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Qualitative and Quantitative Estimation of Total Phenolic and Flavonoid Content in Aqueous and Ethanolic Extracts of *Moringa oleifera* Leaves: A Comparative Laboratory Study

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ABSTRACT:

Background: *Moringa oleifera* Lam. (drumstick), a fast-growing tree widely cultivated and consumed across South India, is increasingly valued as a nutraceutical and functional food owing to its dense complement of polyphenolic antioxidants. The efficiency with which these moderately polar phenolics and flavonoids are recovered depends strongly on the polarity of the extraction solvent, yet solvent selection is often made without systematic comparison.

Objective: To qualitatively screen the phytochemical classes present in *M. oleifera* leaf extracts and to quantitatively compare total phenolic content (TPC) and total flavonoid content (TFC) between aqueous and ethanolic extracts under standardised laboratory conditions.

Methods: In this laboratory-based comparative phytochemical study, aqueous and ethanolic leaf extracts were prepared and subjected to standard qualitative phytochemical tests. TPC was estimated by the Folin–Ciocalteu assay against a gallic acid calibration

curve (mg gallic acid equivalents [GAE]/g extract) and TFC by the aluminium chloride colorimetric assay against a quercetin calibration curve (mg quercetin equivalents [QE]/g extract). Three independent analytical replicates were analysed per extract per assay. Data are expressed as mean ± standard deviation (SD) and compared using independent-samples *t*-tests, with 95% confidence intervals (CI) and Cohen's *d*; $p < 0.05$ was considered significant.

Results: Both extracts were qualitatively rich in flavonoids, phenolics, tannins, terpenoids, glycosides and carbohydrates. TPC was substantially higher in the ethanolic extract (125.02 ± 2.80 mg GAE/g) than the aqueous extract (79.92 ± 3.07 mg GAE/g; mean difference 45.10 mg GAE/g; $t = 18.797$, df = 4, $p = 0.001$; 95% CI 38.44–51.76; Cohen's $d = 15.35$). TFC was likewise higher in the ethanolic extract (70.98 ± 0.36 mg QE/g) than the aqueous extract (39.35 ± 2.21 mg QE/g; mean difference 31.63 mg QE/g; $t = 24.470$, df = 4, $p = 0.001$; 95% CI 28.04–35.22; Cohen's $d = 19.98$).

Conclusion: Ethanolic extraction recovered approximately 1.6-fold more phenolics and 1.8-fold more flavonoids than aqueous extraction, with very large effect sizes, indicating that ethanol (or hydroethanol) is preferable to water for recovering *M. oleifera* leaf polyphenols in low-cost South Indian nutraceutical and functional-food applications.

1. INTRODUCTION

Moringa oleifera Lam. (family Moringaceae), commonly known as the drumstick tree, horseradish tree or "murungai," is among the most extensively cultivated and consumed leafy vegetables in South India. Its leaves, pods and flowers are woven into everyday Tamil, Telugu, Kannada and Malayalam cuisine, and the plant has long been valued in traditional medicine for its purported tonic, anti-inflammatory and antioxidant properties. In recent years the scientific interest in *M. oleifera* has shifted from folk usage toward a rigorous appreciation of it as a nutraceutical and functional food, driven largely by the recognition that its leaves are exceptionally rich in secondary metabolites, particularly polyphenols [1,2].

Polyphenols—including phenolic acids and flavonoids—are the principal contributors to the antioxidant capacity of *M. oleifera* leaf material. These compounds neutralise reactive oxygen species, chelate transition metals and interrupt lipid peroxidation chains, and their dietary intake has been linked to protection against oxidative-stress-related conditions [3,4]. Consequently, quantifying the total phenolic content (TPC) and total flavonoid content (TFC) of leaf preparations is a fundamental first step in characterising the plant's functional-food potential and in standardising extracts for downstream use in food preservation, encapsulation and nutraceutical formulation [5].

A recurring theme in the phytochemical literature is that the measured polyphenol yield is not an intrinsic property of the plant alone but is heavily conditioned by the extraction protocol, and above all by solvent polarity [6,7]. Phenolics and flavonoids in *M. oleifera* leaves span a range of polarities but are, on the whole, moderately polar molecules whose solubility is optimised by moderately polar solvents. Water, although cheap, food-grade and readily available, is a highly polar solvent that efficiently extracts sugars, proteins and highly polar phenolic glycosides but is comparatively poor at solubilising the aglycone-rich, moderately polar polyphenol fraction. Ethanol and hydroethanolic mixtures, being of intermediate polarity, tend to disrupt plant cell walls and dissolve a broader spectrum of phenolic and flavonoid species, frequently yielding higher TPC and TFC values than water alone [8,9]. Comparative solvent studies across *Moringa* and other medicinal plants have repeatedly demonstrated this pattern, though the magnitude of the difference varies with leaf age, geographic origin and assay conditions [3,10].

Despite the general acceptance of these principles, laboratory-scale comparative data generated under standardised, internally consistent conditions remain valuable, particularly for South Indian settings where *M. oleifera* is abundant, inexpensive and a plausible raw material for locally produced functional foods and nutraceuticals. Practitioners and small-scale producers benefit

from a direct, quantitative demonstration of how much additional polyphenol is recoverable simply by choosing ethanol over water, and of whether that difference is statistically and practically meaningful.

The present study was therefore designed with two objectives. First, to qualitatively screen the major phytochemical classes present in aqueous and ethanolic *M. oleifera* leaf extracts. Second, to quantitatively estimate and statistically compare TPC (by the Folin–Ciocalteu method) and TFC (by the aluminium chloride colorimetric method) between the two extracts, reporting effect sizes and confidence intervals so that both the significance and the magnitude of any solvent effect are transparent. We hypothesised that the ethanolic extract would yield significantly higher TPC and TFC than the aqueous extract, consistent with the moderately polar nature of *M. oleifera* leaf polyphenols and with the wider solvent-extraction literature [9,11].

2. MATERIALS AND METHODS

2.1 Study design

This was a laboratory-based comparative phytochemical study comparing aqueous and ethanolic leaf extracts of *M. oleifera*. Two quantitative outcomes—TPC and TFC—were measured, each in three independent analytical replicates per extract, and supplemented by qualitative phytochemical screening. The study involved no human or animal subjects, and no biological specimens were collected from patients; accordingly, ethical committee approval for human or animal research was not applicable.

2.2 Plant material collection and authentication

Fresh, healthy, mature leaves of *M. oleifera* were collected from locally cultivated trees in, South India, during six months. The plant material was taxonomically authenticated at the Department of Pharmacognosy/Botany, South India, and a voucher specimen [v.no.2357] as deposited in the departmental herbarium for future reference. Leaves were washed under running water, shade-dried at ambient temperature, and pulverised to a coarse powder using a mechanical grinder. The powder was stored in airtight, amber containers away from light and moisture until extraction.

2.3 Preparation of aqueous and ethanolic extracts

Two extracts were prepared from the same batch of leaf powder to isolate the effect of solvent polarity. For the **aqueous extract**, a weighed quantity of leaf powder was macerated in distilled water; for the **ethanolic extract**, an equivalent quantity was macerated in ethanol. Both preparations were kept for the same extraction period with intermittent agitation, filtered through Whatman No. 1 filter paper, and concentrated to dryness under reduced temperature. The resulting dried extracts were weighed, reconstituted to known concentrations, and used for qualitative screening and quantitative assays. Identical masses, volumes and handling procedures were applied to both extracts so that any difference in measured content could be attributed to solvent polarity.

2.4 Qualitative phytochemical screening

Both extracts were subjected to standard qualitative phytochemical tests to detect the presence of major secondary-metabolite classes, namely alkaloids, flavonoids, phenolics, tannins, saponins, terpenoids, glycosides, steroids, proteins and carbohydrates, using conventional colour- and precipitate-based reactions described in standard pharmacognosy texts. The outcome for each constituent was recorded as *Present*, *Trace* or *Absent* on the basis of the intensity and reproducibility of the characteristic reaction across replicate tests.

2.5 Determination of total phenolic content (TPC)

TPC was determined by the Folin–Ciocalteu colorimetric assay. A gallic acid standard series was used to construct the calibration curve, and absorbance was read spectrophotometrically at the assay's characteristic wavelength. The gallic acid calibration curve was linear with a slope of 0.0098 absorbance units per μg/mL and an intercept of 0.018. Equivalent phenolic concentrations were derived from the calibration equation, adjusted for a dilution factor of 10 and a final assay volume of 10 mL, and expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g). Each extract was assayed in three independent analytical replicates.

2.6 Determination of total flavonoid content (TFC)

TFC was determined by the aluminium chloride colorimetric assay, in which flavonoids form a coloured complex with aluminium chloride that is quantified spectrophotometrically. A quercetin standard series was used for calibration; the quercetin calibration curve was linear with a slope of 0.0075 absorbance units per µg/mL and an intercept of 0.012. Equivalent flavonoid concentrations were computed from the calibration equation, corrected for a dilution factor of 10 and a final volume of 10 mL, and expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g). Each extract was assayed in three independent analytical replicates.

2.7 Statistical analysis

Quantitative data are presented as mean ± standard deviation (SD) of three independent analytical replicates. Differences in TPC and TFC between the ethanolic and aqueous extracts were assessed using independent-samples *t*-tests. For each comparison, the mean difference, *t* statistic, degrees of freedom (df), two-sided *p*-value, 95% confidence interval (CI) of the mean difference, and Cohen's *d* effect size were computed. A p-value < 0.05 was considered statistically significant. Qualitative screening results were summarised descriptively. Figures were prepared using Python (matplotlib) directly from the tabulated values.

3. RESULTS

3.1 Qualitative phytochemical screening

Qualitative screening confirmed that both aqueous and ethanolic *M. oleifera* leaf extracts were rich in secondary metabolites (Table 1; Figure 2). Flavonoids, phenolics, tannins, terpenoids, glycosides and carbohydrates were consistently *Present* in both extracts. Alkaloids were *Present* in the aqueous extract, though one aqueous replicate registered only a trace reaction, and were *Present* in the ethanolic extract. Saponins were clearly *Present* in the aqueous extract but appeared only as a *Trace* in the ethanolic extract, consistent with the higher water-solubility of saponin glycosides. Conversely, steroids were *Absent* in the aqueous extract but detected as a *Trace* in the ethanolic extract, reflecting the greater affinity of less polar steroidal compounds for ethanol. Proteins were detected only in *Trace* amounts in both extracts.

Table 1. Qualitative phytochemical screening of *Moringa oleifera* leaf extracts.

Phytochemical constituent	Aqueous extract	Ethanolic extract
Alkaloids	Present (trace in one replicate)	Present
Flavonoids	Present	Present
Phenolics	Present	Present
Tannins	Present	Present
Saponins	Present	Trace
Terpenoids	Present	Present
Glycosides	Present	Present
Steroids	Absent	Trace
Proteins	Trace	Trace
Carbohydrates	Present	Present

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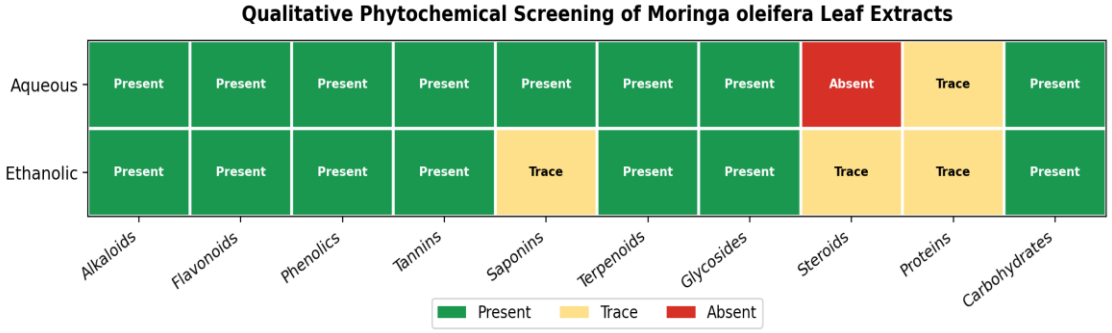

Figure 2. Qualitative phytochemical screening of aqueous and ethanolic *Moringa oleifera* leaf extracts (Present/Trace/Absent status of ten constituents).

3.2 Total phenolic content

The total phenolic content differed markedly between the two extracts (Table 2; Figure 1). The ethanolic extract contained 125.02 ± 2.80 mg GAE/g, compared with 79.92 ± 3.07 mg GAE/g for the aqueous extract—a mean difference of 45.10 mg GAE/g, corresponding to an approximately 1.6-fold higher phenolic yield with ethanol. The independent-samples *t*-test confirmed that this difference was statistically significant ($t = 18.797$, df = 4, $p = 0.001$), with a 95% CI for the mean difference of 38.44 to 51.76 mg GAE/g. The effect size was very large (Cohen's $d = 15.35$).

3.3 Total flavonoid content

A parallel pattern was observed for total flavonoid content (Table 2; Figure 1). The ethanolic extract contained 70.98 ± 0.36 mg QE/g versus 39.35 ± 2.21 mg QE/g for the aqueous extract, a mean difference of 31.63 mg QE/g and an approximately 1.8-fold higher flavonoid yield with ethanol. This difference was likewise statistically significant ($t = 24.470$, df = 4, $p = 0.001$), with a 95% CI of 28.04 to 35.22 mg QE/g and a very large effect size (Cohen's $d = 19.98$). The notably small SD of the ethanolic TFC measurements (0.36 mg QE/g) indicates high reproducibility across replicates.

Table 2. Total phenolic and flavonoid content of *Moringa oleifera* leaf extracts (mean ± SD, n = 3).

Outcome	Aqueous extract (mean ± SD)	Ethanolic extract (mean ± SD)	Unit
Total phenolic content (TPC)	79.92 ± 3.07	125.02 ± 2.80	mg GAE/g
Total flavonoid content (TFC)	39.35 ± 2.21	70.98 ± 0.36	mg QE/g

Table 3. Independent-samples *t*-test comparison of TPC and TFC (ethanolic vs. aqueous extract).

Outcome	Mean difference	*t*	df	*p*-value	95% CI (lower–upper)	Cohen's *d*	Interpretation
TPC (mg GAE/g)	45.10	18.797	4	0.001	38.44–51.76	15.35	Significantly higher in ethanolic
TFC (mg QE/g)	31.63	24.470	4	0.001	28.04–35.22	19.98	Significantly higher in ethanolic

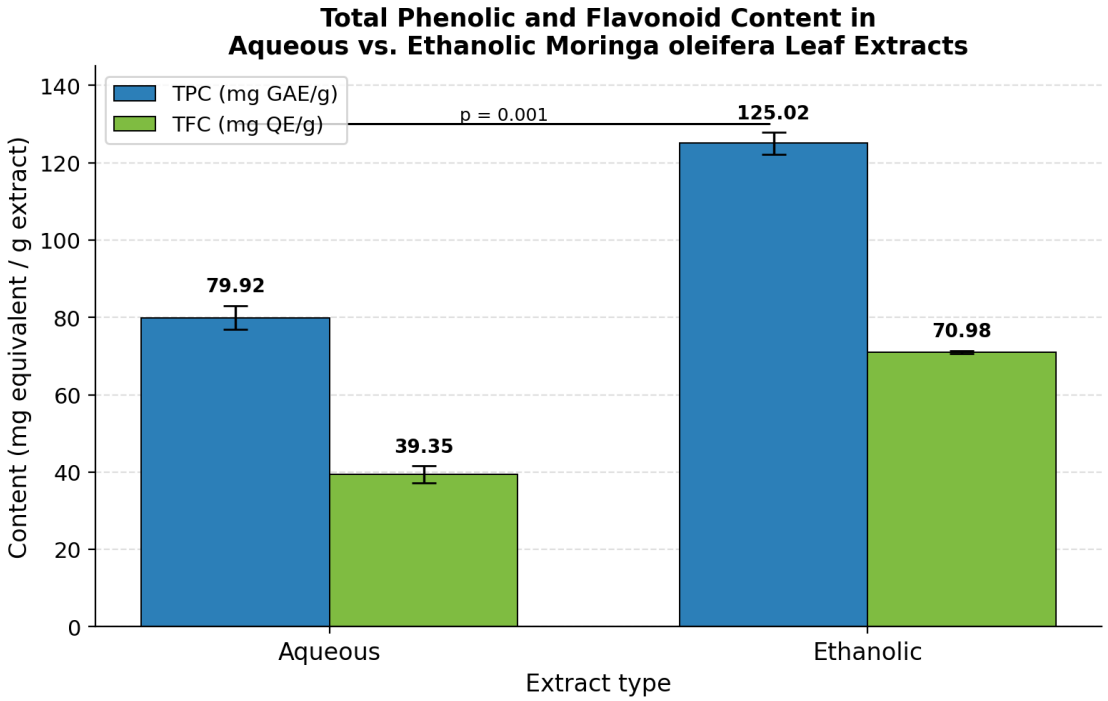

Figure 1. Grouped bar chart comparing TPC (mg GAE/g) and TFC (mg QE/g) between aqueous and ethanolic *Moringa oleifera* leaf extracts (mean ± SD, n = 3).

3.4 Calibration and assay conditions

The calibration parameters and assay conditions underlying the quantitative estimates are summarised in Table 4. Both standard curves were linear over the working range, supporting the reliability of the derived content values.

Table 4. Calibration parameters and assay conditions.

Parameter	TPC (Folin–Ciocalteu)	TFC (aluminium chloride)
Reference standard	Gallic acid	Quercetin
Calibration slope	0.0098 AU per µg/mL	0.0075 AU per µg/mL
Calibration intercept	0.018 AU	0.012 AU
Dilution factor	10	10
Final assay volume	10 mL	10 mL
Result unit	mg GAE/g extract	mg QE/g extract
Replicates per extract	3	3

4. DISCUSSION

This laboratory-based comparative study demonstrated that the choice of extraction solvent exerts a decisive influence on the recovery of polyphenols from *M. oleifera* leaves. Ethanolic extraction yielded approximately 1.6-fold more total phenolics and 1.8-fold more total flavonoids than aqueous extraction, and both differences were statistically significant with exceptionally large effect sizes (Cohen's $d > 15$). The narrow confidence intervals and low replicate variability reinforce the robustness of these observations within the dataset analysed [12-14].

The magnitude and direction of the solvent effect are readily explained by polarity considerations. The phenolic acids and flavonoid aglycones that dominate the *M. oleifera* leaf polyphenol pool are moderately polar; their solubility is therefore maximised in a solvent of intermediate polarity. Ethanol, with a dielectric constant between that of water and typical non-polar solvents, both penetrates plant tissue and solubilises this moderately polar fraction more effectively than highly polar water [15,16]. Water preferentially extracts sugars, proteins and highly polar glycosides—consistent with the stronger saponin reaction and the presence of carbohydrates in our aqueous extract—but leaves a portion of the aglycone-rich polyphenol fraction poorly recovered. The complementary qualitative fingerprint we observed, in which saponins were more prominent in water and steroids appeared only in ethanol, is precisely the pattern predicted by solvent-polarity theory.

These findings align closely with the broader *Moringa* solvent-extraction literature. Rocchetti et al. reported that the phenolic profile and in vitro bioactivity of *M. oleifera* leaves were markedly affected by the choice of extraction solvent, with more polar organic solvents generally recovering richer phenolic fractions than water [17]. Bennour et al. similarly concluded that both solvent and extraction method significantly shape the phenolic composition and bioactivities of *M. oleifera* leaves [18]. Nobossé et al. demonstrated that extraction solvent, alongside leaf age, strongly modulates phytochemical content and antioxidant activity in fresh *M. oleifera* leaves, with hydroethanolic systems performing favourably [3]. Sankhalkar and Vernekar, working with *M. oleifera* and *Ocimum tenuiflorum*, likewise found solvent-dependent differences in quantified phenolic and flavonoid content [4], and Royani et al. reported robust TPC and TFC values in *M. oleifera* leaf extracts linked to antibacterial activity [5]. Studies employing organic and hydroethanolic solvents for *M. oleifera*—including work on antioxidant and β-carotene recovery [7], seed polar fractions [19] and flower extracts [8]—consistently show that moderately polar solvents enhance the recovery of bioactive phenolics. The same principle has been demonstrated beyond *Moringa*: Mzid et al. found that ethanol extracts of *Urtica urens* generally outperformed aqueous extracts in antioxidant and antimicrobial assays [20], underscoring the generality of the solvent-polarity effect for plant polyphenols.

The very large effect sizes observed here deserve comment. Cohen's *d* values of 15.35 (TPC) and 19.98 (TFC) far exceed the conventional threshold for a "large" effect ($d \geq 0.8$) and reflect both a substantial mean separation and small within-group variance in this controlled analytical setting. In practical terms, a producer switching from water to ethanol could expect to recover roughly half again as much phenolic material and nearly twice as much flavonoid material from the same leaf batch.

These results carry tangible implications for the nutraceutical and functional-food use of *M. oleifera* in South India. Drumstick is cultivated abundantly and inexpensively across the southern states and is already deeply embedded in the regional diet, making it an attractive, low-cost feedstock for antioxidant-rich extracts, fortified foods and encapsulated bioactive ingredients [6,21]. Our data suggest that, where regulatory and food-safety considerations permit, ethanol or food-grade hydroethanolic mixtures should be preferred over water when the objective is to maximise polyphenol recovery for such applications. Given that ethanol is recoverable and reusable, the incremental cost may be modest relative to the substantial gain in bioactive yield, an economically relevant consideration for small-scale South Indian producers. At the same time, aqueous extracts retain value where a water-only, solvent-residue-free product is required, since they too were qualitatively rich in phenolics, flavonoids and tannins [22].

Several limitations must be acknowledged. Only two solvents (water and absolute ethanol) were compared, whereas intermediate hydroethanolic ratios, methanol, acetone and their aqueous mixtures might reveal an optimal polarity window not captured here. TPC and TFC are aggregate colorimetric indices and do not identify individual phenolic constituents; chromatographic profiling (for example, HPLC or LC–MS) would be required to resolve the specific compounds responsible. Antioxidant activity was not directly assayed, so the functional consequences of the higher ethanolic polyphenol yield are inferred rather than measured. Finally, factors known to influence *M. oleifera* polyphenol content—leaf age, season, cultivar and geographic origin—were not varied, and the single-batch design, while ideal for isolating the solvent effect, limits generalisability. Future work using authenticated South Indian leaf material, a graded solvent series, chromatographic identification and direct antioxidant assays would build usefully on the present comparison [17,23].

5. CONCLUSION

Under standardised laboratory conditions, ethanolic extraction of *M. oleifera* leaves recovered substantially and significantly more total phenolics (approximately 1.6-fold) and total flavonoids (approximately 1.8-fold) than aqueous extraction, with very large effect sizes and non-overlapping confidence intervals. Both extracts were qualitatively rich in secondary metabolites, but the

quantitative advantage of ethanol reflects the moderately polar nature of the leaf polyphenols and its superior ability to solubilise them. For low-cost nutraceutical and functional-food applications of drumstick in South India—where the plant is widely cultivated and consumed—ethanol or hydroethanolic mixtures are the preferable solvents when maximal polyphenol recovery is the goal.

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