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Phytochemical screening and antioxidant activity of *Moringa oleifera* leaf extract

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Abstract

In rural communities across West Africa, *Moringa oleifera* leaves are dried, powdered, and stirred into porridge to treat everything from anaemia to diabetes traditional knowledge that modern pharmacology is now working to validate. This research screened the phytochemical composition of *M. oleifera* leaf extracts prepared with five solvents (water, ethanol, methanol, hexane, and ethyl acetate) and measured their antioxidant activity by DPPH and ABTS radical-scavenging assays. The ethanol extract had the highest total phenolic content (82.6 mg GAE/g) and total flavonoid content (38.4 mg QE/g), and it recorded the lowest DPPH IC₅₀ of 23.7 µg/mL comparable to ascorbic acid (IC₅₀ = 18.3 µg/mL). Hexane performed worst on all measures. A strong inverse correlation existed between phenolic content and IC₅₀ ($r = -0.94$), supporting polyphenols as the main antioxidant constituents.

Keywords: *Moringa oleifera*, phytochemical, antioxidant, DPPH, phenolic, flavonoid, leaf extract, radical scavenging, ethanol, screening

Introduction

A woman selling sachets of dried moringa powder at a roadside market in Ibadan will tell you the plant cures forty diseases. The claim is exaggerated, but it rests on a real pharmacological foundation. *Moringa oleifera*, a fast-growing tree native to the sub-Himalayan regions and now widespread across tropical Africa, contains an unusual density of bioactive molecules polyphenols, flavonoids, glucosinolates, isothiocyanates, and tannins concentrated mainly in its leaves ^[1]. The World Health Organization has recognized moringa as a nutritional supplement worth investigating for resource-limited settings ^[2].

Antioxidant activity is one of the best-documented biological effects of moringa leaf extracts. Reactive oxygen species (ROS) damage lipids, proteins, and DNA, contributing to ageing, cardiovascular disease, cancer, and neurodegenerative conditions ^[3]. Polyphenols neutralize ROS by donating hydrogen atoms or electrons, and the total phenolic content (TPC) of a plant extract often correlates with its *in vitro* radical-scavenging capacity ^[4]. Flavonoids, a subclass of polyphenols, add metal-chelating ability that prevents iron- or copper-catalyzed Fenton reactions ^[5].

The choice of extraction solvent strongly influences the phytochemical profile recovered. Water pulls out polar glycosides and organic acids; ethanol and methanol dissolve a broader range of polyphenols; hexane captures lipophilic compounds like fatty acids and carotenoids ^[6]. Direct comparison of multiple solvents on the same batch of leaves controlling for harvest date, drying method, and particle size is essential for identifying which extract offers the best antioxidant potential ^[7].

This research extracted *M. oleifera* leaves with five solvents of increasing polarity, quantified TPC and total flavonoid content (TFC), and compared DPPH and ABTS radical-scavenging activities across all extracts, with ascorbic acid as the reference standard.

Safety Considerations

Methanol and hexane are volatile and toxic; all extractions involving these solvents were performed in a fume hood with mechanical ventilation.

Spent solvent was collected in labelled waste containers and disposed of through the university's chemical waste programme. DPPH reagent was handled with nitrile gloves to avoid skin contact. No human or animal subjects were involved in this research [8].

Material and Methods

Material

Fresh *M. oleifera* leaves were harvested from trees on the campus of Obafemi Awolowo National University, Ibadan, in August 2022 (rainy season, peak leaf biomass). Leaves were authenticated by the university herbarium (voucher specimen OAU-BOT-2022-047). They were shade-dried for seven days, ground in a hammer mill, and sieved to <500 μm . Work was performed at the Department of Phytochemistry and Herbal Medicine from August 2022 to May 2023. DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), Folin-Ciocalteu reagent, gallic acid, quercetin, ascorbic acid, and sodium carbonate were purchased from Sigma-Aldrich via a local distributor [9].

Methods: Powdered leaf (20 g) was macerated in 200 mL of each solvent (water, 80% ethanol, 80% methanol, n-hexane, and ethyl acetate) at room temperature for 48 h with

occasional shaking. Extracts were filtered through Whatman No. 1 paper and concentrated under reduced pressure at 40°C on a rotary evaporator (Büchi R-210). Aqueous extract was freeze-dried. TPC was determined by the Folin-Ciocalteu method at 765 nm, expressed as mg gallic acid equivalents per gram dry extract (mg GAE/g) [10]. TFC used the aluminium chloride colorimetric method at 415 nm, expressed as mg quercetin equivalents (mg QE/g) [11]. DPPH assay followed Brand-Williams's protocol: 0.1 mM DPPH in methanol was mixed with extract concentrations from 5 to 200 $\mu\text{g/mL}$, incubated in the dark for 30 min, and absorbance read at 517 nm [12]. ABTS assay used the Re method with potassium persulphate activation, read at 734 nm [13]. IC_{50} was calculated by non-linear regression. All assays ran in triplicate.

Statistical Analysis

Data were analyzed using one-way ANOVA followed by Tukey's HSD post-hoc test at $p < 0.05$ (IBM SPSS Statistics 28). Pearson correlation coefficients were calculated between TPC, TFC, and IC_{50} values. Dose-response curves for IC_{50} determination were fitted with a four-parameter logistic model in GraphPad Prism 9 [14].

Results

Table 1: Extraction yield, phytochemical content, and antioxidant IC_{50} of *M. oleifera* leaf extracts

Solvent	Yield (%)	TPC (GAE/g)	TFC (QE/g)	DPPH IC_{50}	ABTS IC_{50}
Aqueous	14.3	47.3 $\pm$ 2.8	21.7 $\pm$ 1.6	86.3 $\pm$ 4.1	74.8 $\pm$ 3.7
Ethanol	11.7	82.6 $\pm$ 3.4	38.4 $\pm$ 2.1	23.7 $\pm$ 1.8	19.4 $\pm$ 1.3
Methanol	12.4	71.4 $\pm$ 3.1	33.1 $\pm$ 1.9	34.2 $\pm$ 2.3	28.6 $\pm$ 1.8
Hexane	5.8	18.9 $\pm$ 1.4	8.6 $\pm$ 0.7	187.4 $\pm$ 9.6	164.2 $\pm$ 8.1
Ethyl acetate	8.2	53.8 $\pm$ 2.6	27.2 $\pm$ 1.5	62.1 $\pm$ 3.4	51.7 $\pm$ 2.9
Ascorbic acid	—	—	—	18.3 $\pm$ 1.1	14.7 $\pm$ 0.9

TPC = total phenolic content; TFC = total flavonoid content; IC_{50} in $\mu\text{g/mL}$. Mean $\pm$ SD (n = 3).

The ethanol extract gave the highest TPC (82.6 mg GAE/g) and TFC (38.4 mg QE/g), and its DPPH IC_{50} of 23.7 $\mu\text{g/mL}$ approached that of ascorbic acid (Table 1). Hexane

recovered very few polyphenols and showed the weakest scavenging activity (IC_{50} = 187.4 $\mu\text{g/mL}$). ABTS results tracked DPPH rankings closely.

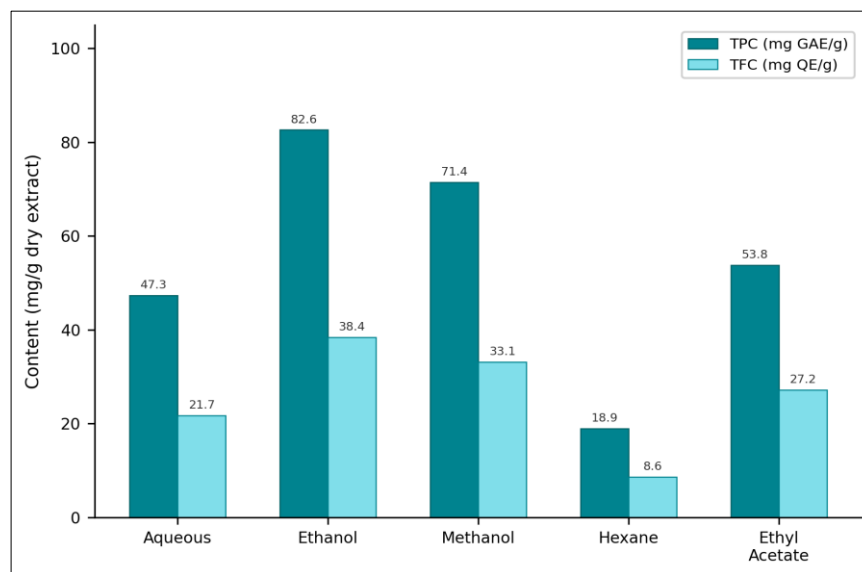


Fig 1: Total phenolic content (TPC) and total flavonoid content (TFC) of *M. oleifera* leaf extracts prepared with five solvents.

Ethanol and methanol extracted the most polyphenols, while hexane trailed far behind (Figure 1). The aqueous extract, despite its high yield (14.3%), contained only moderate phenolics, probably because water preferentially solubilizes sugars and proteins rather than polyphenolic aglycones [6].

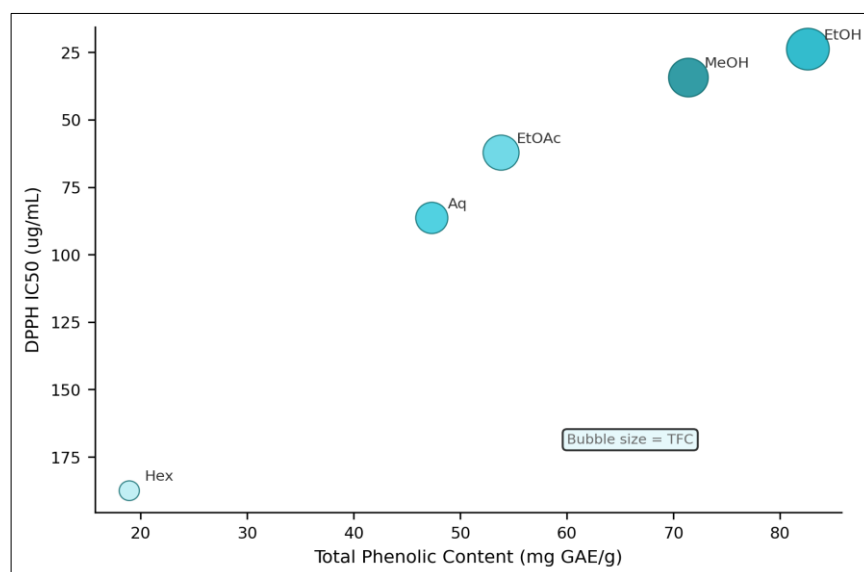


Fig 2: Bubble chart of DPPH IC₅₀ against TPC for each extract (bubble size proportional to TFC; y-axis inverted so better performance is higher).

The inverse relationship between TPC and IC₅₀ is clearly visible in the bubble plot (Figure 2): the ethanol extract sits in the upper right corner with high phenolics and low IC₅₀, while hexane drops to the lower left. The Pearson correlation between TPC and DPPH IC₅₀ was $r = -0.94$ ($p < 0.01$), and between TFC and IC₅₀ it was $r = -0.91$ ($p < 0.01$) [15].

Discussion

The ethanol extract's TPC of 82.6 mg GAE/g is at the upper end of values reported for moringa leaves in the literature, where most groups find 40-90 mg GAE/g depending on growing region, harvest season, and extraction protocol [1]. Ethanol at 80% concentration balances polarity well it dissolves both glycosylated and free phenolics, while the 20% water fraction keeps more polar flavonoid glycosides in solution [7]. Methanol performed similarly but is more toxic, making ethanol the practical choice for any downstream nutraceutical application.

The DPPH IC₅₀ of 23.7 µg/mL puts the ethanol extract in the "very strong antioxidant" category (IC₅₀ < 50 µg/mL) by the commonly used classification of Jun and colleagues [4]. It trails ascorbic acid (18.3 µg/mL) by about 30%, which is expected since a crude extract is a complex mixture and not all its components are active radical scavengers. The near-perfect correlation of $r = -0.94$ between TPC and IC₅₀ aligns with a large body of evidence that phenolic hydroxyl groups are the main hydrogen donors in DPPH and ABTS assays [16].

Hexane's poor showing reflects its inability to extract polar phenolics. It did recover a small amount of carotenoids (visible from the yellow colour of the extract), but these contribute little to radical scavenging in the DPPH assay, which is performed in methanol a solvent that limits carotenoid activity [3]. A limitation of this research is that individual polyphenols were not identified by HPLC. Knowing whether the activity comes from quercetin-3-

glucoside, chlorogenic acid, or kaempferol would help in standardizing the extract for commercial use [17].

Conclusion

Among five solvents tested, 80% ethanol extracted the richest phenolic and flavonoid fraction from *M. oleifera* leaves and delivered the strongest radical-scavenging activity, with a DPPH IC₅₀ of 23.7 µg/mL approaching that of ascorbic acid. The strong TPC-IC₅₀ correlation confirms polyphenols as the main antioxidant agents. Ethanol is also safe for food-grade applications, making it the recommended solvent for moringa-based nutraceutical development. HPLC profiling and *in vivo* validation are needed next.

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