl (DPPH)

The Lee *et al*. (1998) [26] method was used to determine the extracts' DPPH. A vortex mixer was used to thoroughly mix 1 ml of each extract with 2 ml of the DPPH reagent after the extracts had been diluted with methanol. Methanol served as the control substance. Using a spectrophotometer (PD-303UV spectrophotometer, Apel, Saitama, Japan), the absorbance of the mixtures at 518 nm was determined. The following formula was used to estimate the DPPH:

DPPH (%) = $(1\text{-}A_{sample}/A_{control}) \times 100$

Where: A is the observed absorbance.

2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS)

The Re *et al*. (1999) approach was used to estimate the ABTS. ABTS was produced by a 12- to 16-hour dark reaction between aqueous solutions of 7 mM ABTS and 2.4 mM potassium persulfate. This solution was diluted in ethanol (1:89 v/v) before to the experiment and allowed to equilibrate at 30°C. Spectrophotometric measurements at 734 nm revealed an absorbance of 0.700±0.02 (Mod. 4050, Biochrom, Cambridge, UK). After mixing 10 µl of the test sample in ethanol with 1 ml of diluted ABTS solution, the absorbance was precisely measured 30 minutes later at 30 degrees Celsius. For the blank absorbance at 734 nm, the inhibition % was calculated. The radical scavenging activity was measured in micromoles of TE/g, or Trolox equivalents per gram of material.

Ferric reducing antioxidant power (FRAP)

The Yen and Duh (1993) [44] method was used to calculate FRAP. Two milliliters of FRAP working solution and half a milliliter of methanol extract (diluted ten times) were mixed together in a 10-milliliter test tube. After that, distilled water was added to dilute the mixture to 10 ml. The combination was left in the dark for twenty minutes. A spectrophotometer (PD-303UV spectrophotometer, Apel, Saitama, Japan) was used to measure the absorbance of the remaining FRAP solution at 593 nm in comparison to a blank. Trolox equivalents, or micromoles per gram of material (mol TE/g), were used to express the results.

Statistical analyses

The data analysis was conducted using the SAS program, 2000 (SAS Institute Inc, Cary, NC, USA). Significant differences between sample means were found using the LSD test. When a difference was $P \leq 0.05$, it was considered significant. Pearson correlation analysis was used to examine the relationship between the extracts' antioxidant activity and their total phenolic and flavonoid levels.

Results and discussion

Chemical composition, total energy, and minerals of MO parts

As shown in Table 1, the chemical composition of MO leaves, barks, and seeds. Seeds contain significantly ($p \leq 0.05$) more dry matter (94.49%), fat (34.29%), protein (22.67%), and total energy (565.97 Kcal/mol) than other parts of the plant. However, the leaves contained 6.62% ash, whereas the bark had 65.52% carbs. In addition, Table 1 shows that the leaves contained considerably higher quantities of Na (117.99 mg/100gm), Mg (419.57 mg/100gm), K (1898.85 mg/100gm), Ca (2471.10 mg/100gm), S (1046.54 mg/100gm), Mn (5.19 mg/100gm), Fe (7.69 mg/100gm), and Zn (3.43 mg/100gm). The seed was considerably ($p \leq 0.05$) high in P (682.89 mg/100gm). MO chemical composition showed that seeds contained significantly more protein, fat, ash, and total calories than leaves and bark. Furthermore, the leaves of MO had significantly more major and minor minerals than the other parts. The chemical composition of the seeds was similar to that reported for MO by Oluwaniyi *et al*. (2020) [31] and detected for other species leaves (Babiker *et al*, 2018) [11]. The results for the mineral content of the leaves were similar to those published by Al-Juhaimi *et al*. (2017), Babiker *et al*. (2018), and Oluwaniyi *et al*. (2020) [3, 11, 31] for different plant parts. In addition, Hodas *et al*. (2021) [21] found that Moringa leaves have significant quantities of fiber, ash, carbohydrates, total proteins, and lipids. Variations in chemical composition and mineral content in this study and other studies could be attributed to variations in soil composition, climatic characteristics, cultivating conditions, processing, and storage quality, as reported. The nutrient profile of M. oleifera is primarily determined by soil composition, climatic characteristics, cultivating conditions, processing, and storage quality (van Wyk and Prinsloo, 2020). Oluwaniyi *et al*. (2020) [41] discovered that Moringa oleifera leaves and stem bark have significant amounts of crude fiber, moisture, and ash, but the seeds contain comparatively modest amounts of the same parameters but high levels of protein and crude fat. In addition, they found that the leaves had the most macroelements, whereas the seeds contained the most microelements. The leaves also have the highest antioxidant content and activity compared to the other parts.

Table 1: Chemical composition (%) and energy (Kcal/mol), of *Moringa oliefera* parts

Parameters	Plant parts		
	Leaves	Seeds	Bark
Dry matter	90.87 ± 0.61^{b}	94.49 ± 0.74^{a}	92.25 ± 0.89^{c}
Protein	20.42 ± 0.47^{b}	22.67 ± 0.60^{a}	15.43 ± 0.38^{c}
Fat	7.13 ± 0.12^{b}	34.29 ± 0.96^{a}	4.83 ± 0.15^{c}
Carbohydrates	52.54 ± 0.46^{b}	41.67 ± 1.22^{c}	65.52 ± 0.86^{a}
Ash	6.62 ± 0.24^{a}	4.81 ± 0.27^{a}	1.87 ± 0.45^{b}
Total energy	356.01 ± 2.94^{c}	565.97 ± 4.68^{a}	367.27 ± 1.95^{b}

Values are means ± SD of triplicates. Mean values in a row with different superscripts (a, b, c) are significantly different at level $p \leq 0.05$.

Effects of solvent extraction on the total phenolic and flavonoid contents of MO parts

As shown in Table 2 the content total phenolic (TPC) and flavonoids (TFC) of plant parts using different solvent systems were differed between solvents. Among solvents used, absolute acetone extract of MO seeds yielded considerably ($p \leq 0.05$) high TPC (41.54 mg GAE/g DW). In the leaves, aqueous methanol extract exhibited considerably ($p \leq 0.05$) high TPC (35.69 mg GAE/g DW) and absolute methanol (32.97 mg GAE/g DW), followed by aqueous ethanol (50%) which generated significantly ($p \leq 0.05$) higher TPC (32.39 mg GAE/g DW) than other solvents. Leaf extracts exhibited significantly higher TPC ($p \leq 0.05$) than all other parts when using all solvents except absolute acetone, which extracted significantly more TPC from the seed. The absolute acetone extract of MO seeds extracted a significant amount of TFC (4.12 mg Catechin/g DW), but significantly ($p \leq 0.05$) lower than that of the leaves. The aqueous methanol extract showed significantly ($p \leq 0.05$) high TFC in the leaves (12.98 mg Catechin/g DW), followed by aqueous ethanol (12.45 mg Catechin/g DW). Leaf extracts had significantly more TFC than the other parts ($p \leq 0.05$), except for absolute ethanol, which extracted significantly more from the seeds (2.03 mg Catechin/g DW). TPC and TFC concentrations varied considerably ($P \leq 0.05$) across MO parts extracted with various solvents. The current investigation found that aqueous methanol and aqueous ethanol had considerably greater ($p \leq 0.05$) TPC and TFC in the leaves and bark extracts, whereas absolute acetone had significantly higher ($p \leq 0.05$) TPC and TFC in the seeds extract. Variations in extraction yield could be due to the higher extraction rates of phenolics and flavonoids in more polar solvents, such as aqueous methanol/ethanol, compared to absolute methanol/ethanol. This is because different antioxidant molecules have varied chemical characteristics and polarity, and they may or may not dissolve in a given solvent (Ali *et al*. 2019). Mehmood *et al*. (2022) [2, 27] found that solvent composition and polarity had a substantial influence on phenol and antioxidant extraction. Because of interactions (hydrogen bonds) between the polar sites of antioxidant compounds and solvents, validation extractions of these compounds were carried out more frequently in polar solvents, which were more efficient than non-polar solvents. The use of aqueous ethanol/methanol produced MO fractions rich in TPC, TFC, and antioxidant activity. Aqueous methanol was the best solvent for extracting MO components, particularly leaves, followed by aqueous ethanol, based on TPC, TFC, and antioxidant activity data. This may be due to phenolic compounds' high solubility in ethanol and methanol (Gonfa *et al*, 2020). Rajhi *et al*. (2021) [19, 35] found that the flavonoid concentration of *Capparis spinosa* leaves is highest in organic solvents and lowest in aqueous extraction. According to Dhingra *et al*. (2017) [17], the liquid-liquid extraction process can dilute or increase phenolic chemicals in the crude extract, and solvent polarity has a significant effect on extract yields (Ouerghemmi *et al*, 2016) [33].

AlMousa *et al*. (2022) reported that methanol extract contained more TPC with high antioxidant and antibacterial activities compared to other solvents. Although total phenolics (TPC) in MO parts were identified in the sequence leaves > bark > seeds, Nantongo *et al*. (2018) [29] studied the variability of phenolic and alkaloid content in different plant parts of *Carissa edulis* and discovered no change in phenol abundance. However, for the sake of sustainability, leaves are recommended as a medication alternative to the favored root or stem bark. Moreover, Oluwaniyi *et al*. (2020) [31] discovered that MO leaves contain a high concentration of phenolic compounds, which can help prevent the spread of a variety of diseases. In addition, Al-Owaisi *et al*. (2014) [5] discovered that the most polar extract, methanol, had the highest total phenol and flavonoid concentrations when compared to ethyl acetate/chloroform extracts. In contrast to our findings, Al-Dabbas (2017) [1] found considerable quantities of phenolics, flavonoids, and flavonol in all extracts of Moringa peregrina, whether from leaves or seeds.

Table 2: Minerals content (mg/100gm DW) of *Moringa oliefera* parts

Parameters	Plant parts		
	Leaves	Seeds	Bark
Major minerals			
Na	117.99± 5.32^{a}	105.47 ± 4.10^{b}	89.78 ± 6.27^{c}
Mg	419.57 ± 18.73^{a}	282.16 ± 14.36^{b}	156.41 ± 11.81^{c}
P	634.09 ± 57.97^{b}	682.89 ± 76.28^{a}	612.76 ± 15.01^{c}
K	1898.85 ± 31.4^{a}	1330 ± 46.79^{c}	1038 ± 24.85^{b}
Ca	2471.10 ± 48.12^{a}	1707.06 ± 10.03^{b}	1232.79 ± 55.8^{c}
S	1046.54± 28.19^{a}	946.54± 14.31^{b}	746.39± 31.23^{c}
Trace minerals			
Mn	5.19 ± 1.05^{a}	3.34 ± 1.13^{b}	2.45 ± 0.93^{c}
Fe	7.69 ± 5.71^{a}	6.08 ± 2.52^{b}	3,23 ± 3.86^{c}
Cu	1.22 ± 0.18^{a}	1,02 ± 0.21^{a}	0.71 ± 0.14^{b}
Zn	3.43 ± 4.42^{a}	2.08 ± 0.03^{b}	1.12 ± 0.21^{c}
Se	0.36 ± 0.01^{a}	0.41 ± 0.04^{a}	0.23 ± 0.02^{c}
Cd	0.23± 0.01^{c}	0.36± 0.06^{b}	0.47± 0.08^{a}
Ni	0.24± 0.03^{b}	0.26± 0.06^{b}	0.37± 0.08^{a}

Values are means ± SD of triplicates. Mean values in a row with different superscripts (a, b, c) are significantly different at level $p \leq 0.05$.

Effects of solvent extraction on antioxidant activity of MO parts and correlation with TPC and TFC

MO parts antioxidant activity was assessed in the current study utilizing a variety of antioxidant assays, including DPPH, ABTS, and FRAP, as shown in Table 3. As demonstrated, the acetone extract had the highest percentage of DPPH scavenging in the seeds (16.52%), followed by ethanol (15.62%). These percentages showed a strong association with TPC (R = 0.919; Table 4) and TFC (R = 0.817; Table 4). In comparison to other solvents, aqueous methanol extracts produced much higher DPPH (92.72%) in the bark, followed by aqueous acetone (92.63%). The bark's DPPH showed a good connection with TPC (R = 0.981; Table 4) and TFC (R = 0.931; Table 4). The leaves' DPPH levels were higher when extracted with aqueous ethanol (93.34%), followed by aqueous methanol (92.91%), and were found to be positively associated with TPC (R = 0.991; Table 4) and TFC (R = 0.821; Table 4). The ABTS of the bark (18.73 g Trolox/g sample) and leaves (32.75 g Trolox/g sample) were higher in aqueous methanol extracts, whereas the seeds (3.21 g Trolox/g sample) were higher in absolute acetone extracts. The parts' ABTS was favorably correlated with TCP (R = 0.734, 0.811, and 0.878 for the seeds, bark, and leaves, respectively; Table 4) and TFC (R = 0.801, 0.851, and 0.758 for the seeds, bark, and leaves, respectively; Table 4). The FRAP value of the acetone extract of the seeds (3.11 g Trolox/g sample) was higher than that of the other solvents, whereas that of the bark was higher in aqueous methanol (4.04 g Trolox/g sample) and aqueous acetone (4.03 g Trolox/g sample). However, the aqueous ethanol extract had the highest TRAP (9.66 g Trolox/g sample) in leaves. The parts' FRAP was positively correlated with the TCP (R = 0.958, 0.972, and 0.965 for the seeds, bark, and leaves, respectively; Table 4) and TFC (R = 0.861, 0.878, and 0.763 for the seeds, bark, and leaves, respectively; Table 4).

Table 3: Total phenolics (mg Gallic acid /g sample) and flavonoids (mg Catechin /g sample) of *M. oliefera* parts extracted by different solvents

Extraction solvent	Total phenolics			Total flavonoids		
	Seeds	Bark	Leaves	Seeds	Bark	Leaves
Water	17.23 ± 1.16^{dr}	14.07 ± 1.38^{cq}	23.48 ± 2.98^{ep}	1.12 ± 0.23^{er}	2.23 ± 0.21^{dq}	6.14 ± 0.31^{dp}
Methanol	25.67 ± 1.21^{cr}	17.65 ± 2.23^{dq}	32.97± 1.99^{dp}	1.23 ± 0.18^{dq}	1.35 ± 0.12^{eq}	9.56 ± 0.24^{cp}
Ethanol	31.51 ± 1.23^{bq}	18.31 ± 1.47^{fp}	26.46 ± 1.38^{gr}	2.03 ± 0.14^{cp}	1.45 ± 0.25^{fq}	1.18 ± 0.43^{fq}
Acetone	41.54 ± 2.51^{ar}	16.97 ± 4.16^{eq}	21.25 ± 0.66^{fp}	4.12 ± 0.31^{aq}	0.98 ± 0.15^{gr}	5.97 ± 0.37^{dp}
Aqueous methanol (50%)	9.63 ± 0.56^{fr}	20.45 ± 0.42^{aq}	35.69 ± 0.49^{bp}	2.35 ± 0.41^{br}	4.96 ± 0.31^{aq}	12.98 ± 0.34^{bp}
Aqueous ethanol (50%)	8.23 ± 1.19^{fr}	18.11 ± 0.24^{bq}	32.39 ± 1.78^{ap}	0.93 ± 0.08^{fr}	2.97 ± 0.34^{cq}	12.45 ± 0.41^{ap}
Aqueous acetone (50%)	11.71 ± 1.13^{er}	19.61 ± 0.81^{aq}	25.28 ± 1.76^{cp}	1.07 ±0.11^{er}	4.13 ± 0.32^{bp}	3.62 ± 0.56^{eq}

Different letters (a, b, or c) in the same column or (p, q, or r) in a row for each parameter indicate significant differences at $p \leq 0.05$ level-Duncan's multiple range tests.

Table 4: DPPH (%), ABTS, and FRAP (g Trolox/g sample) of *M. oliefera* parts extracted by different solvents

Extraction solvent	DPPH			ABTS		
	Seeds	Bark	Leaves	Seeds	Bark	Leaves
Water	12.17 ± 0.43^{cq}	85.31 ± 0.19^{dp}	85.51 ± 0.87^{dp}	1.91 ± 0.13^{cr}	8.07 ± 0.29^{cp}	5.53 ± 0.26^{fq}
Methanol	13.28 ± 0.56^{cr}	80.61 ± 0.26^{eq}	85.44 ± 0.47^{dp}	1.13 ± 0.46^{er}	2.98 ± 0.16^{eq}	11.25 ± 0.37^{dp}
Ethanol	15.62 ± 1.03^{br}	80.61 ± 0.17^{ep}	68.67 ± 0.39^{eq}	2.54 ± 0.61^{bp}	1.96 ± 0.31^{fq}	0.63 ± 0.11^{gr}
Acetone	16.52 ± 0.42^{adr}	79.42 ± 0.21^{fq}	83.47 ± 0.47^{ap}	3.21 ± 0.16^{aq}	2.23 ± 0.81^{fr}	10.39 ± 0.77^{ep}
Aqueous methanol (50%)	11.37 ± 0.61^{cq}	92.72 ± 0.46^{ap}	92.91 ± 2.55^{bp}	1.92 ± 0.34^{cr}	18.73 ± 0.48^{aq}	32.75 ± 0.68^{ap}
Aqueous ethanol (50%)	$7.88 \pm 0.34^{, er}$	91.39 ± 0.48^{cq}	93.34 ± 0.37^{ap}	1.75 ± 0.42^{dr}	10.77 ± 0.57^{bq}	31.24 ± 0.68^{bp}
Aqueous acetone (50%)	10.87 ± 0.46^{dr}	92.63 ± 0.33^{bp}	90.49 ± 0.88^{cq}	1.21 ± 0.38^{er}	6.11 ± 0.37^{dq}	24.95 ± 0.89^{cp}
Extraction solvent	FRAP					
Water	1.61 ± 0.32^{br}	3.88 ± 0.38^{aq}	6.48 ± 0.79^{dp}			
Methanol	1.83 ± 0.44^{bq}	2.56 ± 0.21^{bq}	7.39 ± 0.91^{cp}			
Ethanol	1.79 ± 0.25^{bq}	2.19 ± 0.25^{cp}	0.94 ± 0.58^{fr}			
Acetone	3.11 ± 0.49^{aq}	2.35 ± 0.19^{cr}	4.07 ± 0.47^{ep}			
Aqueous methanol (50%)	1.24 ± 0.31^{cr}	4.04 ± 0.31^{aq}	9.16 ± 0.26^{bp}			
Aqueous ethanol (50%)	1.19 ± 0.72^{cr}	3.91 ± 0.47^{aq}	9.66 ± 0.51^{ap}			
Aqueous acetone (50%)	1.35 ± 0.29^{cr}	4.03 ± 0.39^{aq}	9.11 ± 0.37^{bp}			

Different letters in the same column or (p, q, or r) in a row for each parameter indicate significant differences at $p \leq 0.05$ level—Duncan's multiple range test.

A number of studies on phenolic substances have revealed potential biological activity, such as antioxidant, antidiabetic, anti-inflammatory, antibacterial, and anticancer activities (Burlacu *et al*, 2020; Rafie *et al*, 2017) [13, 34]. The antioxidant activity of phenolic compounds is mostly owing to their reduced properties, which allow them to act as metal chelators, absorbing and neutralizing free radicals (Tohma *et al*, 2017) [40]. Flavonoids and tannins are considered the most promising polyphenolic compounds found in plant secondary metabolites (Swallah *et al*, 2020) [39]. The antioxidant activity of these extracts was tested using the DPPH, ABTS, and FRAP assays. Polyphenols and flavonoids are some of the plant secondary metabolites identified in crude extracts of MO components. These bioactive chemicals' hydrogen-donating action can cause DPPH solution to discolor (Waheed *et al*, 2014) [42]. The bark and leaf extracts had significantly higher antioxidant activity than the seeds. This difference in antioxidant activity among MO components could be ascribed to the fact that bark and leaves had significantly higher TPC and TFC levels than seeds. In addition, MO parts' antioxidant activity was strongly correlated with TPC and TFC ($R > 7$). The antioxidant activity of aqueous organic solvent extracts was significantly greater than that of other solvents. This is partly because polar solvents are commonly used to extract polyphenols from plant matrices, with aqueous mixtures containing ethanol, methanol, acetone, and ethyl acetate being the most suited solvents (Sasadara *et al*, 2021) [37].

According to Gonfa *et al*. (2020) [19], high molecular weight phenolic complexes may develop when phenolic compounds are extracted in methanol. Additionally, methanol extract exhibited the highest content of polyphenols and antioxidant activity in vegetable waste when compared to ethanol and aqueous extracts (Hagos *et al*, 2023) [20]. The current study supported a previous investigation which showed that methanol and ethanol were efficient at extracting bioactive compounds (Belyagoubi *et al*, 2016) [12]. The maximum extraction efficiency was found in aqueous solvents ($p \leq 0.05$), suggesting that aqueous organic solvents performed better in terms of extraction efficiency than either pure solvents or water. The majority of the phenolic compounds in plant samples may be extracted with an organic solvent and water mixture, and the more polar the solvent, the higher the antioxidant activity of the extract (Al-Dabbas, 2017) [1]. However, it is challenging to find a single solvent that can extract all phenolic chemicals. Antioxidant activity and total phenolic content in wild vegetables were found to be significantly associated (Aryal *et al*, 2019). Carbonell-Capella *et al*. (2015) [8, 14] discovered a high association between total phenolic content and antioxidant activity in stevia-containing beverages that underwent *in vitro* digestion. This discovery is consistent with previous research, which has demonstrated a substantial link between polyphenolic compounds and antioxidant activity.

In a comparable manner Chen *et al*. (2016) [16] discovered a strong association between antioxidant activity and TPC fruits during germination. These findings are congruent with those of Asem *et al*. (2020) [9], who discovered a significant correlation between propolis' total phenolic and flavonoid content and antioxidant activity. Muflihah *et al*. (2021) [28] found that Pearson correlation analysis suggested that the observed TPC and TFC were significant contributors to antioxidant activity, indicating that these molecules play a vital role in antioxidant capacity.

Table 5: Correlation between total phenolics, flavonoids and antioxidant activity (DPPH, ABTS, and RFAP) of *M. oliefera* parts extracted by different solvents

Plant part	DPPH	ABTS	FRAP
Total phenolics			
Seeds	0.919**	0.734*	0.958**
Bark	0.981**	0.811*	0.972**
Leaves	0.991**	0.878*	0.965**
Total flavonoids			
Seeds	0.817*	0.801*	0.861*
Bark	0.931**	0.851*	0.878*
Leaves	0.821*	0.758*	0.763*

* $p \leq 0.05$, ** $p \leq 0.01$

Conclusions

The present study demonstrated that the MO parts differed in chemical composition and mineral concentration as well as the oxidative characteristics. Aqueous organic solvent (50%) extracts of MO parts had higher phenolic and flavonoid contents which accompanied by a higher antioxidant activity. In addition, the mixture of organic solvent and water may facilitate the extraction of all soluble

components in both water and organic solvents. The bark and leaves contained high concentrations of TPC and TFC, as well as significant antioxidant activity. The antioxidant activity of all parts was strongly correlated with TPC and TFC. The current study will undoubtedly assist determine the efficacy of MO bark and leaves as potential sources of minerals, and natural antioxidants whereas the seeds as a potential source of protein and fat for nutraceutical and functional food applications.

Author Contributions

A.H.M.A.: Conceptualization, Methodology, Investigation, Writing-original draft preparation and Data curation; E.E.B.: Supervision, Data curation, Visualisation. Writing-review and editing. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

Data supporting the findings of this study are available from the corresponding author on a reasonable request.

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