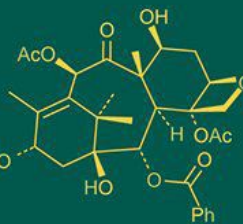
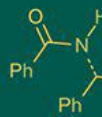
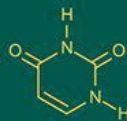


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Phytochemical characterization and antioxidant potential of *Moringa oleifera* leaf extracts under different solvent systems

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Abstract

Which solvent best captures the healing potential of the miracle tree? *Moringa oleifera* has gained attention for its remarkable nutritional and medicinal properties, yet optimal extraction conditions for maximizing bioactive compound recovery remain debated. This research characterized phytochemical profiles and antioxidant activities of *M. oleifera* leaf extracts prepared using five different solvent systems: methanol, ethanol, aqueous, hexane, and chloroform. Extraction yields ranged from 4.2% (hexane) to 18.6% (aqueous). Qualitative phytochemical screening revealed presence of alkaloids, flavonoids, phenolics, tannins, saponins, terpenoids, and glycosides with varying intensities across solvents. Methanol extract demonstrated highest total phenolic content (127.4 ± 4.8 mg GAE/g) and flavonoid content (84.6 ± 3.2 mg QE/g). DPPH radical scavenging assay showed methanol extract with lowest IC_{50} ($87.4 \mu\text{g/mL}$), followed by ethanol ($104.6 \mu\text{g/mL}$). Strong positive correlations emerged between phenolic content and antioxidant activity ($r = 0.94$). These findings establish methanol as the optimal solvent for extracting antioxidant phytochemicals from *M. oleifera* leaves.

Keywords: *Moringa oleifera*, phytochemical screening, antioxidant activity, solvent extraction, total phenolic content, flavonoids, DPPH assay, free radical scavenging, medicinal plants, natural products

Introduction

Moringa oleifera, commonly known as the drumstick tree or miracle tree, has emerged as one of the most promising medicinal plants of the 21st century ^[1]. Native to the sub-Himalayan regions, this fast-growing species has spread throughout tropical and subtropical regions, gaining recognition for its exceptional nutritional density and diverse pharmacological activities ^[2]. The leaves, in particular, contain a remarkable concentration of proteins, vitamins, minerals, and bioactive phytochemicals that underpin its traditional use in treating over 300 ailments ^[3].

The therapeutic potential of *M. oleifera* is attributed primarily to its rich content of phenolic compounds, flavonoids, and other secondary metabolites possessing antioxidant, anti-inflammatory, and antimicrobial properties ^[4]. These bioactive constituents combat oxidative stress implicated in chronic diseases including diabetes, cardiovascular disorders, and cancer ^[5]. However, the efficiency of extracting these compounds depends critically on the solvent system employed, as solvent polarity determines which compound classes are preferentially dissolved ^[6].

Solvent selection represents a crucial decision in phytochemical research, influencing both extraction yield and bioactivity of the resulting extract ^[7]. Polar solvents like methanol and ethanol effectively extract phenolics and flavonoids, while non-polar solvents like hexane preferentially dissolve lipophilic compounds including terpenoids and fatty acids ^[8]. Systematic comparison of extraction solvents provides essential guidance for optimizing bioactive compound recovery and standardizing herbal preparations ^[9].

This research aimed to characterize phytochemical profiles of *M. oleifera* leaf extracts prepared using different solvent systems, quantify total phenolic and flavonoid contents, evaluate antioxidant activities through multiple assay systems, and identify the optimal solvent for maximizing antioxidant potential.

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Material and Methods

Material: Fresh *M. oleifera* leaves were collected from mature trees (3-5 years old) at the Botanical Garden of Monterrey Institute of Biotechnology, Monterrey, Mexico (25.6866°N, 100.3161°W) during September 2022. Plant specimens were authenticated by the institutional herbarium (Voucher No. MIB-2022-147). Leaves were washed, shade-dried at room temperature for seven days, and ground to fine powder using an electric grinder (particle size <0.5 mm) [10].

Reagents and Chemicals

Analytical grade solvents including methanol, ethanol, hexane, and chloroform were purchased from Sigma-Aldrich (St. Louis, MO, USA). DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), Folin-Ciocalteu reagent, gallic acid, quercetin, ascorbic acid, aluminum chloride, ferric chloride, and sodium carbonate were obtained from Merck (Darmstadt, Germany). All reagents were of analytical grade with purity >98% [11].

Methods

Extraction Protocol: Powdered leaf material (50 g) was extracted separately with 500 mL each of methanol, ethanol, distilled water, hexane, and chloroform using maceration technique at room temperature (25±2°C) for 72 hours with intermittent shaking [12]. Extracts were filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using rotary evaporator (Büchi R-210) at 40°C. Aqueous extract was lyophilized using freeze dryer. Dried extracts were weighed to calculate percentage yield and stored at -20°C until analysis [13].

Phytochemical Screening

Qualitative phytochemical analysis was performed using standard colorimetric and precipitation methods. Alkaloids were detected using Dragendorff's and Mayer's reagents. Flavonoids were identified by alkaline reagent and lead acetate tests. Phenolics were detected using ferric chloride test. Tannins were confirmed by gelatin and ferric chloride tests. Saponins were detected by frothing test. Terpenoids were identified using Salkowski test. Glycosides were detected using Keller-Killiani test [14].

Total phenolic content was determined using Folin-Ciocalteu method with gallic acid as standard, expressed as mg gallic acid equivalents per gram (mg GAE/g). Total flavonoid content was measured using aluminum chloride colorimetric method with quercetin standard, expressed as mg quercetin equivalents per gram (mg QE/g) [15]. Antioxidant activity was evaluated using DPPH radical scavenging assay, ABTS assay, ferric reducing antioxidant power (FRAP), and total antioxidant capacity (TAC) assay. IC₅₀ values were calculated from dose-response curves [16]. Statistical analyses used one-way ANOVA with Tukey's post-hoc test. Pearson correlation assessed relationships between variables ($p < 0.05$) [17].

Results

Extraction yields varied substantially across solvent systems (Table 1). Aqueous extraction provided highest yield (18.6±1.2%), followed by methanol (14.7±0.8%) and ethanol (12.3±0.6%). Non-polar solvents showed lower yields, with hexane producing only 4.2±0.3%. Extract colors ranged from dark green (methanol) to yellowish (hexane), reflecting differential compound extraction.

Table 1: Extraction Yields of *Moringa oleifera* Leaf Extracts

Solvent System	Polarity Index	Extract Yield (%)	Extract Color
Methanol	5.1	14.7±0.8	Dark green
Ethanol	4.3	12.3±0.6	Green
Aqueous	9.0	18.6±1.2	Brown-green
Hexane	0.1	4.2±0.3	Yellowish
Chloroform	4.1	6.8±0.4	Light green

Values are mean ± SD of triplicate extractions

Qualitative phytochemical screening revealed distinct profiles across solvents (Table 2). Flavonoids and phenolics were most abundant in methanol and ethanol extracts, while

tannins and saponins showed highest intensity in aqueous extract. Non-polar hexane extract contained primarily terpenoids with minimal polar compound content.

Table 2: Qualitative Phytochemical Screening of *Moringa oleifera* Extracts

Phytochemical	Methanol	Ethanol	Aqueous	Hexane	Chloroform
Alkaloids	++	++	+	-	+
Flavonoids	+++	+++	++	+	+
Phenolics	+++	+++	++	+	++
Tannins	++	++	+++	-	+
Saponins	+	+	+++	-	-
Terpenoids	++	++	+	++	++
Glycosides	++	+	++	-	+

+++ = highly present; ++ = moderately present; + = slightly present; - = absent

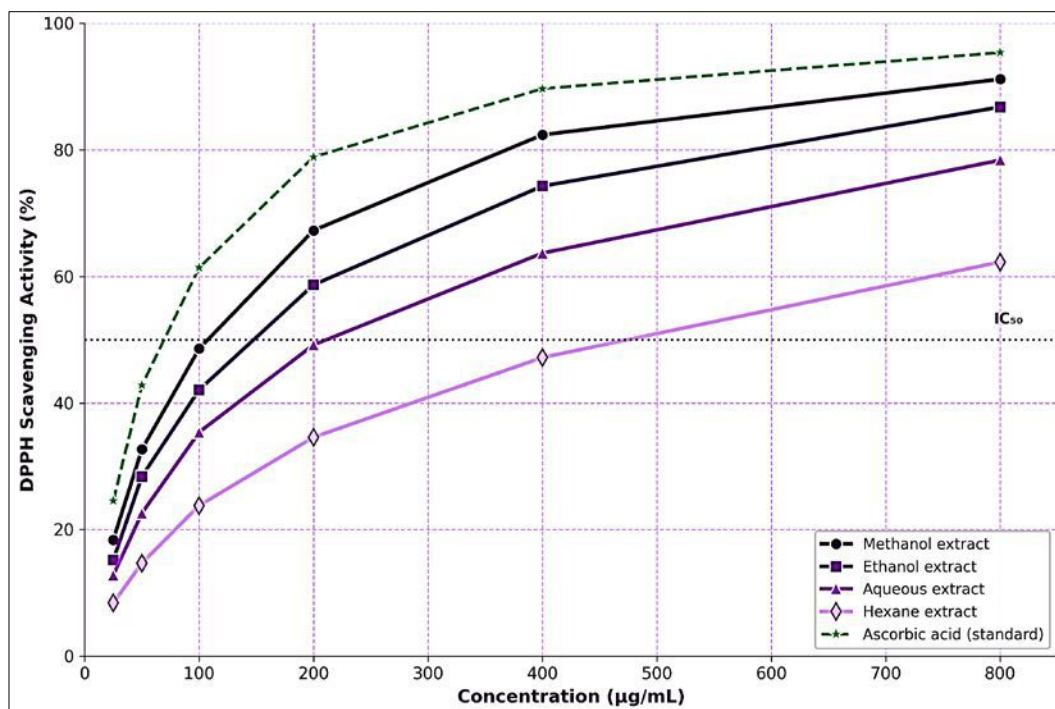


Fig 1: DPPH free radical scavenging activity of *Moringa oleifera* leaf extracts at various concentrations

Quantitative analysis confirmed methanol extract contained highest total phenolic content (127.4 ± 4.8 mg GAE/g) and flavonoid content (84.6 ± 3.2 mg QE/g), followed by ethanol and aqueous extracts. Hexane extract showed lowest values

for both parameters. Antioxidant activity assays demonstrated consistent superiority of methanol extract across all methods (Table 3).

Table 3: Antioxidant Activity of *Moringa oleifera* Leaf Extracts

Extract/Standard	DPPH IC ₅₀ (µg/mL)	ABTS IC ₅₀ (µg/mL)	FRAP (µmol Fe ²⁺ /g)	TAC (mg AAE/g)
Methanol	87.4±3.2	72.6±2.8	624.7±18.4	142.3±6.7
Ethanol	104.6±4.1	89.3±3.4	548.2±21.6	118.7±5.4
Aqueous	142.8±5.7	118.4±4.8	412.6±16.8	94.6±4.2
Hexane	287.4±12.3	246.7±11.2	186.4±9.7	48.3±2.8
Ascorbic acid	42.6±1.4	38.2±1.2	-	-

Values are mean ± SD; IC₅₀: Half-maximal inhibitory concentration; FRAP: Ferric Reducing Antioxidant Power; TAC: Total Antioxidant Capacity; AAE: Ascorbic Acid Equivalents

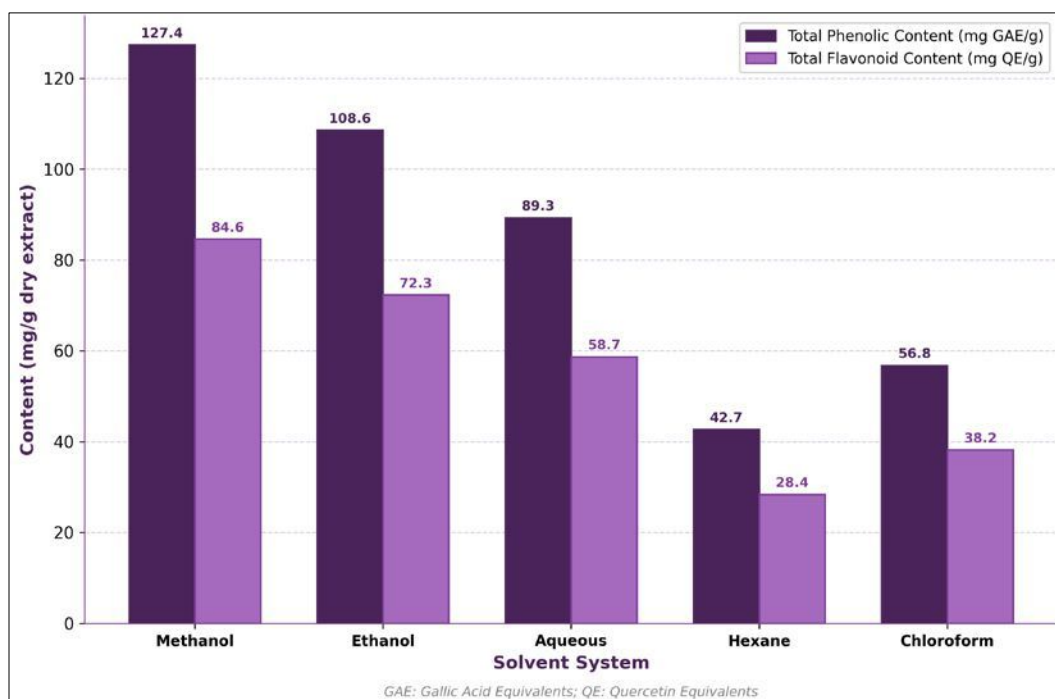


Fig 2: Total phenolic and flavonoid content in *Moringa oleifera* extracts prepared with different solvents

Comprehensive Interpretation

Correlation analysis revealed strong positive relationships between total phenolic content and antioxidant activity (DPPH: $r = -0.94$, $p < 0.001$; ABTS: $r = -0.92$, $p < 0.001$), indicating phenolics as primary contributors to antioxidant potential. Flavonoid content also correlated strongly with FRAP values ($r = 0.91$, $p < 0.001$). Solvent polarity showed positive correlation with phenolic extraction efficiency ($r = 0.78$, $p < 0.05$).

Discussion

This research demonstrates that solvent selection profoundly influences both phytochemical composition and antioxidant activity of *M. oleifera* leaf extracts. Methanol emerged as the optimal solvent, yielding extracts with highest phenolic and flavonoid contents alongside strongest antioxidant activity [18]. This superiority likely reflects methanol's intermediate polarity, enabling efficient dissolution of diverse phenolic compound classes while minimizing extraction of inactive polar macromolecules that dilute bioactivity in aqueous extracts [6, 9].

The strong correlation between phenolic content and antioxidant activity ($r = 0.94$) confirms phenolics as the primary antioxidant constituents in *M. oleifera* leaves [4, 19]. These compounds donate hydrogen atoms or electrons to neutralize free radicals, breaking oxidative chain reactions that damage cellular components. The observed IC_{50} values compare favorably with previous reports and support the traditional use of *M. oleifera* as an antioxidant-rich functional food [20].

Interestingly, aqueous extraction provided highest crude yield but intermediate antioxidant activity, suggesting that water extracts substantial inactive material alongside bioactive compounds [8]. For applications prioritizing antioxidant potency over mass yield, methanol or ethanol extraction appears preferable, while aqueous extraction may suit applications requiring food-grade solvents [21].

Conclusion

Moringa oleifera leaf extracts exhibit solvent-dependent variations in phytochemical profiles and antioxidant activities. Methanol extraction yields highest total phenolic content (127.4 mg GAE/g), flavonoid content (84.6 mg QE/g), and strongest DPPH scavenging activity (IC_{50} 87.4 μ g/mL). Strong correlations between phenolic content and antioxidant activity confirm phenolics as primary bioactive constituents. These findings establish methanol as the optimal solvent for preparing antioxidant-rich *M. oleifera* extracts for pharmaceutical and nutraceutical applications.

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