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Effect of Solvent Polarity on Extraction Yield, Phytochemical Composition, and Antioxidant Activity of *Curcuma xanthorrhiza* Roxb. and *Moringa oleifera* Lam.

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Abstract

The present study investigated the effect of solvent polarity on extraction yield, phytochemical composition, and antioxidant activity of *Curcuma xanthorrhiza* Roxb. rhizomes and *Moringa oleifera* Lam. leaves. Maceration was carried out using ethanol, acetone, ethyl acetate, and n-hexane for 72 hours (1:10 w/v). Extraction yield, total phenolic content (TPC), total flavonoid content (TFC), and DPPH radical scavenging activity were determined. Results demonstrated a clear polarity-dependent trend. Ethanol yielded the highest extract recovery for both species (17.55 ± 0.97% and 22.93 ± 0.65%), while n-hexane showed the lowest yield. Ethanol extracts exhibited the greatest TPC and TFC values, 47.12 mg GAE/g and 6.76 mg QE/g for *C. xanthorrhiza*, and 25.91 mg GAE/g and 4.67 mg QE/g for *M. oleifera*, respectively. Correspondingly, ethanol fractions displayed the strongest antioxidant activity with IC_{50} values of 22.70 and 29.80 mg/mL, indicating an inverse correlation between phenolic load and radical scavenging capacity. The study confirms that solvent polarity is a critical determinant of phytochemical recovery and antioxidant potency. The novelty of this work lies in the first comparative evaluation of *C. xanthorrhiza* and *M. oleifera* extracted under identical solvent systems, providing a rational framework for solvent selection in phytopharmaceutical and nutraceutical applications. Further work should isolate and characterize the active antioxidant constituents from the most potent extracts.

Keywords: antioxidant activity; *Curcuma xanthorrhiza*; extraction yield; *Moringa oleifera*; solvent polarity.

Abbreviations: TPC = total phenolic content; TFC = total flavonoid content; DPPH = 2,2-diphenyl-1-picrylhydrazyl; ABTS = 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); GAE = gallic acid equivalent; QE = quercetin equivalent.

INTRODUCTION

Oxidative stress, largely stemming from the excessive generation of reactive oxygen species (ROS), is widely recognized as a fundamental contributor to cellular damage and a spectrum of chronic diseases, including cancer, diabetes, and cardiovascular disorders. The quest for effective natural antioxidants has thus sparked substantial interest in plant-derived products, which often contain high levels of phytochemicals such as polyphenols and flavonoids capable of neutralizing free radicals and modulate oxidative pathways (El-Sherbiny et al., 2024; Haroen et al., 2025; Lister et al., 2025). Within this context, the exploration of medicinal plants as potential antioxidant reservoirs has gained prominence in the search for safer, sustainable alternatives to synthetic antioxidants.

Curcuma xanthorrhiza Roxb. (Javanese turmeric) and *Moringa oleifera* Lam. are two medicinal plants widely utilized for their diverse bioactive compounds and pronounced antioxidant activities. *C. xanthorrhiza*, prominent in traditional Indonesian medicine, is noted for its curcuminoids, xanthorrhizol, and array polyphenolics that exhibit potent antioxidant, anti-inflammatory, and hepatoprotective properties (Lister et al., 2025; Rosidi et al., 2025; Yusnira & Ediputra, 2025). Beyond its ethnomedicinal role, *C. xanthorrhiza* is integrated into daily life as a culinary spice and beverage ingredient, and it is extensively exploited in pharmacology, functional foods, and cosmeceutical formulations. Aqueous rhizome extracts are rich in primary and secondary metabolites and exhibit a broad spectrum of biological activities, and this attributable to their phytochemical repertoire (Abd Rashid et al., 2022). Likewise, *M. oleifera*, frequently referred to as the miracle tree, similarly boasts a rich profile of phenolics and flavonoids, particularly in its leaves, which correlate strongly with its robust antioxidant, antibacterial, and cytoprotective properties (El-Sherbiny et al., 2024; Haroen et al., 2025; Liga et al., 2025). The leaves were

reported to contain higher levels of minerals, vitamins, and essential phytochemicals than other parts (Garofalo et al., 2024). Although both species are recognized for their antioxidant potential, comparative evaluation under varying solvent polarity conditions remains limited.

Efficient extraction of these phytochemicals is crucial for optimizing the therapeutic potential of plant extracts (Sun et al., 2025). Among various factors influencing extraction yield and composition, solvent polarity is regarded as the most influential. The principle of like dissolves like governs the solubility and yield of different classes of phytochemicals. Polar solvents such as methanol, ethanol, water are particularly effective in extracting hydrophilic compounds such as polyphenols and flavonoids, while semi-polar and non-polar solvents such as ethyl acetate or n-hexane favor lipophilic constituents (Arya et al., 2025; Tourabi et al., 2025). Furthermore, the use of intermediate polarity or mixed-solvent systems has been shown to enhance the simultaneous extraction of a broader spectrum of bioactives, leading to increased yield and bioactivity (Jaglan et al., 2024).

Recent studies explicitly support the crucial role of solvent polarity in maximizing extraction efficiency, antioxidant capacity, and phytochemical content of medicinal plant extracts. For instance, ethanol and ethyl acetate have demonstrated superior extraction of phenolic and flavonoid compounds from *C. xanthorrhiza*, yielding extracts with enhanced free radical scavenging ability (Haroen et al., 2025; Jonathan & Ananingsih, 2025). Similarly, ethanolic and methanolic extracts of *M. oleifera* leaves present higher concentrations of phytochemicals and antioxidant activities in DPPH and ABTS assays compared to less polar extracts (Geleta et al., 2025). Despite their extensive pharmacological use, direct comparative studies under standardized solvent conditions remain scarce

Given these considerations, the present study aims to elucidate the influence of solvent polarity on the extraction yield, antioxidant activity, and phytochemical composition of *C. xanthorrhiza* Roxb. and *M. oleifera* Lam. By conducting a parallel evaluation using solvents of differing polarity, this work provides a comparative understanding of solute–solvent interactions in two phytochemically rich medicinal species, offering valuable insights for future optimization of extraction strategies and phytopharmaceutical development.

MATERIALS AND METHODS

Materials

The rhizomes of *C. xanthorrhiza* Roxb. and the leaves of *M. oleifera* Lam. were collected from Deli Serdang, North Sumatra, Indonesia. Analytical-grade solvents including ethanol, ethyl acetate, acetone, and n-hexane (Merck, Germany) were employed for extraction. All other reagents were of analytical purity, including magnesium powder, ferric chloride ($FeCl_3$) 5%, hydrochloric acid (HCl) 37%, Dragendorff's, Mayer's, and Bouchardat's reagents, Liebermann–Burchard and Salkowski reagents, ammonia solution (25%), and distilled water.

Collection and Identification of Plant Materials

Fresh rhizomes of *C. xanthorrhiza* and leaves of *M. oleifera* were harvested in August 2025 from Kenangan Baru Village, Percut Sei Tuan District, Deli Serdang Regency, North Sumatra, Indonesia (3°36′23.7″N, 98°42′30.2″E). Taxonomic identification was performed in the Herbarium Medanense, Universitas Sumatera Utara, and authenticated by a botanist. Voucher specimens were deposited under accession numbers 1152/MEDA/2025 (*C. xanthorrhiza* Roxb.) and 1153/MEDA/2025 (*M. oleifera* Lam.). The plant materials were washed with tap water to remove debris, air-dried under shade at ambient temperature (25–28 °C) for seven days, and milled to fine powder using a mechanical grinder. The powdered samples were stored in airtight containers at room temperature until further analysis.

Preparation of Plant Extracts

Extraction was performed separately for each plant using maceration according to the method of Harborne, (2008) with minor modifications. Twenty five grams (25 g) of powdered sample were soaked in 250 mL of solvent (ethanol, ethyl acetate, acetone, or n-hexane; 1:10 w/v) in sealed Erlenmeyer flasks for 72 h at room temperature with intermittent agitation. The mixtures were filtered through Whatman No. 1 filter paper, and the filtrates were concentrated under reduced pressure using a rotary evaporator at 40 °C.

To ensure complete removal of residual solvent and prevent oxidation of thermolabile constituents, the semi-solid extracts were further evaporated under a gentle stream of nitrogen gas (N_2). The resulting crude extracts were subsequently air-dried at ambient temperature, weighed, and stored at 4 °C in amber glass vials until further use.

Determination of Extraction Yield

The extraction yield was determined following the method described by Aryanti et al., (2025). The yield was expressed as the percentage ratio between the weight of the dried extract obtained after solvent evaporation and the initial dry weight of the plant material. All yields were calculated on a dry weight basis (w/w) and recorded separately for each solvent type.

Preliminary Phytochemical Screening

Qualitative phytochemical screening was conducted on all solvent extracts of *C. xanthorrhiza* and *M. oleifera* to

identify the major classes of secondary metabolites, including alkaloids, flavonoids, phenolics, terpenoids, steroids, and saponins, following standard procedures with minor modifications (Dubale et al., 2023; Mohamed et al., 2025; Rajkumar et al., 2022).

Detection of alkaloids (Dragendorff's test)
Approximately 0.05 g of each extract was dissolved in 1 mL of methanol and filtered. The filtrate was combined with 2 mL of 1% hydrochloric acid and one drop (≈50 µL) of Dragendorff's reagent. The mixture was gently shaken and allowed to stand for 2 minutes. Formation of an orange-red or reddish-brown precipitate confirmed the presence of alkaloids, resulting from the complexation of bismuth iodide with the nitrogen atom of alkaloid molecules. A reagent blank without extract served as the negative control. The same procedure was also applied using Mayer's and Bouchardat's reagents to confirm the presence of alkaloids through characteristic white-yellowish and orange-brown precipitates, respectively.

Detection of flavonoids (Alkaline reagent test)
A 0.05 g sample of each extract was dissolved in 1 mL of methanol and treated with two drops of 10% sodium hydroxide (NaOH). After 1 minute, several drops of 37% hydrochloric acid (HCl) were added. A transient yellow coloration that disappeared upon acidification indicated the presence of flavonoids, due to the formation and neutralization of flavonoid anions.

Detection of phenolics (Ferric chloride test)
Two milliliters of 15% ferric chloride ($FeCl_3$) solution were added to 1 mL of each extract and mixed thoroughly. The appearance of a dark green color denoted phenolics, whereas a blue-black coloration indicated the presence of tannins.

Detection of triterpenoid (Salkowski reaction)
Five milligrams (5 mg) of extract were dissolved in 1 mL of chloroform (Merck, Germany) and sonicated at 40 kHz for 5 minutes. The solvent was then evaporated to dryness, and 1 mL of concentrated sulfuric acid (96%) was carefully added along the test tube wall. The solution was heated in a water bath at 75 °C for 2 minutes. The formation of a gray or brownish ring at the interface confirmed the presence of terpenoids.

Detection of saponins (Foam test)
An aliquot of 1 mL of extract was dissolved in 8 mL of hot distilled water, filtered, and transferred into a test tube. Two milliliters of hot distilled water were added, and the mixture was vigorously shaken for 30 seconds. Persistent frothing of 1–10 cm in height that lasted for at least 10 minutes and remained stable upon addition of one drop of concentrated HCl indicated the presence of saponins.

Determination of Total Phenolic Content (TPC)
The total phenolic content of each solvent extract was quantified using the Folin–Ciocalteu colorimetric assay with slight modifications of (Mohamed et al., 2025). Approximately 10 mg of the dried extract was dissolved in 10 mL of methanol (99.9 %) and homogenized. The solution was centrifuged at 3000 rpm for 15 min to obtain a clear supernatant. An aliquot of 0.4 mL of this supernatant was mixed with 0.4 mL of Folin–Ciocalteu reagent (diluted 1:1 v/v with distilled water) and allowed to stand for 5 min in the dark. Subsequently, 4.2 mL of sodium carbonate solution (5 % w/v) was added, and the mixture was vortexed for 30 s and incubated for 30 min at room temperature (25 ± 2 °C). The absorbance was measured at 760 nm using a UV–Vis spectrophotometer against a reagent blank.

A standard calibration curve was constructed using gallic acid solutions in the range of 0–140 mg L^{-1} (R^2 = 0.9667). The total phenolic content of each extract was determined from the calibration curve and expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE/g). The concentrations of phenolics were derived from the linear relationship between absorbance and gallic acid concentration using the regression parameters obtained from the standard curve.

Determination of Total Flavonoid Content (TFC)
Total flavonoid content was estimated by the aluminum chloride ($AlCl_3$) colorimetric method. Ten milligrams of each extract were dissolved in 10 mL of ethanol (99.9 %) and homogenized thoroughly. The solution was centrifuged at 3000 rpm for 15 min, and the supernatant was used for color development. An aliquot of 0.5 mL of the supernatant was mixed with 2 mL of distilled water and 0.15 mL of sodium nitrite solution (5 % w/v). After 5 min, 0.3 mL of aluminum chloride solution (10 % w/v) was added, followed by 2 mL of sodium hydroxide (1 % w/v). The total volume was adjusted to 10 mL with distilled water, vortexed, and incubated at room temperature for 30 min. Absorbance was read at 415 nm using a UV–Vis spectrophotometer against a reagent blank containing all reagents except extract.

A standard calibration curve was prepared using quercetin solutions ranging from 0 to 140 mg L^{-1} (R^2 = 0.9825). The total flavonoid content of each extract was calculated from the regression line of the standard curve and expressed as milligrams of quercetin equivalent per gram of extract (mg QE/g). The flavonoid concentrations were derived from the linear relationship between absorbance and quercetin concentration based on the corresponding regression parameters. Pearson correlation coefficients (r) were computed to evaluate linear associations between total phenolic content, total flavonoid content, and antioxidant activity (IC_{50}). The strength and direction of the correlations were interpreted based on standard statistical criteria. Calculations were performed using Microsoft Excel 365 2024.

RESULTS AND DISCUSSION

Extraction Yield of Different Solvents

Extraction yield reflects the overall efficiency of solvent systems in recovering extractable constituents from plant matrices and serves as a preliminary indicator of extraction performance. Since the solubility of phytochemicals varies with solvent polarity, solvents of differing polarity often yield distinct quantities of extractable material. In this study, maceration was conducted using four solvents (ethanol, acetone, ethyl acetate, and n-hexane) to evaluate how solvent polarity influences the total extract recovery from *C. xanthorrhiza* rhizomes and *M. oleifera* leaves.

Extraction yield data obtained from maceration (1:10 w/v, 72 h) revealed a consistent solvent-dependent pattern. Ethanol produced the highest extract recovery for both *C. xanthorrhiza* rhizomes and *M. oleifera* leaves, whereas n-hexane gave the lowest yields (Figure 1). For *C. xanthorrhiza*, mean yields were 17.55 ± 0.97% for ethanol, 15.32 ± 0.77% for ethyl acetate, 15.34 ± 1.14% for acetone, and 9.64 ± 1.63% for n-hexane. While in *M. oleifera*, ethanol extraction yielded 22.93 ± 0.65%, followed by acetone (11.23 ± 5.11%), ethyl acetate (11.07 ± 2.98%), and n-hexane (8.05 ± 0.35%). When compared to n-hexane, ethanol increased extraction recovery by approximately 81.9% for *C. xanthorrhiza* and 184.9% for *M. oleifera*, equivalent to 1.8- and 2.9-fold higher yields, respectively. These data clearly demonstrate that solvent polarity plays a crucial role in determining extraction efficiency, with ethanol being the most effective solvent under maceration conditions.

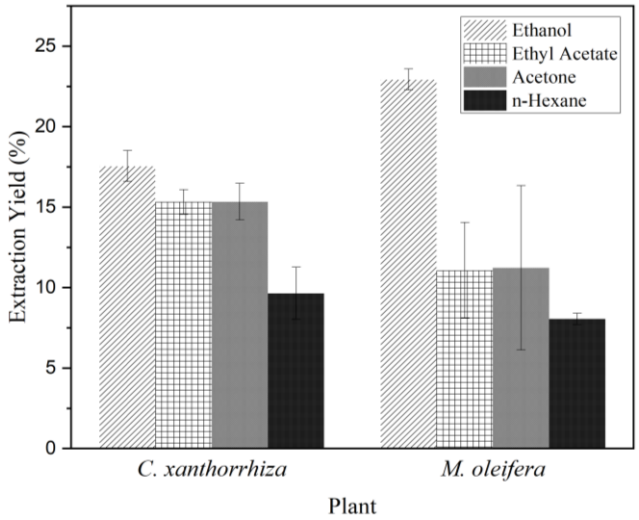

Figure 1. Extraction yield (%) of *Curcuma xanthorrhiza* rhizomes and Moringa oleifera leaves obtained by maceration (1:10 w/v, 72 h) using four solvents of differing polarity. Error bars represent standard deviations of duplicate determinations (n = 2).

This pattern aligns with previous reports showing that polar protic solvents, particularly ethanol and aqueous ethanol mixtures, generally yield higher extract recoveries from both leafy and rhizomatous plant matrices. Such solvents effectively solubilize a wide range of polar and moderately polar compounds while promoting cell-wall swelling and diffusion. Comparative extraction studies on phenolic-rich plants support this observation, confirming that ethanol and other polar solvents often achieve greater total extract weight and phenolic recovery than nonpolar solvents (Alara et al., 2021; Borges et al., 2020).

The observed increase in extraction yield with more polar solvents can be explained by molecular-level interactions between the solvent and plant metabolites. Mechanistically, extraction yield is largely governed by the polarity of the solvent and the strength of solute–solvent interactions (Chathoth et al., 2025; Nawaz et al., 2020). Solvents with high polarity and strong hydrogen-bonding ability are more efficient at dissolving polar phytochemicals such as phenolics, flavonoid glycosides, and other oxygenated secondary metabolites. Conversely, nonpolar solvents tend to extract lipophilic constituents, including fatty acids, terpenes, and hydrocarbons, which are present in smaller quantities in most medicinal plant matrices (Bitwell et al., 2023).

Ethanol, a polar protic solvent, exhibits both hydrogen-bond donor and acceptor properties, enabling it to interact with a wider range of chemical constituents than n-hexane, which is completely nonpolar (Shi et al., 2022). Consequently, ethanol enhances the solvation of diverse phytochemical classes and facilitates diffusion through plant cell walls. Moreover, solvent physical characteristics particularly dielectric constant, viscosity, and surface tension collectively influence the solvent's ability to penetrate the plant matrix and desorb intracellular compounds, thereby determining overall extraction efficiency (Acree & Lang, 2023; Dai & Mumper, 2010; Shi et al., 2022).

Nevertheless, solvent polarity is not the only determinant of yield. Several comparative studies report exceptions in which medium-polarity solvents or even nonpolar solvents produce higher yields for particular matrices or target classes (Alara et al., 2021; Bitwell et al., 2023; Tripathi et al., 2025). For example, acetone or hexane may give larger yields when the sample is rich in lipids, waxes or nonpolar terpenoids, conversely, aqueous ethanol mixtures often outperform absolute ethanol for recovering phenolics because a small fraction of water improves solute diffusivity and matrix swelling (Quitério et al., 2022; Zarrinmehr et al., 2022). These context-dependent outcomes have been observed across crops and medicinal plants and are emphasized in recent comparative reviews. Therefore, while the dominant yields in ethanol solvent are expected have rich phenolic compound in matrices such as *M. oleifera* and *C. xanthorrhiza*, specific compound classes such as volatile oils or nonpolar terpenes could be better recovered by less polar solvents in targeted extractions.

Comparative literature on each species reinforces the results. Reports on *M. oleifera* commonly show that

hydroalcoholic and polar organic solvents produce the highest extract yields and phenolic recovery, with consequent stronger antioxidant activity in DPPH and related assays (Pop et al., 2022). For *Curcuma* species, polar solvent extracts typically yield higher total extract mass and capture curcuminoids and phenolics effectively, although the optimal solvent strength depends on the matrix and target analytes (Budiansyah et al., 2023). These parallels validate the present observations and justify ethanol as an appropriate general-purpose extraction solvent for the dual aims of high yield and recovery of antioxidant phytochemicals.

From a practical standpoint, solvent selection should correspond to the intended extraction goal. For broad recovery of phenolic antioxidants, ethanol in aqueous mixtures between 40 and 80% is preferable because it offers an effective balance of polarity, safety, and compatibility with downstream analysis (Urías-Orona et al., 2017). Studies have shown that water–ethanol mixtures often yield higher total phenolic content and antioxidant activity than absolute ethanol due to improved solute diffusion and matrix swelling. In contrast, nonpolar solvents such as n-hexane or dichloromethane are suitable for isolating volatile oils and fatty acids, although their total extract yields are generally lower and less correlated with antioxidant capacity (Quitério et al., 2022). Extraction efficiency also depends on parameters such as particle size, extraction time, temperature, and solvent-to-solid ratio (Jaglan et al., 2024). Employing optimization techniques such as response surface methodology can help determine the most effective combination of factors for maximizing yield and antioxidant recovery. It should be noted that extraction yield alone is not a direct indicator of bioactive content, therefore, yield data must be interpreted alongside phytochemical and bioactivity analyses. The present results, obtained through maceration at room temperature using organic solvents, may differ under hydroalcoholic or advanced extraction systems such as sonication, Soxhlet, or accelerated solvent extraction.

Phytochemical Composition and Solvent Polarity Effects

Qualitative phytochemical screening was performed to identify the major classes of secondary metabolites present in the extracts and to examine how solvent polarity influenced their distribution. The colorimetric and precipitation assays followed established phytochemical protocols with slight modifications (Alara et al., 2021). These methods remain widely used as preliminary approaches to characterize plant metabolites and to guide further chromatographic and spectrophotometric analysis. The qualitative distribution of secondary metabolites in the extracts of *C. xanthorrhiza* and *M. oleifera* is summarized in Table 1. while the corresponding heatmap visualization (Figure 2) illustrates the solvent-dependent variation in metabolite intensity across phytochemical classes.

Table 1. Preliminary phytochemical screening of C. xanthorrhiza rhizomes and M. oleifera leaves extracted with solvents of varying polarity.

Phytochemical	*M. oleifera*				*C. xanthorrhiza*			
	Ethanol	Ethyl acetate	Acetone	n-Hexane	Ethanol	Ethyl acetate	Acetone	n-Hexane
Flavonoids (yellow color)	++	+	+	–	++	++	++	–
Phenolics (blue color)	++	+	+	–	++	++	++	–
Alkaloids (Mayer, whitish precipitate)	++	+	+	+	+	+	+	+
Alkaloids (Dragendorff, reddish-brown precipitate)	++	++	++	+++	+	+	+	–
Alkaloids (Bouchardat, orange-brown precipitate)	+++	++	+	+	+	+	+	–
Triterpenoids (reddish-brown precipitate)	+++	+++	++	+	++	+	+	+
Saponins (foam test)	+	–	–	–	+	–	–	–

Symbols represent relative intensity: + = low, ++ = moderate, +++ = high, and – = not detected.

Results are based on single determinations and indicate observed distribution trends rather than statistically validated frequencies.

The screening revealed a solvent-dependent pattern of metabolite distribution. Polar ethanol fractions produced the strongest reactions for flavonoids, phenolics, and saponins, while nonpolar n-hexane extracts were largely inactive. Ethanol yielded the most intense coloration for both plants, consistent with the known solubility of phenolic hydroxyl-bearing metabolites in polar protic solvents (Pop et al., 2022). The absence of these classes in n-hexane indicates that nonpolar solvents fail to extract hydrophilic compounds. Similar findings were reported for ethanol and aqueous-ethanol extracts of *Dracaena angustifolia, Moringa* and *Curcuma* species, where polar solvents efficiently solubilized polyphenols and glycosides (Karta et al., 2024; Nurcholis et al., 2023; Pop et al., 2022).

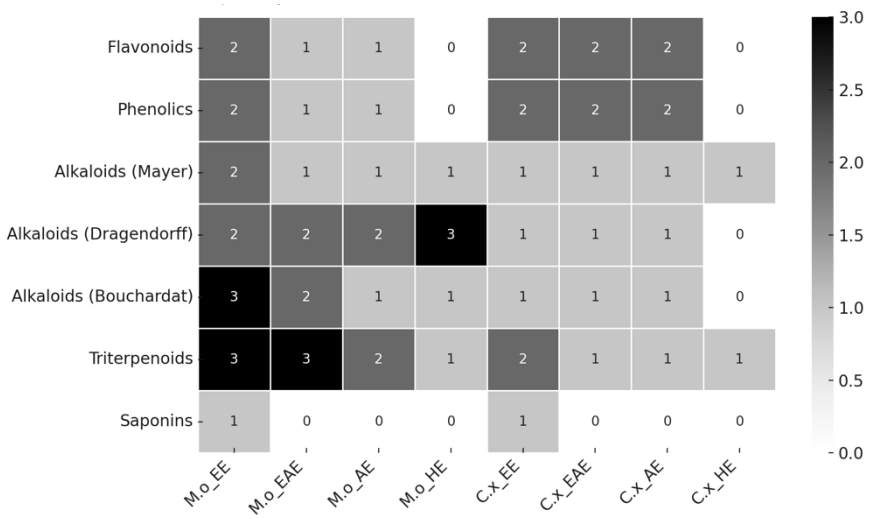

Figure 2. Heatmap visualization of the semi-quantitative phytochemical distribution in *M. oleifera* (M.o) leaves and *C. xanthorrhiza* (C.x) rhizomes extracted with solvents of differing polarity. Grayscale intensity represents metabolite abundance on a qualitative scale; EE: ethanol extract; EAE: ethyl acetate extract; AE: acetone extract; HE: n-hexane extract.

Triterpenoids exhibited moderate to strong reactions across both polar and medium-polarity solvents, consistent with their amphiphilic nature. The mixed solubility of these metabolites explains why ethyl acetate and acetone extracts retained notable triterpenoid presence, while n-hexane yielded only weak reactions. The detection of alkaloids in both polar and medium-polarity fractions suggests a diverse alkaloid profile containing bases of differing polarity. The strong Dragendorff reaction observed in the n-hexane fraction of *M. oleifera* may result from the partial extraction of lipophilic alkaloid free bases or from cross-reactivity with nitrogen-containing terpenoids (Nazar et al., 2020; Ngo et al., 2023).

The selective detection of saponins exclusively in the ethanol extracts is chemically consistent with their polar glycosidic structure, as these compounds require a polar solvent to disrupt cell membranes and release surfactant-active metabolites. Previous work on *Curcuma longa* and *M. oleifera* leaves also demonstrated that ethanol and methanol extracts contain abundant saponins, phenolics, and flavonoids compared with less polar solvents (Fachriyah et al., 2020; Grover et al., 2021; Royani et al., 2023; Wihanto et al., 2023).

These findings confirm that solvent polarity plays a decisive role in determining the recovery of functional phytochemical classes. Polar solvents facilitate the extraction of hydrophilic antioxidant metabolites such as phenolics, flavonoids, and saponins, whereas nonpolar solvents primarily recover lipophilic constituents like terpenes and hydrocarbons. This solvent-dependent distribution pattern establishes a chemical basis for the quantitative variations observed in total phenolic and flavonoid contents presented in the following section. A comprehensive evaluation of these quantitative parameters will further clarify how solvent polarity not only governs extract composition but also modulates antioxidant potency across both species.

Total Phenolic and Flavonoid Contents

Quantitative determination of total phenolic content and total flavonoid content refines the qualitative patterns observed earlier and provides a basis for predicting antioxidant potential. Comparative values for both species and solvents are presented in Table 2 and show a consistent decline in both phenolic and flavonoid totals as solvent polarity decreases, ethanol producing the highest values and n-hexane the lowest. This solvent-dependent trend in class totals is commonly reported in extraction studies and reviews.

For *M. oleifera* leaves the measured total phenolic content was 25.91 mg GAE/g in the ethanol extract, 18.78 mg GAE/g in acetone, 17.03 mg GAE/g in ethyl acetate and 16.98 mg GAE/g in n-hexane. Total flavonoid contents in the same order were 4.67, 4.16, 3.94 and 3.37 mg QE/g. For *C. xanthorrhiza* rhizomes the corresponding phenolic totals were 47.12, 42.62, 37.12 and 35.62 mg GAE/g and flavonoid totals 6.76, 6.14, 5.28 and 4.90 mg QE/g. These profiles confirm that polar ethanol preferentially extracts phenolic and flavonoid constituents under the present maceration conditions (Dirar et al., 2019; Haroen et al., 2022).

To quantify the solvent effect, ethanol versus n-hexane relative increases in total phenolic content are 52.6 percent for *M. oleifera* and 32.3 percent for *C. xanthorrhiza*. Relative increases in total flavonoid content for ethanol versus n-hexane are 38.5 percent for *M. oleifera* and 38.0 percent for *C. xanthorrhiza*. These calculated differences indicate that flavonoid extraction responds similarly to solvent polarity in both species while the bulk phenolic response is proportionally larger in *M. oleifera*. Species specific chemistry and matrix factors commonly explain such proportional differences (Haroen et al., 2022; Yodi et al., 2023).

Table 2. Total phenolic and flavonoid contents of *M. oleifera* leaves and *C. xanthorrhiza* rhizomes extracted using solvents of varying polarity.

Plant	Solvent	Total Phenolic Content (mg GAE/g extract)	Total Flavonoid Content (mg QE/g extract)
M. oleifera	Ethanol	25.91	4.67
	Acetone	18.78	4.16
	Ethyl acetate	17.03	3.94
	n-Hexane	16.98	3.37
C. xanthorrhiza	Ethanol	47.12	6.76
	Acetone	42.62	6.14
	Ethyl acetate	37.12	5.28
	n-Hexane	35.62	4.90

Values are expressed as single determinations. GAE: gallic acid equivalent; QE: quercetin equivalent. The data illustrate solvent-dependent trends rather than statistically validated differences.

The markedly higher absolute TPC and TFC measured in *C. xanthorrhiza* merit specific consideration. Rhizomes of Curcuma species are known to accumulate curcuminoids and other oxygenated phenolic sesquiterpenes that react strongly in the Folin–Ciocalteu and aluminum chloride assays, which can drive elevated spectrophotometric totals compared with leafy matrices (Segneanu et al., 2022; Widyastuti et al., 2020). Variation in cultivar, harvest time, drying and sample preparation further modulates absolute concentrations and can produce interstudy differences in reported totals (Suryani et al., 2022). Consequently elevated spectrophotometric totals in rhizome extracts are chemically plausible but require compound-level confirmation.

Methodological caveats limit the interpretation of spectrophotometric totals. The Folin–Ciocalteu assay measures total reducing capacity and therefore responds to nonphenolic reducing agents such as ascorbic acid and certain oxygenated metabolites, which can inflate apparent phenolic values (Torres et al., 2024). The aluminum chloride colorimetric method is selective for certain flavonoid structural classes and its response varies with substitution pattern and coextractives (Dominguez-López et al., 2024). For these reasons TPC and TFC should be regarded as comparative class-level indicators rather than absolute molar inventories. Confirmatory chromatographic analysis using HPLC with diode array detection and LC–MS is recommended to identify and quantify the individual phenolics and flavonoids responsible for the observed totals.

The practical significance of these quantitative findings lies in guiding solvent selection for targeted phytochemical recovery. Ethanol and aqueous ethanol systems are recommended for the efficient extraction of antioxidant phenolics and flavonoid glycosides, whereas solvents of intermediate polarity may preferentially concentrate certain triterpenoids, and nonpolar solvents tend to recover volatile constituents that contribute little to overall phenolic yield. Prior to formulation or preparative applications, replicate extractions and appropriate statistical validation are essential to confirm the consistency of these patterns and substantiate any comparative claims regarding solvent performance. The following section explores how these quantitative phenolic and flavonoid profiles relate to the antioxidant potency of each extract.

Effects Of Solvents On Antioxidant Activity

The antioxidant activity of all solvent extracts was evaluated using the DPPH radical scavenging assay, and the results are summarized in Table 3. The IC_{50} value, defined as the concentration required to inhibit 50% of DPPH radicals, serves as an inverse indicator of antioxidant potency, where lower IC_{50} denotes stronger activity (Baliyan et al., 2022). IC_{50} values were obtained from linear regression of inhibition versus concentration plots. Under the present maceration conditions, ethanol extracts exhibited the strongest DPPH scavenging for both species, with IC_{50} values of 29.80 mg mL^{-1} for *M. oleifera* and 22.70 mg mL^{-1} for *C. xanthorrhiza*, while n-hexane fractions showed the weakest activity with IC_{50} values near 100 mg mL^{-1} for both species. These solvent trends mirror the TPC and TFC patterns reported above and are consistent with many comparative extraction studies reporting ethanol extracts exhibited the highest antioxidant capacity, whereas nonpolar fractions displayed the weakest response, confirming that solvent polarity substantially influences antioxidant performance (Haroen et al., 2025; Segwatibe et al., 2023).

To examine quantitative relationships between phytochemical load and radical scavenging, Pearson correlation coefficients (r) were calculated between spectrophotometric totals and IC_{50} across the four solvents for each species (Figure 3). For *M. oleifera* the correlation between total flavonoid content and IC_{50} was strong and negative, $r = -0.96$, indicating that higher flavonoid content is associated with lower IC_{50} and therefore greater scavenging potency; the corresponding correlation between total phenolics and IC_{50} was moderately negative, $r = -0.68$. For *C. xanthorrhiza* strong negative correlations were observed as well, $r = -0.85$ for total phenolics versus IC_{50} and $r = -0.89$ for total flavonoids versus IC_{50}. When the data for both species were pooled the correlations weakened, $r = -0.42$ for phenolics and $r = -0.63$ for flavonoids, this attenuation indicates that the strength of these relationships is species-specific, reflecting distinct

phytochemical compositions and antioxidant mechanisms between M. oleifera and C. xanthorrhiza. These results are consistent with previous reports demonstrating inverse correlations between spectrophotometric phenolic metrics and DPPH IC_{50} while emphasizing that the strength of the relationship depends on the chemical identity of the phenolics present (Ouamnina et al., 2024; Rangani et al., 2023).

Table 3. Antioxidant activity (IC_{50}) of *M. oleifera* leaf and *C. xanthorrhiza* rhizome extracts obtained with solvents of varying polarity, and their correlation with total phenolic and flavonoid contents.

Plant	Solvent	IC_{50} (mg/mL)	Antioxidant Activity	r (TPC vs IC_{50})	r (TFC vs IC_{50})
M. oleifera	Ethanol	29.80	Strong	−0.68	−0.96
	Acetone	44.71	Moderate		
	Ethyl acetate	52.90	Moderate		
	n-Hexane	100.00	Weak		
C. xanthorrhiza	Ethanol	22.70	Strong	−0.85	−0.89
	Acetone	30.83	Moderate		
	Ethyl acetate	48.14	Moderate		
	n-Hexane	93.94	Weak		

Lower IC_{50} values indicate higher antioxidant potency. "Antioxidant activity" is classified as: <50 mg/mL = strong, 50–100 mg/mL = moderate, and >100 mg/mL = weak. Pearson correlation coefficients (r) represent linear relationships between total phenolic content (TPC) or total flavonoid content (TFC) and IC_{50} for each species. All values are based on single determinations; correlations are indicative and not inferential due to limited replication.

The inverse correlations depicted in Figure 3 reinforce that phenolic and flavonoid constituents are major contributors to the antioxidant potential of both species. These metabolites act primarily through hydrogen atom transfer and single electron donation, mechanisms that underpin the DPPH radical scavenging assay. Stronger correlations for flavonoids than for total phenolics may reflect the higher reactivity of specific flavonoid subclasses, such as quercetin derivatives in *Moringa* and curcuminoids in *Curcuma*, which possess multiple hydroxyl and methoxy substituents enhancing redox potential (El-Sherbiny et al., 2024; Pop et al., 2022). Although these findings clearly demonstrate a structure–activity association, the correlations are based on single determinations and thus should be considered indicative rather than inferential. Future analyses using replicated extractions and compound-specific antioxidant testing would provide more definitive quantitative confirmation.

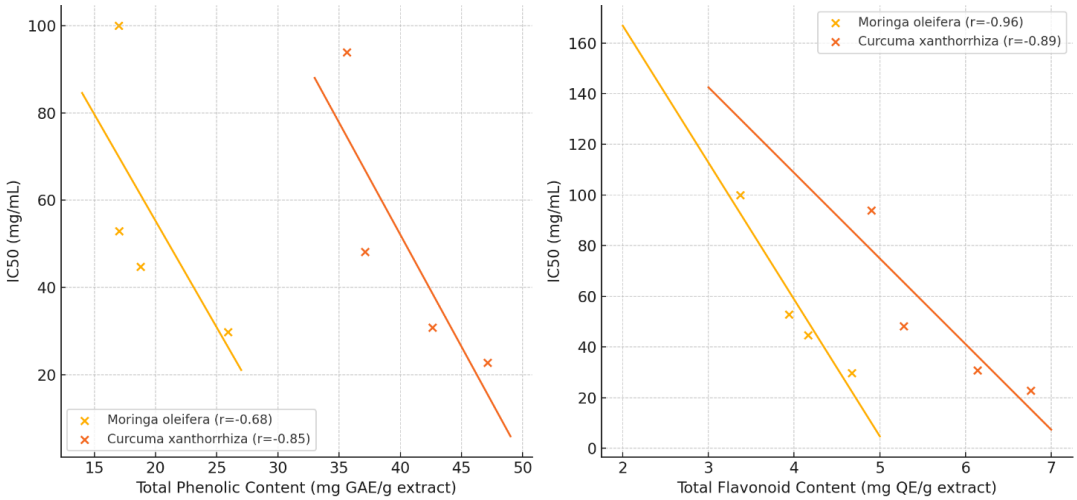

Figure 3. Scatter plots showing the relationships between (a) total phenolic content (TPC) and antioxidant activity (IC_{50}), and (b) total flavonoid content (TFC) and antioxidant activity (IC_{50}) for Moringa oleifera leaves and Curcuma xanthorrhiza rhizomes extracted with solvents of differing polarity.

Beyond simple totals, several factors can explain variance in IC_{50} across solvents and species. First, the DPPH assay measures hydrogen or electron donation in an organic medium and therefore responds differently to structural subclasses of phenolics, flavonoids with ortho-dihydroxy substitution patterns and conjugated systems typically show stronger activity than simple phenolic acids (Gulcin & Alwasel, 2023; Qi et al., 2025). This structural dependence explains why similar TPC values can have different IC_{50} depending on compound identity. Second, nonphenolic antioxidants such as ascorbic acid and certain terpenoids can contribute to DPPH

scavenging and may inflate apparent activity relative to what spectrophotometric TPC predicts (Gulcin, 2025; Setiawan et al., 2023). This is particularly relevant for rhizome extracts that may contain highly active curcuminoids and other oxygenated sesquiterpenes. Third, assay artefacts including solvent–DPPH interactions, differing extract colors, and the fixed DPPH concentration used can influence IC_{50} estimates and complicate cross-study comparisons unless standardized units or the antioxidant activity index are reported (Martinez-Morales et al., 2020). For correct interstudy comparisons, standardization of DPPH concentration and reporting of the antioxidant activity index is recommended.

The pattern observed, therefore supports a mechanistic interpretation in which polar solvent fractions, enriched in flavonoids and phenolic glycosides, achieve greater DPPH scavenging, while nonpolar fractions dominated by hydrocarbons and nonpolar terpenes exhibit weak radical scavenging. However, to move from correlation to causation it is necessary to isolate and test major constituents, for example by HPLC fractionation followed by on-line or off-line DPPH assays, and to perform replicate biological testing. Such targeted work has frequently revealed that a small number of potent molecules, rather than bulk phenolic mass alone, drive antioxidant potency in complex extracts (Al-Shebli & Al-Anbari, 2023; Deepali et al., 2025).

The overall findings indicate that solvent polarity markedly governs antioxidant performance, with polar ethanol extracts exhibiting superior radical scavenging efficiency compared to less polar fractions. These results collectively demonstrate that the distribution and reactivity of antioxidant constituents are strongly solvent-dependent, providing a mechanistic link between extract composition and bioactivity.

CONCLUSIONS

The present study demonstrated that solvent polarity plays a decisive role in determining extraction yield, phytochemical composition, and antioxidant activity in *C. xanthorrhiza* rhizomes and *M. oleifera* leaves. Among the four solvents tested, ethanol consistently produced the highest extract recovery, total phenolic, and total flavonoid contents, while n-hexane yielded the lowest values.The strong solvent-dependent pattern observed across all analyses reflects the chemical affinity between solvent polarity and metabolite solubility. Polar protic solvents such as ethanol effectively extracted hydrophilic antioxidant compounds including phenolics, flavonoids, and saponins, whereas nonpolar solvents preferentially recovered lipophilic terpenes and hydrocarbons. The quantitative data confirmed that higher phenolic and flavonoid concentrations corresponded with stronger antioxidant activity, as indicated by lower IC_{50} values in the DPPH assay, and strong negative correlations between IC_{50} and both TPC and TFC.

These findings highlight ethanol as the most suitable solvent for the broad recovery of antioxidant constituents from both species, offering a balance of extraction efficiency, safety, and bioactive compound selectivity. The results provide a scientific basis for optimizing solvent systems in phytochemical extraction and formulation processes. Future research should focus on compound-level identification through chromatographic and spectroscopic techniques, along with biological validation of the major antioxidant constituents responsible for the observed activity.

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