

Chemical Composition and Oxidative Properties of *Moringa peregrina* Dried-Ground Parts Extracted with Different Solvents

ABSTRACT

Background: *Moringa* species contain extremely high level of antioxidant and low levels of anti-nutritional compounds distributed between different parts of the plant.

Objective: The present study examined the chemical composition, total energy, and oxidative properties of several solvent extracts of *Moringa peregrina* (MP) seeds, bark, and leaves dried-ground.

Methods: The chemical composition, total energy, and mineral content of the MP parts were all determined. The total phenolics, total flavonoids, and antioxidant activity of various solvent extracts were also measured.

Results: The chemical composition differed between parts, with seeds having considerably ($p \leq 0.05$) high levels of protein (19.4%), fat (32.23%), and ash (2.90%). The bark was significantly ($p \leq 0.05$) higher in carbohydrates (76.1%) than the other parts. The leaves had significantly ($p \leq 0.05$) higher levels of Na, Mg, K, Mn, Fe, Zn, and Se. However, the seeds are rich in P and Cu, whereas the bark is rich in calcium. The solvents' efficacy in extracting phenolics and flavonoids from the three parts varied substantially, with aqueous methanol ranking first and the leaves having larger amounts than the other parts. The antioxidant activity was examined using the DPPH, ABTS, and FRAP tests. Leaves had stronger antioxidant activity than other parts. Furthermore, antioxidant activity was strongly associated with total phenolics and flavonoids.

Conclusion: In general, extracts made with aqueous organic solvents produced more total phenolics and flavonoids and had higher antioxidant activity, with leaves demonstrating superior antioxidant properties compared to other parts.

Keywords: *Moringa*, leaves, bark, phenolics, minerals,

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1. INTRODUCTION

Many physiologically active phytochemical compounds are found in plants. Because of safety issues, several of these plant secondary metabolites are valuable sources of natural antioxidants that are favored over synthetic ones [1]. Through a variety of biological

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pathways, the bioactive secondary metabolites have been demonstrated to scavenge free radicals, hence reducing the risk and progression of diseases such as cancer, cardiovascular disease, and neurological disorders [2]. In the arid tropics, Moringa is a wholesome and nutritious food that seems to make it a desirable component for creating sustainable communities [3]. When added as a protein supplement, it enhances the amount of milk produced by goats and ewes that are adapted to hot weather [4]. A nutritional investigation revealed that the high vitamin A concentration of dry Moringa leaf powder compares favorably to that of milk powder in terms of calcium and protein content. Strong antioxidants are also present in the plant's leaves; among these, isothiocyanates stand out for their antibacterial, hypotensive, hypoglycemic, and anticancer qualities [3].

MP is an endangered species of desert tree that is growing in Saudi Arabia and Egypt [5]. The samples of Moringa leaves and seeds had a comparatively high mineral content, particularly calcium. Vitamins C and A are present in the leaves and seeds of MP, with a greater concentration of Vitamin C than A [6]. Patil et al. [7] found that Moringa leaves collected in the hot-wet season had high levels of vitamin A, whereas leaves taken during the cool-dry season had high concentrations of iron and vitamin C. It has also been demonstrated that Moringa contains extremely low levels of anti-nutritional compounds, including oxalate, phytates, tannins, and oligosaccharides [7], this makes Moringa a very promising plant-based source of nutrition.

Many variables affect the extraction of bioactive chemicals from plant material, including solvents, techniques, and extraction times; and for large-scale manufacturing, the ideal extraction methodology should be successful and efficient [8]. The model system used by Al-Dabbas [9], demonstrated that several polar extracts from the leaves and seeds of *M. peregrine* had varying antioxidant properties. These activities are mostly connected to the concentration of flavonoids and phenolic compounds. He also mentioned that *M. peregrina* can prevent undesirable oxidation processes and is thought to be a possible natural antioxidant source. It is possible for different bioactive chemicals with different polarity and chemical properties may not dissolve in a given solvent [8]. Aqueous solutions comprising ethanol, methanol, acetone, and ethyl acetate are the most appropriate of these solvents for recovering polyphenols from a plant matrix. Polar solvents are commonly used for this purpose [10]. The greatest quantities of phenolic chemicals may be extracted from

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flaxseed more successfully using aqueous methanol [10]. MP has the potential to become a significant phytochemical supply in poor nations where malnutrition and famine pose serious issues. Thus, the purpose of this study is to examine the chemical composition and oxidative characteristics of *Moringa peregrina* parts extracted using various solvents.

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2. MATERIALS AND METHODS

2.1. Materials

Moringa peregrina tree parts were collected from an agricultural farm in Khartoum, Sudan, during the 2019-2020 seasons. The leaves, bark, and seeds were collected manually and cleaned of debris and dirt. The parts were air-dried and ground to pass through a 0.1 mm mesh and kept at 4°C until use. All chemicals and reagents used in this study were of analytical grade.

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Comment [Dp10]: Avoid repetition of the sentences, make it like this "*Moringa peregrina* tree parts, including leaves, bark, and seeds, were collected manually from an agricultural farm in Khartoum, Sudan, during the 2019-2020 seasons and cleaned of debris and dirt".

2.2. Chemical composition and total energy determination

Determining the chemical composition and overall energy

The MP parts were freeze-dried to a consistent weight at 105°C to measure their moisture content. The AOAC [11] standard procedures were utilized to assess crude protein, fat, and ash. Carbohydrate (nitrogen-free extract) was calculated by difference. The Atwater factors were used to determine the energy following Osborne and Voogt's [12] description: 1 gram of carbohydrates offers 4 kcal, 1 gram of protein provides 4 kcal, and 1 gram of fat provides 9 kcal.

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2.3. Minerals analysis

According to Amaglo et al. [13], the material was dissolved in a solution of HNO₃ and HCl (1:1). After standing for 72 hours at 24°C, the mixture was filtered through a Millipore vacuum filter (0.45µm). Ultrapure water was used to dilute the filtrate 1:4. The method used for the analysis was ionic liquid chromatography. An electrolytic self-regenerating system, a conductivity detector, and a quaternary gradient pump were installed in the HPLC. Methane-sulfonic acid (25 mmol/L) was used as the elution solvent, with a flow rate of 1.0 ml/min. Analytical purity commercial solutions were used to create the standard dilution for the calibrations, which was then dried at 140°C for four hours and re-suspended in 0.01 mol/L of each HNO₃:HCl (1:1). Mineral levels were expressed as milligrams per kilogram of dry plant matter.

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2.4. Extract preparation

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About 20 g 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 aqueous acetone, for six hours at ambient temperature in an orbital shaker (Gallenkamp, UK). Whatman No. 1 filter paper was used to separate the extracts from the residues. Using the same new solvent, the precipitates were extracted once again and then combined. By using a rotary evaporator (EYELA, SB-651, Rikakikai Co. Ltd. Tokyo, Japan), the combined extracts were concentrated and solvent-free at 45 °C under reduced pressure.

2.5. Determination of total phenolics (TPC)

The Folin-Ciocalteu method, as outlined by Chaovanalikit and Wrolstad [14] was utilized to assess the TPC. About 400 µl of Folin-Ciocalteu reagent was combined with 200 µl of the extract. The control experiment involved the use of methanol. Deionized water was used to dilute the solution to a total volume of 4.6 ml, and it was then well mixed. Exactly 1 ml of a 20% Na₂CO₃ solution was added, mixed right away, and allowed to incubate for two hours after being let to stand at room temperature for ten minutes. Utilizing a spectrophotometer (PD-UV, Apel, Saitama, Japan), the absorbance was measured at 765 nm. The standard employed was 1 mg/ml of gallic acid, and the TPC of the samples was measured in mg GAE/100-gram dry weight. Every measurement was done three times, and the presented values were means ± SD.

2.6. Determination of total flavonoid content (TFC)

The Kim *et al.* [15] method was applied to calculate the TFC of the extract. A mixture consisting of 1 mL of methanolic extract, 300 µL of 5% NaNO₂ solution, and 300 µL of 10% aluminum chloride was incubated for 5 minutes at 25°C. Next, 2 mL of 1 mol/L sodium hydroxide was added. After adding H₂O to bring the volume to 10 mL, the solution was well vortexed. At 510 nm, the absorbance was measured. Various catechin concentrations were used to create a calibration curve (R² = 0.974). A milligram of catechin equivalents (CE) per gram of sample (DW) was the unit of measurement for TFC.

2.7. Antioxidant activity determination

2.7.1. 2, 2-diphenyl-1-picrylhydrazyl (DPPH)

The DPPH of the extracts was calculated using Lee *et al.*'s [16] technique. After diluting the extracts with methanol, the DPPH reagent (2 mL) and 1 mL of each extract were

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thoroughly combined with a vortex mixer. The control substance was methanol. Mixtures' absorbance at 518 nm was measured using a spectrophotometer (PD-303UV spectrophotometer, Apel, Saitama, Japan). The DPPH was estimated with the following formula:

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

Where: A is the observed absorbance.

2.7.2. 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS)

The ABTS was estimated using the Re et al. [17] technique. Aqueous solutions of 7 mM ABTS and 2.4 mM potassium persulfate were reacted for 12–16 hours at room temperature in the dark to generate ABTS. Before the experiment, this solution was diluted in ethanol (1:89 v/v) and allowed to equilibrate at 30°C, with a result of an absorbance of 0.700 ± 0.02 at 734 nm, as measured spectrophotometrically (Mod. 4050, Biochrom, Cambridge, UK).

Thirty minutes later at thirty degrees Celsius, the absorbance was measured exactly after 10 µl of the test sample in ethanol was mixed with 1 ml of diluted ABTS solution. The inhibition percentage for the blank absorbance at 734 nm was computed. The Trolox equivalents per gram of sample, or micromoles of TE/g, were used to quantify the radical scavenging activity.

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2.7.3. Ferric reducing antioxidant power (FRAP)

FRAP was calculated using the method described by Yen and Duh [18]. In a 10 mL test tube, 0.5 mL of methanol extract (diluted ten times) and 2 mL of FRAP working solution were combined. The combination was then diluted to 10 mL with distilled water. Twenty minutes were spent with the mixture in the dark. At 593 nm, the absorbance of the remaining FRAP solution was measured using a spectrophotometer (PD-303UV spectrophotometer, Apel, Saitama, Japan) against a blank. The findings were expressed using Trolox equivalents, or micromoles per gram of material (µmol TE/g).

2.8. Statistical analyses

The statistical analysis system, SAS program, 2000 (SAS Institute Inc., Cary, NC, USA) was used for the statistical analysis. Using the LSD test, significant variances between sample means were identified. A difference was deemed significant when it was $P \leq 0.05$. The association between the total phenolic and flavonoid contents and the antioxidant activity of the extracts was examined using Pearson correlation analysis.

3. RESULTS AND DISCUSSION

3.1. Chemical composition, minerals, and total energy

Table 1 lists the chemical composition of the MP leaves, bark, and seeds. In comparison to other parts, the seeds have significantly ($p \leq 0.05$) higher levels of fat (32.23%), protein (19.40%), ash (2.91%), and total energy (527.55 Kcal/mol). According to the MP mineral data (Table 1), the leaves exhibited significantly ($p \leq 0.05$) greater concentrations of Na (1102 mg/kg), Mg (4258 mg/kg), K (8575 mg/kg), Mn (36.22 mg/kg), Fe (519 mg/kg), Zn (115.01 mg/kg), and Se (11.52 mg/kg). But whilst the bark is significantly ($p \leq 0.05$) rich in Ca (9634 mg/kg), the seeds are rich in P (6682 mg/kg) and Cu (25 mg/kg). The chemical composition of MP revealed that seeds included substantially more protein, fat, ash, and total calories than leaves and bark. Furthermore, the leaves of MP had much more major and minor minerals than the other parts. The chemical composition of the seeds was comparable to that reported for MP by Al-Dabbas et al. [19] and that observed for MP leaves [20]. The findings for the mineral composition of the leaves were similar to those reported by Al-Juhaimi et al. [21], Babiker et al. [20], and Hassanein [22].

3.2. Effects of solvent extraction on the total phenolic and flavonoid contents of MP parts

Table 2 displays the results of the total phenolic contents (TPC) and flavonoids (TFC) of plant parts utilizing various solvent systems. Among the plant parts, absolute acetone extract of MP seeds gave significantly ($p \leq 0.05$) high TPC (39.79 mg GAE/g DW), aqueous methanol extract showed significantly ($p \leq 0.05$) high TPC in the bark (205.45 mg GAE/g DW), and aqueous ethanol (50%) gave significantly ($p \leq 0.05$) higher TPC in leaf extract (392.39 mg GAE/g DW), followed by aqueous methanol (350.69 mg GAE/g DW). The leaf extracts had significantly ($p \leq 0.05$) higher TPC than other parts when using all solvents except 100% ethanol, which extracted significantly ($p \leq 0.05$) more TPC from the bark. The absolute acetone extract of MP seeds extracted considerably ($p \leq 0.05$) high TFC (4.34 mg Catechin/g DW), whereas the aqueous methanol extract showed significantly ($p \leq 0.05$) high TFC in the bark (5.16 mg Catechin/g DW) and leaves (13.48 mg Catechin/g DW). When utilizing all solvents, leaf extracts contained significantly ($p \leq 0.05$) more TFC than other parts, except for 100% ethanol, which extracted significantly ($p \leq 0.05$) more from the bark. TPC and TFC concentrations differed significantly ($P \leq 0.05$) amongst MP

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parts extracted using different solvents. The current study's findings demonstrated that in comparison to other solvent extracts, aqueous methanol, and aqueous ethanol had significantly ($p \leq 0.05$) higher TPC and TFC in the leaves and **park** extracts, while absolute acetone had a significantly ($p \leq 0.05$) higher TPC and TFC the seeds' extract. This may be the result of the increased extraction rates of phenolics and flavonoids in more polar solvents, like aqueous methanol/ethanol, as compared to absolute methanol/ethanol. This is because different antioxidant molecules have different chemical properties and polarities, and they might or might not dissolve in a particular solvent [23].

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According to Mehmood et al. [24], solvent composition and polarity significantly influenced the extraction of phenols and antioxidants. Due to the interactions (hydrogen bonds) between the polar sites of antioxidant compounds and solvents, validation extractions of these compounds were performed more often in polar solvents, which were more efficient than in non-polar solvents. The application of aqueous ethanol/methanol resulted in MP fractions that were rich in TPC and TFC, as well as antioxidant activity. Aqueous methanol was the best solvent for extracting MP components, particularly leaves, followed by aqueous ethanol, according to TPC, TFC, and antioxidant activity data. This could be owing to phenolic chemicals' high solubility in ethanol and methanol [25]. On the other hand, Rajhi et al. [26] discovered that the flavonoid concentration of **Capparis spinosa** leaves is highest in organic solvents and lowest in aqueous extraction. According to Dhingra et al. [27], the liquid-liquid extraction method can dilute or boost phenolic compounds in the crude extract, and solvent polarity has a major impact on extract yields [28].

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AlMousa et al. [29] discovered that methanol extract contained more TPC with high antioxidant and antimicrobial activity than the other solvents. Although total phenolics (TPC) in MP parts were found in the order leaves > bark > seeds, Nantongo et al. [30] investigated the variability of phenolic and alkaloid content in different plant parts of **Carissa edulis** and found no difference in phenol abundance between the plant parts. However, for sustainability, leaves are recommended as a replacement form of medicine instead of the preferred root or stem bark. In addition, Al-Owaisi et al. [31] revealed that MP leaves contain a high concentration of phenolic chemicals, which can play an important role in preventing the advancement of numerous diseases. Furthermore, they

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found that methanol, the most polar extract, had the highest total phenol and flavonoid concentration compared to ethyl acetate/chloroform extracts [31]. In contrast to our findings, Al-Dabbas [9] identified significant levels of phenolics, flavonoids, and flavonol in all extracts, whether from leaves or seeds.

3.3. Effects of solvent extraction on antioxidant activity of MP parts and correlation with TPC and TFC

The antioxidant activity of MP parts antioxidants was measured in the current study using several antioxidant assays, including DPPH, ABTS, and FRAP, as indicated in Table 3. As shown, acetone extract had the highest percentage of DPPH scavenging in the seeds (14.62%), followed by ethanol (12.64%). These percentages had a good positive correlation with TPC ($R = 0.923$; Figures 1) and TFC ($R = 0.747$; Figures 2). When compared to other solvents, aqueous methanol extracts produced noticeably higher DPPH (90.71%) in the bark, followed by aqueous acetone (90.66%). There was a positive correlation between the bark's DPPH and TPC ($R = 0.974$; Figures 1) and TFC ($R = 0.929$; Figures 2). The leaves DPPH levels were greater when extracted with aqueous ethanol (91.45%), followed by aqueous methanol (90.85%), and were shown to be favorably linked with TPC ($R = 0.986$; Figures 1) and TFC ($R = 0.789$; Figures 2). The ABTS of the bark (18.23 g Trolox/g sample) and leaves (32.25 g Trolox/g sample) were greater in aqueous methanol extracts, whereas the seeds (2.71 g Trolox/g sample) were higher in acetone extracts. The parts' ABTS was positively linked with TCP ($R = 0.701, 0.798$, and 0.862 for the seeds, bark, and leaves, respectively; Figure 1) and TFC ($R = 0.765, 0.827$, and 0.707 for the seeds, bark, and leaves, respectively; Figure 2). The FRAP value of the acetone extract of the seeds (2.61 g Trolox/g sample) was higher than that of the other solvents, whereas that of the bark was higher in aqueous methanol (3.54 g Trolox/g sample) and aqueous acetone (3.53 g Trolox/g sample). However, the aqueous ethanol extract produced the highest TRAP (9.16 g Trolox/g sample) in leaves. The parts' FRAP was favorably linked with the TCP ($R = 0.928, 0.961$, and 0.970 for the seeds, bark, and leaves, respectively; Figure 1) and TFC ($R = 0.834, 0.871$, and 0.721 for the seeds, bark, and leaves, respectively; Figure 2).

Some investigations on phenolic compounds have demonstrated their potential biological activity, including antioxidant, antidiabetic, anti-inflammatory, antibacterial, and

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anticancer properties [32,33]. The antioxidant activity of phenolic compounds is mostly due to their reduced characteristics, which enable them to act as metal chelators and absorb and neutralize free radicals [34]. Flavonoids and tannins are regarded as the most promising polyphenolic chemicals among plant secondary metabolites [35]. The antioxidant activity of such extracts was examined using DPPH, ABTS, and FRAP assays. Polyphenols and flavonoids are among the plant secondary metabolites found in crude extracts of MP components. These bioactive compounds can discolor DPPH solution due to their hydrogen-donating activity [36]. The bark and leaf extracts had much stronger antioxidant activity than the seeds. This variance in antioxidant activity amongst MP components could be attributed to the fact that both bark and leaves had much greater TPC and TFC levels than seeds. Furthermore, the antioxidant activity of MP sections was highly linked with TPC and TFC ($R > 7$). The antioxidant activity of aqueous organic solvent extracts was much higher than that of other solvents. This is primarily because polar solvents are widely employed to extract polyphenols from plant matrices, with aqueous combinations containing ethanol, methanol, acetone, and ethyl acetate being the most appropriate solvents [37]. According to Gonfaet al.[25], when phenolic chemicals are extracted in methanol, high molecular weight phenolic complexes can develop. Furthermore, as compared to ethanol and aqueous extracts, methanol extract had the highest polyphenol concentration as well as antioxidant activity in vegetable waste [38]. The present finding supports a prior study that demonstrated methanol and ethanol to be effective at extracting bioactive chemicals [39]. Aqueous solvents had the highest extraction efficiency ($p \leq 0.05$), indicating that aqueous organic solvents outperformed pure solvents or water in terms of extraction efficiency. The majority of the phenolic components of plant samples can be extracted using a mixture of organic solvent and water, and the more polar the solvent, the greater the antioxidant activity of the extract [9]. However, it is difficult to obtain a single solvent capable of extracting all phenolic compounds. Antioxidant activity and total phenolic content in wild vegetables showed a significant association [40].

Carbonell-Capella et al. [41] found a strong correlation between the total phenolic content and antioxidant activity in beverages that contained stevia and were subjected to *in vitro* digestion. This finding is in line with other research that also found a strong correlation

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between polyphenolic chemicals and antioxidant activity. Similarly, Chen et al. [42] found a high correlation between antioxidant activity and TPC fruits during germination. These findings are consistent with those of Asem et al. [43], who discovered a substantial association between propolis' total phenolic and flavonoid content and its antioxidant capabilities. Muflihah et al. [44] demonstrated that Pearson correlation analysis revealed that the detected TPC and TFC were major contributors to antioxidant activity, showing that these molecules play an important role in antioxidant capacity.

4. CONCLUSIONS

The current investigation revealed that the MP parts differed in terms of chemical composition and mineral content. Aqueous organic solvent (50%) extracts of MP parts had stronger antioxidant activity and phenolic and flavonoid concentrations. Furthermore, this could be due to the combination of organic solvent and water, which makes it easier to extract all soluble components in both water and organic solvents. The bark and leaves included high levels of TPC and TFC, as well as considerable antioxidant activity. The antioxidant activity of all components was highly associated with TPC and TFC. The current research will surely help determine the potency of MP bark and leaves as prospective sources of protein, minerals, and natural antioxidants for nutraceutical and functional food applications.

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REFERENCES

1. van Wyk AS, Prinsloo G. Health, safety and quality concerns of plant-based traditional medicines and herbal remedies. *South African Journal of Botany*, 2020; 133, 54-62.
2. Karthikeyan A, Joseph A, Nair BG. Promising bioactive compounds from the marine environment and their potential effects on various diseases. *Journal of Genetic Engineering and Biotechnology*, 2022; 20(1), 14.
3. Olson ME, Sankaran RP, Fahey JW, Grusak MA, Odee D, Nouman W. Leaf protein and mineral concentrations across the “Miracle Tree” genus *Moringa*. *PloS one*, 2016; 11(7), e0159782.

4. Babiker EE, Juhaimi FA, Ghafoor K, Abdoun KA. Comparative study on feeding value of Moringa leaves as a partial replacement for alfalfa hay in ewes and goats. *Livestock Science*, 2017; 195, 21-26.
5. Farahat EA, Refaat AM. Predicting the impacts of climate change on the distribution of Moringa peregrina (Forssk.) Fiori-A conservation approach. *Journal of Mountain Science*, 2021; 18(5), 1235-1245.
6. Asghari G, Palizban A, Bakhshaei B. Quantitative analysis of the nutritional components in leaves and seeds of the Persian Moringa peregrina (Forssk.) Fiori. *Pharmacognosy Research*, 2015; 7(3), 242.
7. Patil SV, Mohite BV, Marathe KR, Salunkhe NS, Marathe V, Patil VS. Moringa tree, gift of nature: a review on nutritional and industrial potential. *Current Pharmacology Reports*, 8(4), 2022; 262-280.
8. Jha AK, Sit N. Extraction of bioactive compounds from plant materials using combination of various novel methods: A review. *Trends in Food Science and Technology*, 2022; 119, 579-591.
9. Al-Dabbas MM. Antioxidant activity of different extracts from the aerial part of Moringa peregrina (Forssk.) Fiori, from Jordan. *Pakistan J of pharmaceutical sciences*, 2017; 30(6).
10. Wu Y, Gao H, Wang Y, Peng Z, Guo Z, Ma Y, Zhong Q. Effects of different extraction methods on contents, profiles, and antioxidant abilities of free and bound phenolics of Sargassum polycystum from the South China Sea. *Journal of Food Science*, 2022; 87(3), 968-981.
11. AOAC. Official Methods of Analysis. (18th ed.). Association of Official Analytical Chemists, Arlington, VA, USA, 2006.
12. Osborne DR, Voogt P. Calculation of caloric value. In: *Analysis of nutrients in foods*. New York, Academic Press, 1978; pp: 23-34
13. Amaglo NK, Bennett RN, Lo Curto RB, Rosa EA, Lo Turco V, Giuffrida A, Timpo GM. Profiling selected phytochemicals and nutrients in different tissues of the multipurpose tree Moringa oleifera L., grown in Ghana. *Food Chemistry*, 2010; 122, 1047-1054.

14. Chaovanalikit A, Wrolstad RE. Total anthocyanins and total phenolics of fresh and processed cherries and their antioxidant properties. *Journal of Food Science*, 2004; 69, 67-72.
15. Kim DO, Jeong SW, Lee CY. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chemistry*, 2003; 81(3), 321-326.
16. Lee SJ, Yoon BD, Oh HM. Rapid method for the determination of lipid from the green alga *Botryococcus braunii*. *Biotechnology Techniques*, 1998; 12(7), 553-556.
17. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, 1999; 26, 1231-1237.
18. Yen GC, Duh PD. Antioxidative properties of methanolic extracts from peanut hulls. *Journal of the American Oil Chemists' Society*, 1993; 70(4), 383-386.
19. Al-Dabbas MM, Ahmad RA, Ajo RY, Abulaila KH, Akash MU, Al-Ismail KH. Chemical composition and oil components in seeds of *Moringa peregrina* (Forssk) Fiori. *Crop Research*, 2010; 40 (1), 2.
20. Babiker EE, Juhaimi FA, Ghafoor K, Abdoun KA. Effect of drying methods on nutritional quality of young shoots and leaves of two *Moringa* species as non-conventional fodders. *Agroforestry Systems*, 2018; 92(3), 717-729.
21. Al-Juhaimi FA, Ghafoor K, Ahmed IM, Babiker EE, Özcan MM. Comparative study of mineral and oxidative status of *Sonchus oleraceus*, *Moringa oleifera* and *Moringa peregrina* leaves. *Journal of Food Measurement and Characterization*, 2017; 11(4), 1745-1751.
22. Hassanein AMA. Nutritional, chemical and molecular characterisation of *Moringa oleifera* Lam. and *Moringa peregrina* (Forssk.) Fiori genotypes. *The Journal of Horticultural Science and Biotechnology*, 2018; 93(5), 500-509.
23. Ali A, Chua BL, Chow YH. An insight into the extraction and fractionation technologies of the essential oils and bioactive compounds in *Rosmarinus officinalis* L.: past, present and future. *TrAC Trends in Analytical Chemistry*, 2019; 118, 338-351.
24. Mehmood A, Javid S, Khan MF, Ahmad KS, Mustafa A. In vitro total phenolics, total flavonoids, antioxidant and antibacterial activities of selected medicinal plants using different solvent systems. *BMC chemistry*, 2022; 16(1), 64.

25. Gonfa T, Teketle S, Kiros T. Effect of extraction solvent on qualitative and quantitative analysis of major phyto-constituents and in-vitro antioxidant activity evaluation of *Cadaba rotundifolia* Forssk leaf extracts. *Cogent Food & Agriculture*, 2020; 6(1), 1853867.
26. Rajhi I, Hernandez-Ramos F, Abderrabba M, Ben Dhia MT, Ayadi S, Labidi J. Antioxidant, Antifungal and Phytochemical Investigations of *Capparis spinosa* L. *Agriculture*, 2021; 11(10), 1025.
27. Dhingra N, Kar A, Sharma R, Bhasin S. In-vitro antioxidative potential of different fractions from *Prunus dulcis* seeds: Vis a vis antiproliferative and antibacterial activities of active compounds. *South African Journal of Botany*, 2017; 108, 184-192.
28. Ouerghemmi S, Sebei H, Siracusa L, Ruberto G, Saija A, Cimino F, Cristani M. Comparative study of phenolic composition and antioxidant activity of leaf extracts from three wild *Rosa* species grown in different Tunisia regions: *Rosa canina* L., *Rosa moschata* Herrm. and *Rosa sempervirens* L. *Industrial Crops and Products*, 2016; 94, 167-177.
29. AlMousa LA, AlFaris NA, Alshammari GM, ALTamimi JZ, Alsyadi MM, Alagal RI, Yahya MA. Antioxidant and antimicrobial potential of two extracts from *Capparis spinosa* L. and *Rumex nervosus* and molecular docking investigation of selected major compounds. *Saudi Journal of Biological Sciences*, 2022; 29(8), 103346.
30. Nantongo JS, Odoi JB, Abigaba G, Gwali S. Variability of phenolic and alkaloid content in different plant parts of *Carissa edulis* Vahl and *Zanthoxylum chalybeum* Engl. *BMC Research Notes*, 2018; 11(1), 1-5.
31. Al-Owaisi M, Al-Hadiwi N, Khan SA. GC-MS analysis, determination of total phenolics, flavonoid content and free radical scavenging activities of various crude extracts of *Moringa peregrina* (Forssk.) Fiori leaves. *Asian Pacific Journal of Tropical Biomedicine*, 2014; 4(12), 964-970.
32. Burlacu E, Nisca A, Tanase C. A comprehensive review of phytochemistry and biological activities of *Quercus* species. *Forests*, 2020; 11(9), 904.
33. Rafie H, Soheila H, Grant E. *Rosmarinus officinalis* (rosemary): a novel therapeutic agent for antioxidant, antimicrobial, anticancer, antidiabetic, antidepressant,

neuroprotective, anti-inflammatory and anti-obesity treatment. Journal of Herbal Medicine, 2017; 3(2), 8.

34. Tohma H, Gülçin İ, Bursal E, Gören AC, Alwasel SH, Köksal E. Antioxidant activity and phenolic compounds of ginger (*Zingiber officinale* Rosc.) determined by HPLC-MS/MS. Journal of Food Measurement and Characterization, 2017; 11(2), 556-566.
35. Swallah MS, Sun H, Affoh R, Fu H, Yu H. Antioxidant potential overviews of secondary metabolites (polyphenols) in fruits. International Journal of Food Science, 2020; 2020, 19081686.
36. Waheed I, Ahmad M, Syed NH, Ashraf R. Investigation of phytochemical and antioxidant properties of methanol extract and fractions of *Ballota limbata* (Lamiaceae). Indian Journal of Pharmaceutical Sciences, 2014; 76(3), 251-256.
37. Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S, Ju Y H. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. Journal of food and Drug Analysis, 2014; 22(3), 296-302.
38. Hagos M, Chandravanshi BS, Redi-Abshiro M, Yaya EE. Determination of total phenolic, total flavonoid, ascorbic acid contents and antioxidant activity of pumpkin flesh, peel and seeds. Bulletin of the Chemical Society of Ethiopia, 2023; 37(5), 1093-1108.
39. Belyagoubi L, Belyagoubi-Benhammou N, Atik-Bekkara F, Coustard JM. Effects of extraction solvents on phenolic content and antioxidant properties of *Pistacia atlantica* Desf fruits from Algeria. International Food Research Journal, 2016; 23(3). 948-953.
40. Aryal S, Baniya MK, Danekhu K, Kunwar P, Gurung R, Koirala, N. Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. Plants, 2019; 8(4), 96.
41. Carbonell-Capella JM, Buniowska M, Esteve MJ, Frigola A. Effect of *Stevia rebaudiana* addition on bioaccessibility of bioactive compounds and antioxidant activity of beverages based on exotic fruits mixed with oat following simulated human digestion. Food Chemistry, 2015; 184, 122-130.

42. Chen Z, Yu L, Wang X, Gu Z, Beta T. Changes of phenolic profiles and antioxidant activity in canaryseed (*Phalaris canariensis* L.) during germination. *Food Chemistry*, 2016; 194, 608-618.
43. Asem N, Abdul Gapar NA, Abd Hapit NH, Omar EA. Correlation between total phenolic and flavonoid contents with antioxidant activity of Malaysian stingless bee propolis extract. *Journal of Apicultural Research*, 2020; 59(4), 437-442.
44. Muflihah YM, Gollavelli G, Ling YC. Correlation study of antioxidant activity with phenolic and flavonoid compounds in 12 Indonesian indigenous herbs. *Antioxidants*, 2021; 10(10), 1530.

Table 1. Chemical composition (%), energy (Kcal/mol), and Minerals content (mg/kg DW) of *Moringa peregrina* parts.

Parameters	Plant part		
	Leaves	Seeds	Bark
<i>Chemical composition</i>			
Moisture	9.83 ± 0.21 ^a	5.51 ± 0.30 ^c	7.75 ± 0.21 ^b

Protein	11.41 ± 0.27 ^b	19.40 ± 0.60 ^a	9.23 ± 0.38 ^b
Fat	6.03 ± 0.12 ^b	32.23 ± 0.96 ^a	5.13 ± 0.15 ^b
Carbohydrates	70.51 ± 0.06 ^b	39.97 ± 1.42 ^c	76.12 ± 0.76 ^a
Ash	2.32 ± 0.20 ^a	2.91 ± 0.20 ^a	1.97 ± 0.15 ^b
Total energy	381.87 ± 3.42 ^c	527.55 ± 6.57 ^a	387.49 ± 2.67 ^b
<i>Major minerals</i>			
Na	1102 ± 5.32 ^a	805 ± 4.10 ^b	784 ± 6.27 ^c
Mg	4258 ± 18.73 ^a	2822 ± 14.36 ^b	1564 ± 11.81 ^c
P	1262 ± 37.97 ^b	6682 ± 76.28 ^a	625 ± 15.01 ^c
K	8575 ± 30.4 ^a	6330 ± 46.79 ^c	5038 ± 24.85 ^b
Ca	9063 ± 48.12 ^b	1707 ± 10.03 ^c	9634 ± 55.8 ^a
<i>Traceminerals</i>			
Mn	36.22 ± 1.05 ^a	33.3 ± 1.13 ^b	22.12 ± 0.93 ^c
Fe	519.00 ± 5.71 ^a	290 ± 2.52 ^c	423.00 ± 3.86 ^b
Cu	13.22 ± 0.78 ^b	25 ± 0.91 ^a	7.11 ± 0.54 ^c
Zn	115.01 ± 4.42 ^a	72 ± 3.03 ^b	31.12 ± 1.9 ^c
Se	11.52 ± 0.06 ^a	7.41 ± 0.04 ^b	3.13 ± 0.02 ^c

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

Comment [Dp32]: Mention it Only on one page, do not divide it in two pages

Table 2. Total phenolics (mg Gallic acid /g sample) and flavonoids (mg Catechin /g sample) of *M. peregrina* parts extracted by different solvents.

Extraction solvent	Total phenolics			Total flavonoids		
	Seeds	Bark	Leaves	Seeds	Bark	Leaves
Water	15.78 ± 1.56 ^{dr}	143.07 ± 1.38 ^{cq}	234.48 ± 2.98 ^{ep}	1.18 ± 0.31 ^{er}	2.83 ± 0.02 ^{dq}	6.33 ± 0.16 ^{dp}
Methanol	23.77 ± 1.15 ^{cr}	97.65 ± 2.23 ^{dq}	288.14 ± 1.99 ^{dp}	1.88 ± 0.28 ^{dq}	1.87 ± 0.06 ^{eq}	9.81 ± 0.25 ^{cp}
Ethanol	29.61 ± 1.19 ^{bq}	48.31 ± 1.47 ^{fp}	26.46 ± 1.38 ^{gr}	2.11 ± 0.05 ^{cp}	1.62 ± 0.06 ^{fq}	1.37 ± 0.02 ^{fq}
Acetone	39.79 ± 4.71 ^{ar}	70.97 ± 4.16 ^{eq}	211.25 ± 0.66 ^{fp}	4.34 ± 0.12 ^{aq}	1.01 ± 0.06 ^{gr}	6.14 ± 0.06 ^{dp}
Aqueous methanol (50%)	8.41 ± 0.73 ^{fr}	205.45 ± 0.42 ^{aq}	350.69 ± 0.49 ^{bp}	2.63 ± 0.36 ^{br}	5.16 ± 0.11 ^{aq}	13.48 ± 0.31 ^{bp}
Aqueous ethanol (50%)	7.39 ± 1.49 ^{fr}	188.11 ± 0.24 ^{bq}	392.39 ± 1.78 ^{ap}	0.96 ± 0.12 ^{fr}	3.07 ± 0.27 ^{cq}	12.85 ± 0.18 ^{ap}
Aqueous acetone (50%)	10.62 ± 1.58 ^{er}	200.61 ± 0.81 ^{aq}	310.28 ± 1.76 ^{cp}	1.17 ± 0.18 ^{er}	4.46 ± 0.17 ^{bp}	3.82 ± 0.42 ^{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.

Comment [Dp33]: Also. Mention the values of Gallic acid for the comparison as it is a standard used in the experiment

Comment [Dp34]: Mention the graph of this table, as the graph has been mentioned is about the correlation of TPC and TFC with antioxidant activities

Table 3. DPPH (%), ABTS, and FRAP (g Trolox/g sample) of *M. peregrina* parts extracted by different solvents.

Extraction solvent	DPPH			ABTS		
	Seeds	Bark	Leaves	Seeds	Bark	Leaves
Water	10.87 ± 0.46 ^{cq}	83.19 ± 0.24 ^{dp}	83.01 ± 0.91 ^{dp}	1.41 ± 0.09 ^{cr}	7.57 ± 0.04 ^{cp}	4.93 ± 0.05 ^{fq}
Methanol	11.08 ± 0.88 ^{cr}	78.81 ± 0.29 ^{eq}	83.51 ± 0.16 ^{dp}	0.63 ± 0.54 ^{er}	2.48 ± 0.02 ^{eq}	10.75 ± 0.09 ^{dp}
Ethanol	12.64 ± 1.18 ^{br}	78.22 ± 0.08 ^{ep}	66.41 ± 0.13 ^{eq}	2.04 ± 0.56 ^{bp}	1.46 ± 0.04 ^{fq}	0.13 ± 0.01 ^{gr}
Acetone	14.62 ± 0.35 ^{adr}	77.62 ± 0.08 ^{fq}	81.76 ± 0.29 ^{ap}	2.71 ± 0.06 ^{aq}	1.73 ± 0.02 ^{fr}	9.89 ± 0.08 ^{ep}
Aqueous methanol (50%)	9.27 ± 0.52 ^{cq}	90.71 ± 0.31 ^{ap}	90.85 ± 2.12 ^{bp}	1.42 ± 0.14 ^{cr}	18.23 ± 0.12 ^{aq}	32.25 ± 0.42 ^{ap}
Aqueous ethanol (50%)	5.92 ± 0.76 ^{er}	89.47 ± 0.31 ^{cq}	91.45 ± 0.41 ^{ap}	1.25 ± 0.17 ^{dr}	10.27 ± 0.29 ^{bq}	30.74 ± 0.52 ^{bp}
Aqueous acetone (50%)	8.99 ± 0.75 ^{dr}	90.66 ± 0.45 ^{bp}	87.16 ± 0.97 ^{cq}	0.71 ± 0.08 ^{er}	5.61 ± 0.74 ^{dq}	24.45 ± 0.94 ^{cp}
Extraction solvent	FRAP					
Water	1.11 ± 0.18 ^{br}	3.38 ± 0.18 ^{aq}	5.98 ± 0.06 ^{dp}			
Methanol	1.33 ± 0.52 ^{bq}	2.06 ± 0.06 ^{bq}	6.89 ± 0.38 ^{cp}			
Ethanol	1.29 ± 0.07 ^{bq}	1.69 ± 0.07 ^{cp}	0.44 ± 0.07 ^{fr}			
Acetone	2.61 ± 0.14 ^{aq}	1.85 ± 0.08 ^{cr}	3.57 ± 0.21 ^{ep}			
Aqueous methanol (50%)	0.74 ± 0.18 ^{cr}	3.54 ± 0.03 ^{aq}	8.66 ± 0.03 ^{bp}			
Aqueous ethanol (50%)	0.69 ± 0.19 ^{cr}	3.41 ± 0.04 ^{aq}	9.16 ± 0.07 ^{ap}			
Aqueous acetone (50%)	0.85 ± 0.14 ^{cr}	3.53 ± 0.04 ^{aq}	8.61 ± 0.08 ^{bp}			

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

Comment [Dp35]: Same as above, mention the values of standard, Trolox

Comment [Dp36]: Mention the graph of this table, as the graph has been mentioned is about the correlation of TPC and TFC with antioxidant activities

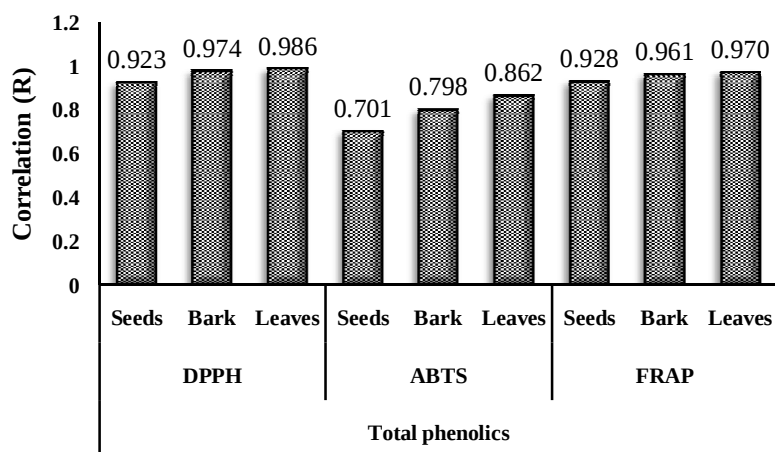


Figure 1. Correlation between total phenolics and antioxidant activity (DPPH, ABTS, and RFAP)

Comment [Dp37]: Also, values of the standard must be shown in the graph

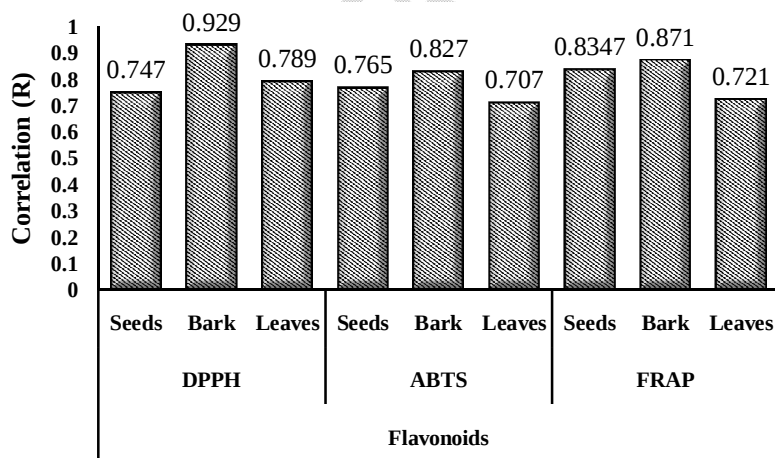


Figure 2. Correlation between total flavonoids and antioxidant activity (DPPH, ABTS, and RFAP)

Comment [Dp38]: Correct it