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Comparative investigation of antioxidant content and activity of different parts of *Moringa peregrina* extracted by different solvent systems

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Summary

A comparative study evaluated the total polyphenol content (*TPC*), total flavonoid content (*TFC*), and antioxidant activity of *Moringa peregrina* (MP) seeds, bark, and leaves extracted using different solvents. Antioxidant activity was assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and ferric reducing antioxidant power (FRAP) assays. Significant variations were observed among plant parts and solvent systems. Leaves consistently showed the highest *TPC* and *TFC*, followed by bark and seeds. Absolute ethanol extracts yielded high *TPC* and *TFC* in leaves and bark, while aqueous methanol produced high *TPC* in bark. The strongest DPPH scavenging activity was found in leaves (91.6 %), followed by bark (90.7 %) and seeds (19.3 %). Ethanol extracts also showed the highest ABTS and FRAP antioxidant activity values, particularly in leaves and bark. Antioxidant activity was strongly correlated with phenolic and flavonoid contents regardless of the solvent used. Overall, MP leaves and bark demonstrated superior antioxidant potential, suggesting their possible therapeutic value in disease prevention and management.

Keywords

Moringa peregrina; polyphenols; ethanol; antioxidant activity

Moringa is a member of the Moringaceae family, which includes 10 to 13 species, including *peregrina* [1], which is widely grown in tropical locations across Africa and Asia, including Saudi Arabia [2]. In addition to their nutritional value, these plants are used in traditional medicine [3]. Moringa has also been found to contain extremely low quantities of anti-nutritional compounds such as oxalate, phytate, tannins, and oligosaccharides [4]. Moreover, it contains many physiologically active phytochemical compounds, and due to safety concerns, many of these plant secondary metabolites are valuable sources of natural antioxidants that outperform synthetic ones [5]. These beneficial secondary metabolites have been shown to scavenge free radicals via various biological pathways, thereby reducing the risk and progression of cancer, cardiovascular disease, and neurological illnesses [6].

In addition, *Moringa peregrina* (MP) has been shown to possess several pharmaceutical properties, including hypolipidaemic, hypoglycaemic, anti-inflammatory, analgesic, and antioxidant activities [7], as well as anticancer, antioxidant, anti-inflammatory, and antiarthritic activities [8]. Al-Khalasi et al. [1] used qualitative analysis

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to identify phytochemicals in MP extracts, and agar diffusion to evaluate the antifungal activity of methanol and water extracts of leaves, seeds, and roots. They found that the extracts tested showed varying antifungal activity against *Candida kruzei*, with the leaf extract being the most effective and exhibiting the lowest inhibitory concentration (MIC). A study discovered that MP leaf ethanolic extract possesses anti-obesity, hyperlipidaemic, and hepatoprotective activities in rats fed a high-fat diet [9]. According to ALQETHAMI and ALDHEBIANI [10], MP is widely grown in Saudi Arabia because it is used as a traditional medicine, and most MP research has been limited in scope, focusing on specific parts of the plant rather than the whole plant. Furthermore, they determined that location and climate have a major impact on the content of primary and secondary metabolites in plants; therefore, MP plants grown in Saudi Arabia should exhibit biological activities that differ from those grown in other regions.

It has been reported that the use of various polar solvents in extraction had a significant impact on the total polyphenol content (*TPC*), total antioxidant capacity (*TAC*), and antioxidant activity of the obtained extracts from plants, as well as the plant's richness in secondary metabolites and phenolic contents [11]. Furthermore, they demonstrated that the plant's aqueous extracts had the highest concentrations of phenolic compounds and the most significant antioxidant activity in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and *TAC* assays. 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 [12]. Aqueous methanol is more effective at extracting phenolic compounds from flaxseed [12]. MP has the potential to become a substantial source of phytochemicals in impoverished countries where starvation and famine are serious problems. Thus, a comparative investigation of the total phenolics, total flavonoids, and antioxidant activity of MP seed, bark, and leaves extracted with various solvent systems was conducted.

MATERIALS AND METHODS

Plant sample collection, authentication, and preparation

The whole MP plant, including leaves, seeds, and bark, was taken from a local farm in Riyadh, Saudi Arabia. A voucher specimen (No. 15642) has been deposited in the Herbarium of the Pharmacognosy Department (College of Pharmacy, King Saud University, Riyadh, Saudi Arabia). The samples were cleaned, washed, and sun-dried.

Preparation of solvent extracts

MP leaf, bark, and seed extracts for phytochemical analysis and antioxidant activity determination were prepared by mixing 5.0 g of sample with 25 ml of each absolute ethanol (100%), aqueous ethanol (80%, 60%, 40%, and 20%), absolute methanol (100%), and aqueous methanol (50%). We selected 50% aqueous methanol because it is widely reported as an effective standard solvent for extracting a broad range of phenolic compounds. In contrast, multiple ethanol concentrations (80%, 60%, 40%, and 20%) were used to assess how different solvent polarities influence extraction efficiency. The mixtures were left on the shaker for 24 h. After that, the resulting mixture was centrifuged at 3.14×10^3 rad·s^{-1} for 15 min (Rotouni II, K. Schneider, Zurich, Switzerland). The supernatants were filtered using Whatman No. 41 filter paper (Whatman, Maidstone, United Kingdom). The supernatants were adjusted to 25 ml using the appropriate solvents and stored at –20 °C for subsequent analysis.

Phytochemical analysis

Total polyphenol content

The total polyphenol content (*TPC*) of MP extracts was measured using the Folin-Ciocalteu technique [13]. A test tube was filled with 2.5 ml of distilled water and 0.1 ml of sample extracts, followed by 0.1 ml of undiluted commercially available Folin–Ciocalteu reagent (Sigma-Aldrich, St. Louis, Missouri, USA). The mixture was then left to react for 6 min. Then, 0.5 ml 20% Na_2CO_3 was added. The absorbance at 760 nm was measured after 30 min at room temperature (22 ± 2 °C) using a PD-303 UV spectrophotometer (Apel, Saitama, Japan). A blank sample was prepared with 0.1 ml of each solvent used (water, Folin–Ciocalteu reagent, and Na_2CO_3, except for the extract. The polyphenol amount was determined using the gallic acid calibration curve (Eq. 1).

$$y = 0.0078x + 0.1861 \ (R^2 = 0.9926) \quad (1)$$

The results were measured using the formula (Eq. 2).

$$TPC = C \times \frac{V}{m} \quad (2)$$

where *TPC* is expressed as grams of gallic acid equivalents (GAE) per kilogram of dry extract,

C is the concentration of gallic acid established from the calibration curve (in grams per litre), V is the volume of extract (in litres), and m is the weight of the plant extract (in kilograms).

Total flavonoid content

The total flavonoid content (*TFC*) of MP extracts was measured using the aluminium chloride colourimetric technique, as described by Baba and Malik [14]. In brief, 50 μl of crude extract or standard solution was mixed with 4 ml of distilled water and 0.3 ml of 5% $NaNO_2$ solution. Five minutes later, 0.3 ml of 10% $AlCl_3{\cdot}6H_2O$ solution was added, and the mixture was allowed to stand for 6 min. Thereafter, 2 ml of 1 mol·l^{-1} NaOH solution was added, and the mixture was diluted to 10 ml with distilled water. The mixtures were mixed and allowed to stand for 15 min before absorbance was measured at 510 nm using a spectrophotometer PD-303 UV (Apel). The *TFC* was determined from a catechin calibration curve, and the results were expressed as grams of catechin equivalent (CE) per kilogram of dry weight. The equation for the catechin calibration curve was (Eq. 3).

$$y = 0.0067x + 0.012 \ (R^2 \approx 0.99) \quad (3)$$

Antioxidant activity

DPPH assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity of MP extracts was evaluated as described by Akillioglu and Karakaya [15]. Incubate 50 ml of extracts with a 950 μl solution of 0.08 mmol·l^{-1} DPPH (Sigma-Aldrich) in methanol for 5 min. The absorbance of the reaction mixture was measured at 515 nm exactly 5 min later using a spectrophotometer PD-303 UV (Apel). Ethanol was used to calibrate the spectrophotometer. The absorbance of the DPPH radical in the absence of samples served as the control. All determinations were made in triplicate. The DPPH antioxidant activity (AA_{DPPH}) was represented as the percentage of inhibition calculated using (Eq. 3) and expressed as (Eq. 4):

$$AA_{\mathrm{DPPH}} = 100 - \left(\frac{A_s}{A_b} \times 100\right) \quad (4)$$

where A_s is the absorbance of the sample and A_b is the absorbance of the blank.

ABTS assay

The 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) activity estimation was done as described by Gouveia and Castilho [16]. Aqueous reagents of 7 mmol·l^{-1} ABTS (Sigma-Aldrich) and 2.4 mmol·l^{-1} potassium persulfate were reacted for 12–16 h at 24 °C in the dark to generate the ABTS radical cation ($ABTS^{\bullet+}$) reagent. The reagent was diluted in ethanol (1 : 89 v/v) and allowed to equilibrate at 30 °C, resulting in an absorbance of 0.700 ± 0.02 at 734 nm, as measured spectrophotometrically (Model 4050, Biochrom, Cambridge, United Kingdom). After mixing 10 μl of the test sample in ethanol with 1 ml of diluted ABTS solution, the absorbance was measured exactly 30 min later at 30 °C. The percentage inhibition of the blank absorbance at 734 nm was calculated. The results of antioxidant activity (AA_{ABTS}) were expressed as grams of Trolox equivalent (TE) per kilogram of dry weight, based on the Trolox calibration curve (Eq. 5).

$$y = 0.8286x + 0.2101 \ (R^2 = 0.996) \quad (5)$$

FRAP assay

The ferric reducing antioxidant power (FRAP) assay was performed according to the method described by Benzie and Strain [17]. In a 10 ml test tube, 0.5 ml of the methanolic extract (diluted tenfold) was mixed with 2.0 ml of the freshly prepared FRAP working solution, and the mixture was made up to 10 ml with distilled water. The reaction mixture was incubated in the dark for 20 min. The absorbance was measured at 593 nm using a spectrophotometer PD-303UV (Apel) against a reagent blank. The results of antioxidant activity (AA_{FRAP}) were expressed as grams of Trolox equivalents (TE) per kilogram of dry weight, based on the Trolox calibration curve (Eq. 5).

Gas chromatography-mass spectrometry

The gas chromatography-mass spectrometry (GC-MS) analysis of the ethanolic extracts of MP parts was performed using an Agilent 7890B GC system coupled with a mass selective detector 5977B MSD (Agilent Technologies, Santa Clara, California, USA). The instrument was equipped with an Innowax FSC capillary column (60 m × 0.25 mm, 0.25 μm film thickness; Agilent Technologies), and helium was used as the carrier gas at a constant flow rate of 0.8 ml·min^{-1}. A 0.1 μl aliquot of the sample was injected at a split ratio of 40 : 1. The GC oven temperature was programmed as follows: held at 60 °C for 10 min, increased to 220 °C at 4 °C·min^{-1}, held for 10 min, and then raised to 240 °C at 1 °C·min^{-1}. The injector and transfer line temperatures were maintained at 250 °C and 280 °C, respectively. Mass spectrometric detection was carried out at 70 eV over a mass range of *m/z* 35–450. Individual phy-

tochemical compounds in MP were identified by comparing their mass spectra with those in the Adams library [18] and by matching their retention times and retention indices (*RI*) with those of authentic reference standards and n-alkanes (C_8–C_{30}) under identical analytical conditions [19].

Statistical analysis

Throughout the investigation, three samples were prepared, and each sample was measured three times. The triplicate samples and measurement data were statistically evaluated using one-way ANOVA (SAS software version 9.4, SAS Institute, Cary, North Carolina, USA). Duncan's multiple-range test (DMRT) was used to compare means, with significance set at $p \leq 0.05$. The association between *TPC*, *TFC*, and antioxidant activity was analysed using Pearson correlation at $P \leq 0.01$, and $P \leq 0.05$.

RESULTS AND DISCUSSION

Extraction of phenolics and flavonoids

Tab. 1 shows the *TPC* (expressed as GAE) and *TFC* (expressed as CE) of MP sections extracted with various solvent systems. Absolute ethanol yielded significantly ($p \leq 0.05$) the maximum *TPC* (403.02 g·kg^{-1}) from the leaves, followed by 50% aqueous ethanol (393.69 g·kg^{-1}) and aqueous methanol (352.57 g·kg^{-1}). Additionally, both aqueous methanol (205.45 g·kg^{-1}) and pure ethanol (200.51 g·kg^{-1}) yielded significantly high ($p \leq 0.05$) *TPC* from the bark. In comparison to other solvents, absolute methanol (23.77 g·kg^{-1}) yielded the maximum *TPC* from seeds ($p < 0.05$), followed by water extract (11.94 g·kg^{-1}). Leaf extracts had significantly higher *TPC* levels ($p < 0.05$) than the other parts across all solvents compared to the bark and seeds.

According to Tab. 1, both absolute ethanol (13.17 g·kg^{-1}) and aqueous methanol (13.48 g·kg^{-1}) extracted significant amounts of *TFC* from the leaves ($p \leq 0.05$). Furthermore, pure ethanol (5.92 g·kg^{-1}) and aqueous methanol (5.16 g·kg^{-1}) extracted significantly more *TFC* from the bark ($p \leq 0.05$). The seeds' *TFC* increased significantly ($p \leq 0.05$) after extraction with absolute ethanol (4.95 g·kg^{-1}) and 40% aqueous methanol (4.58 g·kg^{-1}). Leaf extracts exhibited significantly higher *TFC* levels ($p \leq 0.05$) compared to other

Tab. 1. The total polyphenols and total flavonoids contents of *Moringa peregrina* leaves, seeds, and bark extracted by different solvents.

Solvents	Seeds	Bark	Leaves
TPC [g·kg^{-1}]			
Water	11.94 ± 0.97^{br}	98.30 ± 1.51^{eq}	239.97 ± 2.88^{gp}
Ethanol (100%)	10.23 ± 0.49^{br}	200.51 ± 1.81^{bq}	403.02 ± 1.81^{ap}
Aqueous ethanol (80%)	6.41 ± 0.18^{cr}	117.98 ± 1.39^{dq}	309.21 ± 2.68^{ep}
Aqueous ethanol (60%)	6.62 ± 0.64^{cr}	179.56 ± 0.84^{dq}	338.46 ± 2.21^{dp}
Aqueous ethanol (50%)	7.39 ± 1.49^{cr}	188.11 ± 0.24^{cq}	393.69 ± 1.78^{bp}
Aqueous ethanol (40%)	7.47 ± 0.18^{cr}	88.52 ± 0.49^{fq}	135.97 ± 1.06^{hp}
Aqueous ethanol (20%)	7.15 ± 0.37^{cr}	164.67 ± 0.37^{eq}	348.14 ± 2.13^{cp}
Methanol (100%)	23.77 ± 1.15^{ar}	97.65 ± 2.23^{gq}	288.14 ± 1.99^{fp}
Aqueous methanol (50%)	8.41 ± 0.73^{cr}	205.45 ± 0.42^{aq}	352.57 ± 0.49^{cp}
TFC [g·kg^{-1}]			
Water	1.49 ± 0.54^{br}	2.61 ± 0.13^{dq}	7.41 ± 0.31^{dp}
Ethanol (100%)	4.95 ± 0.58^{ar}	5.92 ± 0.35^{aq}	13.17 ± 0.06^{ap}
Aqueous ethanol (80%)	1.27 ± 0.74^{cr}	3.07 ± 0.23^{cq}	9.24 ± 0.16^{cp}
Aqueous ethanol (60%)	1.34 ± 0.42^{cr}	3.24 ± 0.11^{cq}	10.17 ± 0.04^{bp}
Aqueous ethanol (50%)	0.96 ± 0.12^{fr}	3.07 ± 0.27^{cq}	12.85 ± 0.18^{ap}
Aqueous ethanol (40%)	4.58 ± 0.44^{aq}	4.95 ± 0.23^{bq}	10.23 ± 0.37^{bp}
Aqueous ethanol (20%)	1.11 ± 0.01^{cr}	2.46 ± 0.04^{dq}	3.11 ± 0.06^{ep}
Methanol (100%)	1.88 ± 0.28^{cq}	1.87 ± 0.06^{eq}	9.81 ± 0.25^{cp}
Aqueous methanol (50%)	2.63 ± 0.36^{br}	5.16 ± 0.11^{aq}	13.48 ± 0.31^{ap}

Values are means ± standard deviation of triplicates. Mean values in a column with different superscript letters (a–f) are significantly different at the level $p \leq 0.05$. The superscript letters (p, q, and r) indicate the significant difference between plant parts in the rows at level $p \leq 0.05$.
TPC – total polyphenol content (expressed as grams of gallic acid equivalents per kilogram dry weight), *TFC* – total flavonoid content (expressed as grams of catechin equivalents per kilogram dry weight).

parts, regardless of the solvent used. *TPC* and *TFC* contents differed significantly ($p \leq 0.05$) across MP parts extracted using different solvents.

The study found that aqueous methanol, ethanol, and absolute ethanol had significantly ($p \leq 0.05$) higher *TPC* and *TFC* in the leaves and bark extracts, while absolute methanol had a significantly ($p \leq 0.05$) higher *TPC* in the seeds extract. This could be owing to increased extraction rates of phenolics and flavonoids in more polar solvents, such as aqueous methanol/ethanol, as compared to pure methanol/ethanol. This is because distinct antioxidant molecules have different chemical properties and polarities, and they may or may not dissolve in a given solvent. According to Mehmood et al. [20], solvent composition and polarity significantly affect the extraction of phenols and antioxidants. They also discovered that, due to interactions (hydrogen bonds) between the polar sites of antioxidant compounds and solvents, validation extractions of these substances were performed more frequently in polar solvents, which were more effective than non-polar solvents. As a result, the application of aqueous ethanol/methanol yielded MP fractions with high *TPC*, *TFC*, and antioxidant activity.

TPC, *TFC*, and antioxidant activity data showed that pure ethanol was the most effective solvent for extracting MP part components, particularly from leaves, followed by aqueous methanol and ethanol. This could be due to the high solubility of phenolic compounds in ethanol and methanol [21]. However, Rajhi et al. [22] found that the flavonoid content of *Capparis spinosa* leaves is highest in organic solvents and lowest in aqueous extracts. According to Dhingra et al. [23], the liquid-liquid extraction method can either dilute or enhance phenolic compounds in the crude extract, and solvent polarity significantly affects extract yields. In addition, AlMousa et al. [24] found that the methanol extract had higher *TPC* and more potent antioxidant and antibacterial properties than extracts obtained with other solvents.

Although the total phenolics (*TPC*) in MP parts described in the current study were in the order leaves > bark > seeds, Al-Owaisi et al. [25] reported that MP leaves are rich in phenolic compounds, which can help prevent the progression of various illnesses. In addition, they discovered that methanol, the most polar extract, had the highest total phenol and flavonoid content compared with ethyl acetate/chloroform extracts.

Antioxidant activity

The antioxidant activity of the different MP parts was assessed in the current study using three antioxidant assays, including DPPH, ABTS, and FRAP, as illustrated in Tab. 2. The aqueous methanol extract exhibited a significantly greater ($p \leq 0.05$) percentage of DPPH scavenging in the seeds (19.3 %) compared to pure ethanol (15.6 %). The percentage of AA_{DPPH} in the seeds had a significant positive correlation with *TPC* ($R = 0.934$; $p \leq 0.01$) and *TFC* ($R = 0.758$; $p \leq 0.05$). Aqueous methanol extracts produced much more AA_{DPPH} in the bark than other solvents (90.7 %), followed by 50% aqueous ethanol (89.5 %). The bark's AA_{DPPH} was significantly ($p \leq 0.01$) and strongly related to the *TPC* ($R = 0.985$) and *TFC* ($R = 0.949$). The AA_{DPPH} levels in the leaves were highest when extracted with 50% aqueous ethanol (91.6 %), followed by aqueous methanol (90.9 %). These levels were significantly and positively linked with the leaves' *TPC* ($R = 0.989$; $p \leq 0.01$) and *TFC* ($R = 0.786$; $p \leq 0.05$). The correlation coefficients are presented in Tab. 3.

AA_{ABTS} levels in seeds were significantly higher ($p \leq 0.05$) when extracted with aqueous methanol (1.42 g·kg^{-1}), followed by 50% aqueous ethanol (1.30 g·kg^{-1}). The seeds' AA_{ABTS} had a significant ($p \leq 0.05$) correlation with their *TPC* ($R = 0.722$) and *TFC* ($R = 0.776$). The bark's AA_{ABTS} levels were highest when extracted with 50% aqueous methanol (18.23 g·kg^{-1}), followed by 50% aqueous ethanol (10.27 g·kg^{-1}), with a significant ($p < 0.05$) correlation with the *TPC* ($R = 0.798$) and *TFC* ($R = 0.839$). Moreover, the leaves's AA_{ABTS} values were highest when extracted with absolute ethanol (44.95 g·kg^{-1}), followed by 60% aqueous ethanol (33.54 g·kg^{-1}) and 50% aqueous methanol (32.25 g·kg^{-1}). There was a significant ($p \leq 0.05$) correlation with the *TPC* ($R = 0.868$) and *TFC* ($R = 0.728$).

The 100% methanol extract of the seeds (1.36 g·kg^{-1}) had the highest AA_{FRAP} value among the solvents, followed by 50% aqueous methanol (0.76 g·kg^{-1}) and 50% aqueous ethanol (0.71 g·kg^{-1}). The seeds' AA_{FRAP} showed a significant association with its *TPC* ($R = 0.949$; $p \leq 0.01$) and *TFC* ($R = 0.846$; $p \leq 0.05$). In contrast, the bark's AA_{FRAP} was greater in absolute ethanol (5.01 g·kg^{-1}) followed by 60% aqueous ethanol (4.21 g·kg^{-1}), with a significant association with *TPC* ($R = 0.965$; $p \leq 0.01$) and *TFC* ($R = 0.883$; $p \leq 0.05$). The 100% ethanol extract had the highest AA_{FRAP} (13.61 g·kg^{-1}) in leaves, with a significant association with the *TPC* ($R = 0.977$; $p \leq 0.01$) and *TFC* ($R = 0.754$; $p \leq 0.05$).

Phenolic compounds have been shown to exhibit biological activities, including antibacte-

Tab. 2. Antioxidant activities of *Moringa peregrina* leaves, seeds, and bark extracted by different solvents.

Solvents	Seeds	Bark	Leaves
***AA*$_{DPPH}$ [%]			
Water	9.4 ± 0.7 ^{cr}	79.6 ± 0.5 ^{bq}	82.4 ± 0.5 ^{bp}
Ethanol (100%)	15.6 ± 0.1 ^{bq}	79.4 ± 0.2 ^{bp}	78.8 ± 0.3 ^{cp}
Aqueous ethanol (80%)	6.6 ± 0.5 ^{dr}	76.2 ± 0.5 ^{cp}	69.1 ± 0.6 ^{dq}
Aqueous ethanol (60%)	10.1 ± 0.9 ^{cr}	79.0 ± 0.3 ^{bp}	73.0 ± 0.3 ^{cq}
Aqueous ethanol (50%)	5.9 ± 0.8 ^{dr}	89.5 ± 0.3 ^{aq}	91.6 ± 0.4 ^{ap}
Aqueous ethanol (40%)	11.2 ± 1.0 ^{cr}	71.8 ± 1.3 ^{dp}	67.4 ± 0.5 ^{eq}
Aqueous ethanol (20%)	5.6 ± 0.5 ^{dr}	69.8 ± 0.6 ^{dp}	68.0 ± 0.8 ^{eq}
Methanol (100%)	11.2 ± 0.9 ^{cr}	78.9 ± 0.3 ^{bq}	83.8 ± 0.2 ^{bp}
Aqueous methanol (50%)	19.3 ± 0.5 ^{ar}	90.7 ± 0.3 ^{ap}	90.9 ± 2.1 ^{ap}
***AA*$_{ABTS}$ [g·kg^{-1}]			
Water	0.07 ± 0.01 ^{cr}	4.14 ± 0.05 ^{eq}	10.85 ± 0.15 ^{ep}
Ethanol (100%)	0.05 ± 0.01 ^{cr}	9.08 ± 0.12 ^{cq}	44.95 ± 0.41 ^{ap}
Aqueous ethanol (80%)	0.02 ± 0.00 ^{dr}	4.69 ± 0.08 ^{eq}	28.51 ± 1.11 ^{cp}
Aqueous ethanol (60%)	0.04 ± 0.00 ^{cr}	7.09 ± 0.21 ^{dq}	33.54 ± 0.21 ^{bp}
Aqueous ethanol (50%)	1.30 ± 0.17 ^{ar}	10.27 ± 0.29 ^{bq}	30.74 ± 0.52 ^{bp}
Aqueous ethanol (40%)	0.02 ± 0.01 ^{dr}	5.04 ± 0.09 ^{eq}	19.93 ± 0.68 ^{dp}
Aqueous ethanol (20%)	0.05 ± 0.01 ^{cr}	1.33 ± 0.01 ^{fq}	0.09 ± 0.01 ^{ep}
Methanol (100%)	0.63 ± 0.54 ^{br}	2.48 ± 0.02 ^{eq}	10.75 ± 0.09 ^{dp}
Aqueous methanol (50%)	1.42 ± 0.14 ^{ar}	18.23 ± 0.12 ^{aq}	32.25 ± 0.42 ^{bp}
***AA*$_{FRAP}$ [g·kg^{-1}]			
Water	0.07 ± 0.01 ^{cr}	1.32 ± 0.02 ^{eq}	7.62 ± 0.31 ^{ep}
Ethanol (100%)	0.07 ± 0.01 ^{cr}	5.01 ± 0.02 ^{aq}	13.61 ± 0.25 ^{ap}
Aqueous ethanol (80%)	0.04 ± 0.01 ^{dr}	2.58 ± 0.07 ^{dq}	9.11 ± 0.51 ^{cp}
Aqueous ethanol (60%)	0.04 ± 0.00 ^{dr}	4.21 ± 0.12 ^{bq}	9.95 ± 0.03 ^{cp}
Aqueous ethanol (50%)	0.71 ± 0.09 ^{br}	3.48 ± 0.14 ^{cq}	9.36 ± 0.37 ^{cp}
Aqueous ethanol (40%)	0.06 ± 0.00 ^{cr}	3.74 ± 0.01 ^{cq}	11.34 ± 0.23 ^{bp}
Aqueous ethanol (20%)	0.03 ± 0.00 ^{dq}	0.93 ± 0.06 ^{fp}	0.74 ± 0.01 ^{fp}
Methanol (100%)	1.36 ± 0.54 ^{ar}	2.16 ± 0.07 ^{dq}	6.92 ± 0.48 ^{ep}
Aqueous methanol (50%)	0.76 ± 0.08 ^{br}	3.56 ± 0.13 ^{cq}	8.69 ± 0.13 ^{dp}

Values are means ± standard deviation of triplicates. Mean values in a column with different superscript letters (a–f) significantly differ at a level of $p \leq 0.05$. The superscript letters (p, q, and r) indicate the significant difference between plant parts in the rows at a level of $p \leq 0.05$.
AA$_{DPPH}$ – antioxidant activity determined by DPPH assay, *AA*$_{ABTS}$ – antioxidant activity determined by ABTS assay (expressed as grams of Trolox equivalents), *AA*$_{FRAP}$ – antioxidant activity determined as ferric reducing antioxidant power assay (expressed as grams of Trolox equivalents).

Tab. 3. Pearson correlation *R* between total phenolics, total flavonoids, and antioxidant activities.

Plant part	AA$_{DPPH}$	AA$_{ABTS}$	AA$_{FRAP}$
Total polyphenol content			
Seeds	0.934 **	0.722 *	0.949 **
Bark	0.985 **	0.798 *	0.965 **
Leaves	0.989 **	0.868 *	0.977 **
Total flavonoid content			
Seeds	0.758 *	0.776 *	0.846 *
Bark	0.949 **	0.839 *	0.883 *
Leaves	0.786 *	0.728 *	0.754 *

* – $p \leq 0.05$, ** – $p \leq 0.01$
AA$_{DPPH}$ – antioxidant activity determined by DPPH assay, *AA*$_{ABTS}$ – antioxidant activity determined by ABTS assay, *AA*$_{FRAP}$ – antioxidant activity determined as ferric reducing antioxidant power.

rial, anti-inflammatory, antioxidant, antidiabetic, and anticancer effects [25]. According to Tohma et al. [26], the reduced properties of phenolic compounds, which enable them to function as metal chelators and absorb and neutralise free radicals, are primarily responsible for their antioxidant action. Among plant secondary metabolites, flavonoids and tannins are considered the most promising polyphenolic compounds [27]. Using DPPH, ABTS, and FRAP tests, the antioxidant activity of MP parts extracts was investigated. Among the plant secondary metabolites identified in crude extracts of MP parts are flavonoids and polyphenols.

The difference in antioxidant activity amongst MP parts may be explained by the fact that leaves and bark had significantly higher *TPC* and *TFC* contents than seeds. Additionally, there was a strong correlation ($R > 0.7$) between the antioxidant activity of MP parts *TPC* and *TFC*. Compared to other solvents, the antioxidant activity of aqueous organic solvent extracts was significantly higher. This is mainly because polar solvents are frequently used to extract polyphenols from plant matrices; the best solvents for this purpose include aqueous mixtures comprising ethanol and acetone [28]. Gonfa et al. [21] reported that high-molecular-weight phenolic complexes can form during the extraction of phenolic compounds in methanol. Furthermore, the methanol extract showed the highest polyphenol content and antioxidant activity among the vegetable waste extracts, compared with ethanol and aqueous extracts.

The current study supports the findings of a previous study [29], which showed that methanol and ethanol are efficient in extracting bioactive compounds. The results showed that aqueous organic solvents performed better in terms of extraction efficiency than pure solvents or water, with aqueous solvents exhibiting the best extraction performance ($p < 0.05$). However, it is challenging to find a single solvent that can extract all phenolic compounds. Antioxidant activity and *TPC* in wild vegetables were found to be significantly associated. Carbonell-Capella et al. [30] discovered a high association between *TPC* and antioxidant activity in stevia-containing beverages that underwent in vitro digestion. This finding is consistent with previous research demonstrating a strong link between polyphenolic compounds and antioxidant activity. Similarly, Chen et al. [31] found a strong relationship between antioxidant activity and *TPC* in fruits during germination. Muflihah et al. [32] reported that Pearson's correlation analysis revealed that observed *TPC* and *TFC* were significant contributors to antioxidant activity, suggesting that these molecules play a crucial role in determining antioxidant capacity.

GC-MS profile

In the studies presented above, ethanol extracts yielded higher *TPC* and *TFC* levels than those obtained with methanol or water. As a result, the ethanol extracts of the MP parts were analysed using GC-MS. The parts of MP contained 14, 8, and 9 phytochemical substances, as listed in Tab. 4–6, respectively, together with their chemical compositions. Fig. 1–3 depict the total ion chromatograms of the extracts.

As shown, the phytochemicals in the seed extract (Tab. 4) accounted for 94.5 % of the overall peak area, whereas those in the leaf and bark extracts accounted for 99.9 % and 99.0 %, respectively. D-Allose, with a peak area percentage of 44.6 %, was the most abundant component in the seed extract, followed by benzeneacetonitrile, 4-hydroxy- (17.8 %) and 5-(hydroxyl-methyl)-2-furancarboxaldehyde (8.8 %).

The major phytochemical compounds of the MP bark extract (Tab. 5) were triterpenoid sterols, 25-vinyl-27-norcholesta-5,24(*Z*)-dien-3β-ol (34.2 %), and 9,19-cyclolanost-23-ene-3,25-diol, 3-acetate, (3β,23*E*)- (33.4 %), followed by isopropyl isothiocyanate (15.1 %) and nonanoic acid (6.2 %) .

The predominant phytochemical components in the leaf extract (Tab. 6) were isopropyl isothiocyanate (50.9 %), D-allose (23.3 %), (*Z*)-valerenyl acetate (12.6 %), and 4-hydroxy-2,5-dimethyl-furan-3(H)-one (4.5 %).

Concisely, the data showed that the seed was primarily composed of sugar derivatives (57.2 %), aryl aldehydes (12.2 %), and other organic compounds.

Because location and climate significantly influence the composition of plants' primary and secondary metabolites, we predicted that MP plants grown in Saudi Arabia would exhibit biological activities distinct from those grown in other regions [10]. The mass spectrum was compared to the database library, and a substantial percentage of the matches were positive, as was the complex structure. This research will aid in identifying substances that can be used in body products, medications, and pharmaceuticals and that have therapeutic value. For example, isopropyl isothiocyanate, found in reasonable percentages in leaf (50.9 %), bark (15.1 %), and seed (5.6 %) extracts, along with other isothiocyanate compounds, has been reported to inhibit bacterial growth and the lytic development of *stx*-harbouring prophages

Tab. 4. List of chemical compounds detected in the ethanol extract from seeds of *Moringa peregrina* by the GC-MS analysis.

Compound	Chemical formula	*MW* [g·mol^{-1}]	*RT* [min]	Area [%]
Pyrrolidinecarboxamide	$C_5H_{10}N_2O$	114.15	4.34	0.7
Isopropyl isothiocyanate	C_4H_7NS	101.17	4.68	5.6
2-Furancarboxaldehyde (furfural)	$C_5H_4O_2$	96.08	4.77	1.8
5-Methyl furfural	$C_6H_6O_2$	110.11	6.80	1.0
3-Acetyldihydro-2(3H)-furanone	$C_6H_8O_3$	128.13	7.38	0.6
Isosorbide dinitrate	$C_6H_8N_2O_8$	236.14	8.77	0.1
Levoglucosenone	$C_6H_6O_3$	126.11	9.22	1.3
α-L-Galactopyranose	$C_6H_{12}O_6$	180.16	9.41	0.9
2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	$C_6H_8O_4$	144.12	9.83	0.5
1,4:3,6-Dianhydro-α-D-glucopyranose	$C_6H_8O_4$	144.12	10.88	2.4
5-(Hydroxymethyl)-2-furancarboxaldehyde	$C_6H_6O_3$	126.11	11.14	8.8
D-Allose	$C_6H_{12}O_6$	180.16	15.07	44.6
Benzeneacetonitrile, 4-hydroxy-	C_8H_7NO	133.15	14.66	17.8
1,6-Anhydro-β-D-glucofuranose	$C_6H_{10}O_5$	162.14	16.34	7.9

MW – molecular weigh, *RT* – retention time.

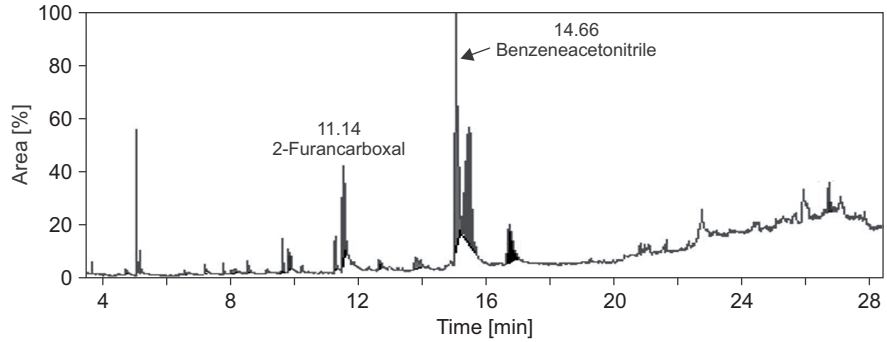

Fig. 1. Total ionic chromatogram of the extract from seeds of *Moringa peregrina*.

[33]. Isothiocyanates, such as phenethyl isothiocyanate and benzyl isothiocyanate, have been shown in animal studies to prevent or lessen the risk of cancer caused by carcinogens. However, D-allose, an uncommon aldohexose, was detected at high levels in seed (44.6 %) and leaf (23.3 %) extracts. It was thought to have an inhibiting effect on human ovarian cancer cells [34].

Despite its relatively low quantity in MP seeds and leaves, the unsaturated cyclic chemical molecule 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, a result of the sugar-amino Maillard reaction, has high antioxidant activity [35]. As previously stated, the seeds contained significant levels of aryl aldehydes (12.2 %), primarily 2-furan-carboxaldehyde (furfural, 1.8 %), 5-methyl furfural (1.0 %), and 5-(hydroxylmethyl)-2-furancarboxaldehyde (8.8 %). Furfural has been shown to be carcinogenic in laboratory animals and mutagenic in single-cell organisms; however, there is no evidence on human subjects [36]. Therefore, excessive ingestion of the plant's seeds may be harmful. The seed also contained 1.3 % levoglucosenone. Several studies have evaluated the cytotoxic effects of 4-substituted levoglucosenone derivatives, such as dihydrolevoglucosenone derivatives, levoglucosenone-derived isoxazolidines, and 4-sulphurated levoglucosenone compounds, against different tumour cell lines [37].

Moringa's bark and leaves contain a large number of diterpenoid and triterpenoid hydrocarbons and alcohols with known biological activity. Interestingly, the bark of MP contains a nucleoside, cytidine (4.3 %). In addition to its role as a pyrimidine component of RNA, cytidine has been shown to regulate neuronal-glial glutamate cycling, which has sparked interest as a possible glutamatergic antidepressant [38]. *Moringa* species are valued for their high nutritional content and diverse bioactive compounds, supporting their po-

Tab. 5. List of chemical compounds detected in the ethanol extract from bark of *Moringa peregrina* by the GC-MS analysis.

Compound	Chemical formula	*MW* [g·mol^{-1}]	*RT* [min]	Area [%]
Isopropyl isothiocyanate	C_4H_7NS	101.17	4.71	15.1
Cytidine	$C_9H_{13}N_3O_5$	243.20	14.22	4.3
Nonanoic acid	$C_9H_{18}O_2$	158.23	14.90	6.2
9-Octadecenoic acid	$C_{18}H_{34}O_2$	282.47	16.42	0.1
1-Hexyl-2-nitrocyclohexane	$C_{12}H_{23}NO_2$	213.32	20.15	0.1
1,5,9-Trimethyl-12-(1-methylethyl)-4,8,13-cyclotetradecatriene	$C_{20}H_{34}O_2$	306.50	23.10	5.5
9,19-Cyclolanost-23-ene-3,25-diol, 3-acetate, (3β,23*E*)-	$C_{32}H_{52}O_3$	484.00	24.19	33.4
25-Vinyl-27-norcholesta-5,24(*Z*)-dien-3β-ol	$C_{28}H_{44}O$	396.00	25.40	34.2

MW – molecular weigh, *RT* – retention time.

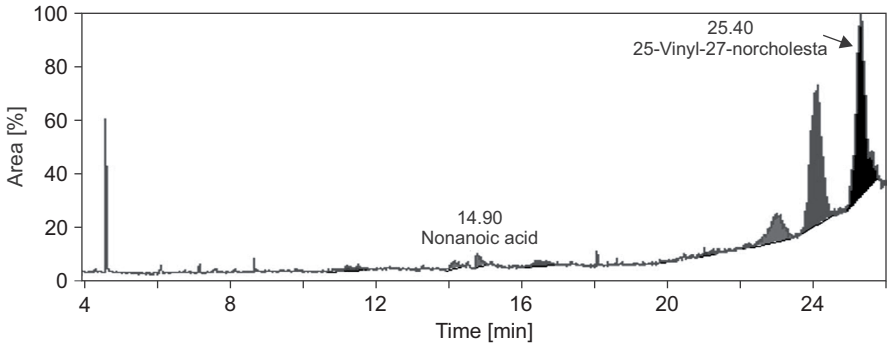

Fig. 2. Total ionic chromatogram of the extract from bark of *Moringa peregrina*.

Tab. 6. List of chemical compounds detected in the ethanol extract from leaves of *Moringa peregrina* by the GC-MS analysis.

Compound	Chemical formula	*MW* [g·mol^{-1}]	*RT* [min]	Area [%]
Isopropyl isothiocyanate	C_4H_7NS	101.17	4.69	50.9
Piperazine adipate	$C_{10}H_{20}N_2O_4$	232.28	6.21	1.4
2-Methyl-2-hexenoic acid	$C_7H_{12}O_2$	128.17	8.19	1.9
4-Hydroxy-2,5-dimethylfuran-3(H)-one	$C_6H_8O_3$	128.13	8.82	4.5
Deoxy-D-arabitol	$C_5H_{12}O_4$	136.15	9.47	0.6
2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	$C_6H_8O_4$	144.12	9.85	3.0
D-Allose	$C_6H_{12}O_6$	180.16	14.89	23.3
Neophytadiene	$C_{20}H_{38}$	278.50	18.19	1.4
(*Z*)-Valerenyl acetate	$C_{17}H_{26}O_2$	262.40	25.84	12.6

MW – molecular weigh, *RT* – retention time.

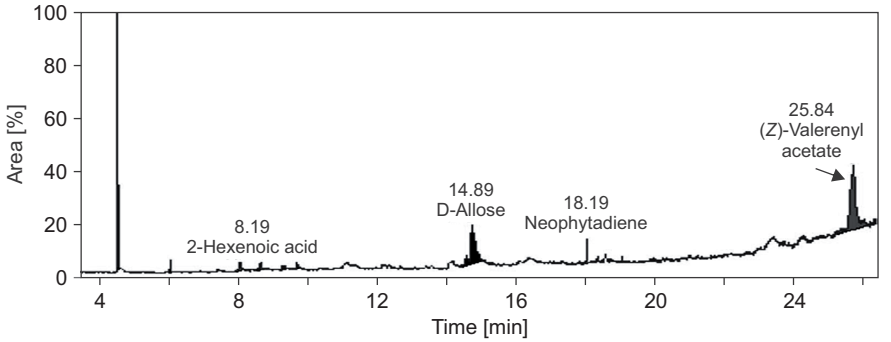

Fig. 3. Total ionic chromatogram of the extract from leaves of *Moringa peregrina*.

tential food and medicinal applications; however, safety, quality, and dosage considerations remain essential when plant materials are used for health-related purposes [3–5].

CONCLUSIONS

The current study discovered that *TPC, TFC* contents and antioxidant activity varied across MP parts. Aqueous methanol (50%) solvent extracts of MP parts contained high phenolic and flavonoid contents, as well as significantly high antioxidant activity. Furthermore, a combination of organic solvents and water may aid in the extraction of all soluble components from both phases. The bark and leaves contained high content of *TPC* and *TFC*, as well as significant antioxidant activity. The antioxidant activity of all components was strongly associated with *TPC* and *TFC*. The current study will undoubtedly help assess the potency of MP bark and leaves as natural antioxidant sources for nutraceutical and functional food applications. Based on this study's findings, it can be concluded that MP parts, particularly leaves and bark powder, contain chemical compounds with pharmacological and nutritional relevance. However, it is recommended that additional research be conducted to isolate and purify the bioactive constituents in MP parts using various extraction solvents, to characterise their molecular structures, formulas, and weights, and to assess their safety (toxicity) for human and other animal use.

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