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Influence of Extraction Methods on Phytochemical Composition, Antioxidant, and Antibacterial Activities of *Monolluma quadrangula* from Yemen: A GC-MS Analysis

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Abstract: *Monolluma quadrangula* (Forssk.) Plowes [syn. *Caralluma quadrangula* (Forssk.) N.E.Br.] is a medicinal plant widely distributed throughout the Arabian Peninsula, particularly in Yemen, Saudi Arabia, and Oman, where it has traditionally been used to treat various ailments, including diabetes and gastric ulcers. This study aimed to evaluate the effects of three extraction methods Soxhlet extraction, ultrasound-assisted extraction, and maceration on the phytochemical composition, antioxidant potential, and antibacterial activity of methanolic extracts of *M. quadrangula*. To the best of our knowledge, this is the first study to comprehensively compare these three extraction methods for *M. quadrangula* collected from Yemen, providing baseline data on its phytochemical profile and biological activities. Phytochemical screening was performed to detect major secondary metabolites, while bioactive compounds were identified using gas chromatography mass spectrometry (GC–MS). Total phenolic and flavonoid contents were determined spectrophotometrically. Antioxidant activity was evaluated using DPPH, ABTS, and ferric reducing power assays, and antibacterial activity was assessed using the broth microdilution method. Phytochemical screening confirmed the presence of terpenoids, alkaloids, and saponins in all extracts, whereas tannins and carotenoids were not detected. GC–MS analysis identified numerous

constituents, including phenolic compounds, terpenoids, alcohols, and organic acids. The Soxhlet extract exhibited the highest extraction yield (18.3%) and total phenolic content (51.6 ± 0.05 mg GAE/g extract), whereas the ultrasound-assisted extract showed the highest flavonoid content (43.8 ± 0.02 mg RE/g extract). All extracts demonstrated antioxidant activity, with the ultrasound-assisted extract showing the strongest activity according to the DPPH, ABTS, and FRAP assays, with values of 169.27 ± 1.19 μg/mL, 1404.69 ± 2.16 μg/mL, and 47.46 ± 0.02 mg TE/g extract, respectively. In the antibacterial assays, the Soxhlet extract showed notable inhibitory activity against *Escherichia coli* CIP 53126 and *Pseudomonas aeruginosa* CIP 82118, whereas the ultrasound-assisted extract showed the greatest activity against *Bacillus subtilis* CIP 5262, with a minimum inhibitory concentration (MIC) of 15.6 mg/mL. Overall, the extraction method significantly influenced the phytochemical composition and biological activities of the extracts. Soxhlet and ultrasound-assisted extraction produced the most promising results for different measured parameters, particularly extraction yield, phenolic content, flavonoid content, antioxidant activity, and antibacterial activity. These findings provide baseline evidence supporting further investigation of this species as a potential source of natural antioxidant and antibacterial agents. Future studies should focus on isolating and characterizing the principal bioactive constituents, elucidating their mechanisms of action, and evaluating their safety and efficacy in relevant biological models before potential pharmaceutical or nutraceutical applications are considered.

Keywords: *Monolluma quadrangula*; GC–MS; extraction methods; antioxidant activity; antibacterial activity

1. Introduction

Medicinal plants have played a central role in traditional healthcare for centuries and remain important sources of bioactive compounds with therapeutic potential [1]. The potential of plant-derived materials as natural sources of antioxidants has attracted increasing attention, particularly in response to concerns regarding the safety of synthetic antioxidants and the growing demand for naturally derived alternatives [2]. Naturally occurring phenolic compounds, particularly phenolic acids and flavonoids, have received considerable attention because of their antioxidant properties and potential role in mitigating oxidative stress [3,4].

Oxidative stress results from an imbalance between the production of reactive oxygen species and the capacity of antioxidant defenses to counteract them and has been associated with aging and several chronic disorders [3]. The extraction process is a critical step in recovering phenolic and other bioactive constituents from plant materials. Conventional approaches, such as maceration and Soxhlet extraction, and intensified techniques, such as ultrasound-assisted extraction (UAE), can differ in extraction efficiency and in the phytochemical profiles of the resulting extracts [5–7].

Monolluma quadrangula (Forssk.) Plowes [syn. *Caralluma quadrangula* (Forssk.) N.E.Br.] belongs to the family *Apocynaceae* and is a leafless succulent species distributed in the Arabian Peninsula, particularly in Yemen, Saudi Arabia, and Oman [8–10]. The genus *Caralluma* is recognized for its diverse phytochemical profile, particularly pregnane glycosides, which represent an important class of secondary metabolites reported from several species of the genus [9,10]. In *M. quadrangula*, phytochemical investigations have led to the isolation and structural characterization of acylated pregnane glycosides using mass spectrometric and nuclear magnetic resonance (NMR) analyses [11]. These findings highlight pregnane glycosides as characteristic constituents of this species and support further investigation of their contribution to its biological properties. Previous investigations have demonstrated several biological activities of *M. quadrangula*. A hydroethanolic extract of *M. quadrangula* was reported to improve glucose tolerance and insulin sensitivity in a type 2 diabetes model and to modulate glucose-metabolizing enzymes, antioxidant defenses, and adiponectin [12]. In addition, methanolic extracts and isolated pregnane glycosides from *M. quadrangula* demonstrated antibacterial and antibiofilm activities against methicillin-resistant *Staphylococcus aureus* and multidrug-resistant *Acinetobacter baumannii* [13]. The differential antibiofilm activities observed among the isolated pregnane glycosides, together with the reported structure activity relationships, suggest that specific structural features of these constituents may contribute to the inhibition of biofilm formation and detachment [13]. Understanding the phytochemical basis and potential molecular mechanisms underlying the antibacterial activity of *M. quadrangula* is important for elucidating the contribution of its bioactive constituents to their activity against clinically relevant and multidrug-resistant bacterial pathogens [13]. These findings highlight the importance of investigating the phytochemical basis underlying the biological activities of *M. quadrangula*, particularly its potential against clinically relevant bacterial pathogens.

The three extraction approaches considered in the present study differ in their underlying extraction principles. Maceration is a conventional technique based on prolonged contact between plant material and solvent, whereas Soxhlet extraction involves repeated solvent reflux and continuous contact with freshly condensed solvent, facilitating relatively exhaustive extraction. In contrast, ultrasound-assisted relies on acoustic cavitation and associated mechanical effects to enhance solvent penetration, disrupt plant tissues, and improve mass transfer, thereby facilitating the release and recovery of intracellular bioactive compounds [6,7,14]. Comparing these approaches under the same experimental conditions can therefore help determine whether differences in extraction principles influence phytochemical recovery and the resulting antioxidant and antibacterial activities.

Although different extraction approaches have been applied to *Caralluma* species, comparative evidence on how extraction technique influences phytochemical recovery and the resulting antioxidant and antibacterial activities remains limited, particularly for *M. quadrangula*. To the best of our knowledge, no comprehensive comparative study has evaluated Soxhlet, ultrasound-assisted, and maceration extraction of *M. quadrangula* from Yemen while integrating phytochemical screening, total phenolic and flavonoid contents,

antioxidant assays, antibacterial activity, and GC–MS profiling. We hypothesized that the extraction method would differentially influence the recovery of phytochemical constituents and, consequently, the antioxidant and antibacterial activities of *M. quadrangula* extracts, with ultrasound-assisted expected to favor the recovery of flavonoid-rich constituents through enhanced tissue disruption and mass transfer, whereas Soxhlet extraction was expected to promote more exhaustive recovery of solvent-soluble constituents [6,7,14]. Accordingly, the present study aimed to compare Soxhlet, ultrasound-assisted, and maceration extraction methods for methanolic extracts of *M. quadrangula* from Yemen by evaluating extraction yield, phytochemical screening, total phenolic and flavonoid contents, antioxidant activities using DPPH, ABTS, and FRAP assays, antibacterial activity, and GC–MS profiles.

2. Materials and Methods

2.1. Materials Chemicals

DPPH (purity >97%), ABTS (purity ≥ 98%; Product Code 1002624974), Folin–Ciocalteu reagent (catalog no. 03870). Rutin, catechin, Trolox, and potassium persulfate were purchased from Sigma. Trichloroacetic acid, ferric chloride, aluminum chloride, sodium acetate, and sodium carbonate were obtained from Loba Chemie. Hydrochloric acid, potassium ferricyanide, phosphate buffer solution, and sulfuric acid were also used in the study. All chemicals were of analytical grade and used without further purification. Trolox was used as the reference antioxidant standard.

2.2. Collection of Plant Material

The aerial parts of *Monolluma quadrangula* were collected from Bani Hawr, Maswar District, Amran Governorate, Yemen (15.639645° N, 43.698402° E), during the collection period (late July to mid-August 2019), the study area experienced moderate summer temperatures and seasonal rainfall. Historical climatic data for the Amran region indicated a mean temperature of approximately 23.7 °C and 116.13 mm of precipitation in July 2019 . The collection site was situated in an open mountainous habitat. At the time of collection, the plants were at the flowering stage. The plants exhibited a succulent and densely branched habit, with erect to ascending, green, fleshy, distinctly four-angled stems bearing small, reduced leaves and tuberculate projections along the stem margins. Numerous yellow, five-lobed, star-shaped flowers were present mainly toward the apical portions of the branches. These morphological characteristics were consistent with the diagnostic features of *M. quadrangula*. The botanical identification of the plant material was confirmed by Ahmed Y. Mubarak, Department of Chemistry, Faculty of Applied Sciences and Humanities, Amran University, Yemen. The collected aerial parts were dried at room temperature in a shaded and well-ventilated area and protected from direct sunlight. After complete drying, the material was ground into a fine powder using an electric grinder and stored under appropriate conditions until further use.

2.3. Preparation of Organic Extracts

M. quadrangula was extracted using three different methods: Soxhlet extraction, hot maceration, and ultrasound-assisted extraction (UAE).The dried plant material was ground into a fine powder using an electric grinder without subsequent sieving. Following extraction and concentration, the extracts were stored at 4 °C in the dark and used within 48 h.

2.3.1. Soxhlet extraction

The powdered plant material (60 g) was extracted with 450 mL of 80% methanol using a Soxhlet apparatus for 6 h at 60 °C, corresponding to a solvent-to-sample ratio of 7.5:1 (mL/g). [15]. The solvent was removed using a rotary evaporator (Heidolph G 1, Germany) at 40–50 °C. The resulting extract was filtered using Whatman No. 4 filter, with a nominal particle retention of 20–25 µm.

2.3.2. Maceration extraction

M. quadrangula powder (60 g) was extracted by hot maceration at 50 °C using 450 mL of 80% methanol, corresponding to a solvent-to-sample ratio of 7.5:1 (mL/g) [16]. The extract was filtered twice using Whatman No. 4 filter paper (pore size: 20–25 µm), and concentrated using a rotary vacuum evaporator (Heidolph G 1, Germany) at 40–50 °C.

2.3.3. Ultrasonic-assisted extraction

The powdered plant material (45 g) was separately extracted using an ultrasonic processor (UP100H, Hielscher, Germany) equipped with a 7-mm-diameter probe operating at 24 kHz. The probe was immersed approximately 1.9 cm into the extraction medium. UAE was performed for 10 min using continuous sonication (cycle 1) at 100% amplitude [17]. The temperature of the extraction mixture was monitored during sonication and reached a maximum of 40 °C.

2.4. Phytochemical screening (qualitative)

The presence of the investigated phytochemical groups was assessed visually based on characteristic colour changes, precipitate formation, or foam development, depending on the test applied. The response intensity was recorded as absent (−), weak (+), moderate (++), or strong (+++). The results were considered preliminary qualitative indications and were not interpreted as quantitative or confirmatory evidence of the tested phytochemical groups.

2.4.1. Testing for alkaloids

A test tube containing 100 mg of the extracts and 3 mL of 1% sulfuric acid is used. Everything must be boiled for 5 minutes at 100 °C in a water bath. Meyer's reagent is applied in 5 drops after chilling and filtering. Alkaloids are present when a precipitate that is white forms [18].

2.4.2. Testing for saponins

2.5 g of extracts and 5 mL of boiling distillate water were combined in a test tube, and the mixture was rapidly agitated. The presence of numerous saponins is indicated by the creation of a stable and long-lasting froth for half an hour [19].

2.4.3. Testing for terpenoids

For the detection of terpenoids, 0.5 g of the extract was mixed with 2 mL of chloroform, followed by careful addition of 3 mL of concentrated H_2SO_4 to form a separate layer. The appearance of a reddish-brown colour at the interface indicated the presence of terpenoids [20].

2.4.4. Testing for carotenoids

For the qualitative screening of carotenoids, a separate portion of 20 g of *M. quadrangula* powdered plant material was used.The powder was mixed with 150 mL of distilled water and filtered. Then, 3 mL of HCl and 3 mL of H_2SO_4 were added to the filtrate. The appearance of a blue-green colour indicated the presence of carotenoids..

2.5. Determination of Extraction Yield and Secondary Metabolites of M. quadrangula

2.5.1. Determination of extraction yield

After solvent evaporation, the obtained extract was weighed, and the extraction yield was calculated and expressed as a percentage (%, w/w) based on the ratio of the weight of the dried extract to the initial weight of the powdered plant material used for extraction, according to the following equation:

Extraction yield (%, w/w) = [Weight of dried extract (g) / Weight of powdered plant material (g)] × 100

2.5.2. Determination of total phenolic content

The total phenolic content (TPC) of the methanolic extracts was determined using the Folin–Ciocalteu method [21]. Briefly, 0.5 mL of the methanolic extract or gallic acid standard solution was mixed with 2.5 mL of diluted Folin–Ciocalteu reagent (1:10), followed by the addition of 4 mL of sodium carbonate solution (7.5%, w/v). The mixture was incubated at 45 °C for 30 min, and the absorbance was measured at 765 nm.

A calibration curve was constructed using gallic acid standards. The total phenolic content was calculated from the calibration curve and expressed as mg gallic acid equivalents (GAE) per g of extract. The assay was performed in triplicate (n = 3).

2.5.3. Determination of flavonoid content

The flavonoid content was determined using the $AlCl_3$ colorimetric method [22]. Briefly,1 mL of the methanolic extract or rutin standard solution was mixed with 200 µL of

$AlCl_3$ solution (10%, w/v), 200 µL of sodium acetate (CH_3COONa) solution (1 mol/L), and 5.6 mL of distilled water. The mixture was incubated at 25 °C for 30 min, and the absorbance was measured at 415 nm against a reagent blank. A calibration curve was prepared using rutin standard solutions. The flavonoid content was calculated from the calibration curve and expressed as milligrams of rutin equivalents per gram of extract (mg RE/g extract). The assay was performed in triplicate (n = 3).

2.5.4. Determination. of condensed tannins content

The condensed tannin content was determined using the vanillin assay with some modifications [23]. Briefly, 0.05 mL of the extract was mixed with 3 mL of vanillin solution (4%, w/v) prepared in methanol and 1.5 mL of concentrated HCl. The mixture was incubated at 25 °C for 15 min, and the absorbance was measured at 500 nm using a UV–Vis spectrophotometer, which corresponds to the absorption maximum of the coloured complex formed during the vanillin–HCl reaction against a reagent blank [23]. A calibration curve was prepared using catechin as the standard. The condensed tannin content was calculated from the calibration curve and expressed as milligrams of catechin equivalents per gram of extract (mg CE/g extract). The assay was performed in triplicate (n = 3).

For all spectrophotometric assays, reagent blanks were prepared under the same conditions as the corresponding samples. The blanks contained all reagents required for each assay, but no plant extract. The methanolic extract was replaced with an equal volume of methanol. The corresponding reagent blanks were used as references for absorbance measurements.

2.6. Antioxidant Activity

Trolox was used as the positive control and reference antioxidant standard in the DPPH radical-scavenging, ABTS radical-scavenging, and ferric reducing power assays to evaluate the antioxidant activity of the methanolic extracts of *M. quadrangula*. The free-radical scavenging activity of the extracts was assessed using the DPPH and ABTS assays, which are based on the reduction of stable radicals by antioxidant compounds present in the extracts. For both assays, antioxidant activity was evaluated in terms of the half-maximal inhibitory concentration IC_{50},defined as the concentration of extract required to inhibit 50% of radical activity. Lower IC_{50} values indicate stronger radical-scavenging activity, and the IC_{50} values were expressed as µg/mL. Ferric-reducing antioxidant power was evaluated using the ferric reducing power assay, and quantitative calculations were performed using the calibration equation obtained with Trolox as the reference standard.

2.6.1. Activity assessment by Radical DPPH

The radical-scavenging activity of the methanolic extracts against DPPH radicals was evaluated according to the reported method, with appropriate modifications. Briefly, 2.5 mL of the extracts at different concentrations (20–1000 µg/mL) was mixed with 0.5 mL of DPPH solution (0.2 mM). The mixtures were incubated for 30 min at 25 °C in the dark [24]. The

absorbance was measured at 517 nm against a methanol blank, while the DPPH solution without extract (DPPH + methanol) was used as the control. Trolox was used as the reference antioxidant standard (0.3-6 µg/mL). The DPPH radical-scavenging activity (RSA%) was calculated according to the following equation [25]:

$$\text{Scavenging effect for DPPH (\%)} = [(A_C - A_S) / A_C] \times 100$$

where A_C is the absorbance of the control (DPPH + methanol), and A_S is the absorbance of the test solution (sample). All measurements were performed in triplicate.

2.6.2. Activity assessment by radical ABTS

The ABTS radical cation decolorization assay was performed according to the method described by Aqil *et al*. [23]. Briefly, a stock solution of ABTS (2 mM) was mixed with 70 mM potassium persulfate stock solution (v/v), and the resulting solution was stored in the dark for 24 h at 25 °C to generate the ABTS•+ radical cation. Methanol was then added to the ABTS•+ solution to obtain an absorbance of 0.7 ± 0.2 at 734 nm. Subsequently, 2 mL of the resulting ABTS•+ solution was added to 200 µL of the methanolic extract at different concentrations (1200–4000 µg/mL). The mixture was thoroughly mixed, and the absorbance was measured at 734 nm after 1 min of reaction. Trolox was used as the reference antioxidant standard (1.5–30 µg/mL). All measurements were performed in triplicate.

The ABTS radical-scavenging activity was calculated using the following equation [25]:

$$\text{ABTS radical-scavenging activity (\%)} = (A_C - A_S)/A_C \times 100$$

Ac=absorbance of the negative control (ABTS•+ solution without extract)

As= absorbance of the test solution (ABTS•+ + extract)

2.6.3. IC_{50} calculation

The IC_{50} values were determined by linear interpolation from plots of radical-scavenging activity (%) versus extract concentration. For each experimental replicate, the two extract concentrations that bracketed 50% radical-scavenging activity were selected. A linear equation (Y = AX + B) was established between these two points, where Y represents radical-scavenging activity (%) and X represents extract concentration. The IC_{50} value was calculated by setting Y = 50 and solving the equation for X .The calculation was performed for each replicate, and the final IC_{50} value was expressed as mean ± standard deviation ($n=3$) and reported in µg/mL

2.6.4. Ferric reducing power assays

The ferric reducing power of the methanolic extracts was determined according to the reported method, with appropriate modifications [26]. Briefly, 0.25 mL of the methanolic extract was mixed with 1.5 mL of phosphate buffer (0.2 M, pH 6.6) and 1.5 mL of 1% (w/v) potassium

ferricyanide [$K_3Fe(CN)_6$]. The mixture was thoroughly mixed and incubated for 20 min at 50 °C in a water bath. Subsequently, 1.5 mL of trichloroacetic acid (10%) was added, and the mixture was centrifuged at 3000 rpm for 10 min. Then, 1.25 mL of the resulting supernatant was mixed with 0.25 mL of an aqueous ($FeCl_3$) solution (0.1%) and allowed to stand for 30 min at room temperature. The absorbance was then measured at 700 nm.Trolox (20–400 µg/mL) was used as the reference standard to construct the calibration curve, and the reducing power was expressed as milligrams of Trolox equivalents per gram of methanolic extract (mg TE/g extract) [26] .

The calibration curves used for the standard-based quantitative phytochemical and antioxidant assays, including the reference standards, concentration ranges, regression equations, and coefficients of determination (R^2), are summarized in Table 1.

Table 1. Calibration curves used for the standard-based quantitative phytochemical and antioxidant assays.

Assay	Reference standard	Concentration range	Calibration equation	R^2	Calculation of results
Total phenolic content	Gallic acid	10–320 µg/mL	Y = 0.0049x − 0.0469	R^2 = 0.9996	Calculated as mg GAE/g extract
Flavonoid content	Rutin	6–150 µg/mL	Y = 0.0053x − 0.0216	R^2 = 0.9990	Calculated as mg RE/g extract
Condensed tannin content	Catechin	100–500 µg/mL	Y = 0.0009x − 0.0037	R^2 = 0.9976	Calculated as mg CE/g extract
Ferric reducing power assays	Trolox	20–400 µg/mL	Y = 0.004x − 0.0254	R^2 = 0.9982	Calculated as mg TE/g extract

2.7. Antibacterial Effectiveness

2.7.1. Microbial strains and growth conditions

The methanolic extracts were tested against four bacterial strains, including the Gram-positive bacteria *Staphylococcus aureus* CIP 483 and *Bacillus subtilis* CIP 5262, and the Gram-negative bacteria *Escherichia coli* CIP 53126 and *Pseudomonas aeruginosa* CIP 82118. The bacterial strains were obtained from the Collection of the Pasteur Institute (CIP). The bacteria were maintained at 4 °C and revived by incubation in tryptic soy broth (TSB; casein-peptone soymeal-peptone broth) at 37 °C for 24 h before testing.

2.7.2. Minimum inhibitory concentration (MIC)

The minimum inhibitory concentrations (MIC) of the extracts were determined using a modified broth microdilution method based on previously reported procedures [27,28]. Bacterial inocula were prepared from broth cultures and adjusted to a turbidity equivalent to 0.5 McFarland, corresponding to approximately 2×10^7 CFU/mL.The bacterial density was also checked by measuring the optical density at 600 nm (OD600) before the assay.

The extract stock solution was prepared at a concentration of 500 mg/mL.Serial two-fold dilutions yielded final tested extract concentrations ranging from 0.488 to 250 mg/mL.Briefly, 200 µL of the extract stock solution was initially placed in the first well as the extract control. TSB was added to the extract control, while bacterial inoculum was omitted. Subsequently, 100 µL was transferred successively from one well to the next to prepare the serial dilutions. Tryptic soy broth (TSB) containing the bacterial inoculum was added to wells 2–12. Wells 2–11 contained the serially diluted extracts, while well 12, containing TSB and bacterial inoculum without extract, served as the bacterial growth control. The extracts were dissolved in sterile water.The plates were covered and incubated at 37 °C for 24 h.Following incubation, resazurin solution (10 mg dissolved in 10 mL of sterile water) was added to each well as an indicator of bacterial growth and incubated at 37 °C for 30–60 min. The MIC was defined as the lowest concentration of the extract that prevented visible bacterial growth upon visual inspection before the addition of resazurin. The MIC determination was subsequently confirmed using the resazurin indicator, with the absence of a color change indicating inhibition of bacterial metabolic activity.The MIC assay was performed in three independent experiments.

2.8. GC-MS Analysis

The chemical composition of the methanolic extract of *M. quadrangula* was characterized by gas chromatography–mass spectrometry (GC–MS) at the UR PAF Food Technology Laboratory, CRRA Rabat, INRA Morocco. A Clarus® 580 gas chromatograph (PerkinElmer) coupled to a Clarus® SQ8S mass spectrometer (PerkinElmer) was used for the analysis. Separation was achieved using a 5MS capillary column (5% diphenyl/95% dimethylpolysiloxane; 30.0 m × 0.25 mm). Helium (He) was used as the carrier gas. A 1 µL aliquot of the diluted methanolic extract was injected in split mode at a split ratio of 20:1. The injector temperature was maintained at 250 °C. The oven temperature programme was initiated at 55 °C and held for 0.40 min, followed by an increase at 25 °C/min to 200 °C, then at 8 °C/min to 280 °C, and finally at 25 °C/min to 300 °C, where it was held for 2 min. The total analysis time was approximately 19 min.

Mass spectra were recorded in electron-impact ionization mode at 70 eV. The transfer line temperature was maintained at 250 °C, whereas the ion-source temperature was 200 °C. A solvent delay of 2.00 min was applied. Mass spectra were acquired in full-scan mode over an m/z range of 50–620. Tentative identification of the detected constituents was performed by comparing the acquired mass spectra with the National Institute of Standards and Technology library (NIST MS, 2011) using the NIST Mass Spectral Search Program for the NIST/EPA/NIH Mass Spectral Library. The library contains more than 62,000 reference mass spectra. The names, molecular weights, and proposed structures of the detected constituents were assigned according to the corresponding NIST library matches. The reverse match factor (R.Match) values obtained from the NIST library search were recorded for the detected compounds [29,30].

Retention time (RT), peak area, peak-area percentage (Area %), and R.Match values were recorded for the detected compounds. The relative abundance of each constituent was

expressed as the percentage of its individual peak area relative to the total chromatographic peak area. As no internal standard or compound-specific calibration curves were used, the GC–MS results were considered semi-quantitative and the peak-area percentages should be interpreted as relative abundances rather than absolute concentrations.

2.9. Statistical Analysis

Data were expressed as mean ± standard deviation (SD) of three replicates (n=3). Statistical analyses were performed using GraphPad Prism Differences among the experimental groups were assessed using one-way analysis of variance (ANOVA), followed by Tukey's multiple-comparisons test. A value of $p<0.05$ was considered statistically significant.

3. Results

3.1. Phytochemical Screening

The phytochemical screening of *M. quadrangula* methanolic extracts revealed the presence of alkaloids, saponins, and terpenoids, as indicated by the formation of a white precipitate, stable froth, and reddish-brown coloration, respectively. In contrast, carotenoids were not detected (Table 2).

Table 2. Phytochemical screening of extracts.

Method	Alkaloids	Saponins	Terpenoids	Carotenoids
Soxhlet	+++	++	++	—
Ultrasonic-assisted	+++	++	++	—
Maceration	++	++	++	—

(−) absence of the phytochemical ; (+) low abundance ; (++) moderate abundance ; (+++) high abundance

3.2. Total Polyphenols, Flavonoids, Tannins Content and Extract Yields

The extraction yields of *M. quadrangula* were obtained using Soxhlet extraction, ultrasound-assisted extraction, and maceration with 80% methanol. Among the investigated extraction techniques, Soxhlet extraction produced the highest yield (18.3%). In addition to Soxhlet extraction, ultrasound-assisted extraction and maceration yielded 6.5% and 10.5%, respectively. The total phenolic content, flavonoid content, and tannins condensed content of the methanolic extracts were determined using spectrophotometric assays and expressed as milligrams of gallic acid equivalent (GAE), rutin equivalent (RE), and catechin equivalent (CE) per gram of extract, respectively.

The results revealed significant differences in phytochemical contents among the extraction methods employed. The Soxhlet extract exhibited the highest total phenolic content (51.60 ± 0.05 mg GAE/g extract), followed by the ultrasonic-assisted extract (34.66 ± 0.03 mg GAE/g extract) and the maceration extract (30.30 ± 0.01 mg GAE/g extract). In

contrast, the ultrasonic-assisted extract showed the highest flavonoid content (43.80 ± 0.02 mg RE/g extract), whereas Soxhlet and maceration extracts yielded considerably lower values of 15.25 ± 0.02 and 13.33±0.57 mg RE/g extract, respectively. In addition, condensed tannins were not detected in any of the investigated *M. quadrangula* extracts (absorbance readings at 500 nm were negative or negligible in all extracts). Accordingly, tannins are reported as "ND" in Table 3, where "ND" indicates that tannins were not detected under the conditions of the quantitative vanillin–HCl assay. The obtained values are summarized in Table 3.

Table 3. Yield, total polyphenols, flavonoids and tannins content *M. quadrangula* extracts.

Method	Total of Phenolic content (mg GAE / g extract)	Flavonoids content (mgRE/g extract)	Condensed tannins (mgCE/g extract)	Yield (%)
Soxhlet	51.6±0.05[a]	15.25±0.02[b]	ND	18.3%
Ultrasonic-assisted	34.66±0.03[b]	43.8±0.02[a]	ND	6.5 %
Maceration	30.3±0.01[c]	13.33±0.57[c]	ND	10.5%

Different superscript letters indicate significant differences among extraction methods (Tukey's multiple comparisons test, $p < 0.05$).

ND : indicates that tannins were not detected in the extracts, mg GAE / g extract : mg equivalent of Gallic acid per gram of extract ; mg RE/g extract : mg equivalent of Rutin per gram of extract, mgCE/g extract : mg equivalent of Catechin per gram of extract.

3.3. Antioxidant Activity of M. quadrangula Extracts

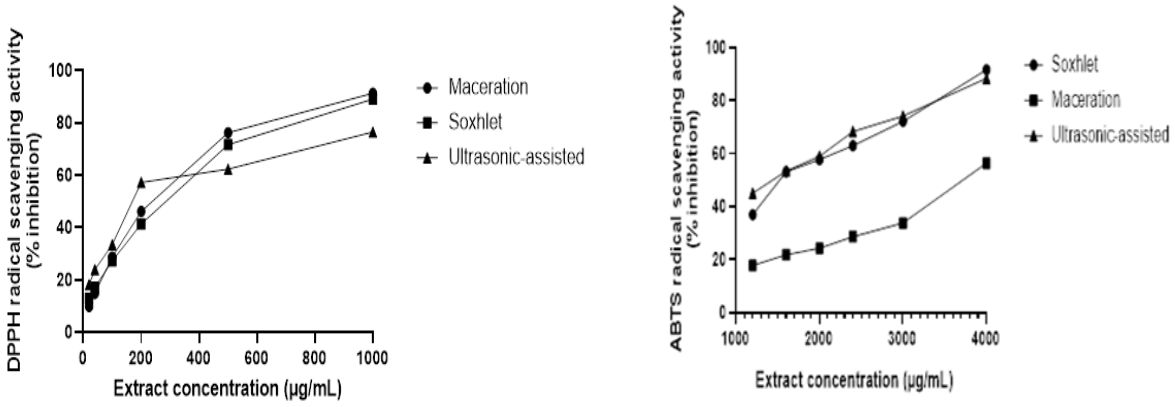
The antioxidant activities of the methanolic extracts of *Monolluma quadrangula*, as determined by the DPPH, ABTS, and ferric reducing power assays. The DPPH and ABTS results are expressed as IC_{50} values, whereas the ferric reducing power assays results are expressed as mg TE/g extract. The ultrasonic-assisted extract exhibited the strongest radical scavenging activity, with IC_{50} values of 169.27 ± 1.19 µg/mL and 1404.69 ± 2.16 µg/mL against DPPH and ABTS radicals, respectively. The Soxhlet extract showed intermediate activity, with IC_{50} values of 237.84 ± 5.36 µg/mL for DPPH and 1519.90 ± 2.54 µg/mL for ABTS, whereas the maceration extract displayed the lowest activity, with IC_{50} values of 283.66 ± 2.43 µg/mL and 3776.75 ± 4.76 µg/mL for DPPH and ABTS, respectively (Table 4).

Table 4. Antioxidant capacity of free radical scavenging DPPH, ABTS and FRAP in extracts *M. quadrangula.*

Method	DPPH IC50 (µg /mL)	ABTS IC50 (µg/mL)	Ferric reducing power assays (mgTE/g extract)
Soxhlet	237.84±5.36[b]	1519.9±2.54[b]	36.21±0.03[b]
ultrasonic-assisted	169.27±1.19[c]	1404.69±2.16[c]	47.46±0.02[a]
Maceration	283.66±2.43[a]	3776.75±4.76[a]	21.4±0.04[c]
Trolox	2.01±0.65	13.40±0.81	_

Different superscript letters indicate significant differences among extraction methods (Tukey's multiple comparisons test, $p < 0.05$).

The dose–response curves showed a concentration-dependent increase in radical-scavenging activity for all three extracts (Figure 1). The extracts exhibited progressively higher inhibition with increasing extract concentration, although differences in the response profiles were observed among the extraction methods. Overall, the curves illustrate the concentration-dependent nature of the antioxidant activity of the extracts.



(a) (b)

Figure 1. Dose–response curves of radical-scavenging activity of methanolic extracts obtained by different extraction methods: (a) DPPH assay; (b) ABTS assay.

The reducing power of the extracts was further evaluated using the ferric reducing power assay, which measures the ability of antioxidants to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). The results revealed that the ultrasonic-assisted extract exhibited the highest ferric reducing capacity, reaching 47.46 ± 0.02 mg TE/g extract, whereas the maceration extract showed the lowest activity (21.40 ± 0.04 mg TE/g extract).

3.4. Antibacterial Activity

Among the tested extracts, the Soxhlet extract exhibited the strongest antibacterial effect against *Pseudomonas aeruginosa* CIP 82118 and *Escherichia coli* CIP 53126, with growth inhibition observed at a MIC value of 15.6 mg/mL. The ultrasonic-assisted extract showed the highest activity against *Bacillus subtilis*, exhibiting the same MIC value (15.6 mg/mL). In contrast, the maceration extract demonstrated the weakest antibacterial activity, particularly against *Staphylococcus aureus* CIP 483, and *Bacillus subtilis* CIP 5262, with a MIC value of 250 mg/mL (Table 5).

Table 5. Minimum inhibitory concentration (MIC) of *M. quadrangula* extracts**.**

	Minimum inhibitory concentration (mg/mL)		
Strains	**Soxhlet**	**Ultrasonic-assisted**	**Maceration**
***Pseudomonas aeruginosa* CIP 82118**	15.6	31.25	125
***Bacillus subtilis* CIP 5262**	31.25	15.6	250
***Escherichia coli* CIP 53126**	15.6	31.25	125
***Staphylococcus aureus* CIP 483**	31.25	62.5	250

3.5. Chemical Composition of Extracts by GC-MS Analysis

The methanolic extract (maceration, ultrasound-assisted extract, Soxhlet) of *M. quadrangula* was analyzed by GC-MS. The chromatogram obtained is shown in (Figure 2, 3 and 4).

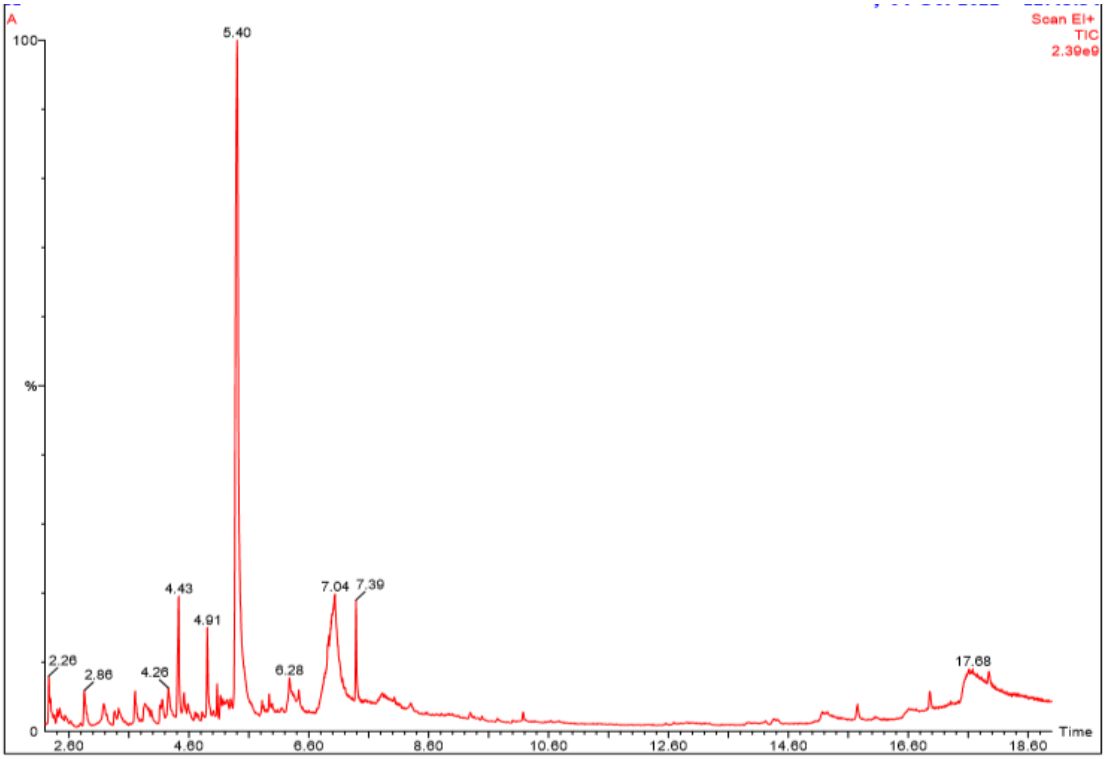


Figure 2. GC–MS chromatogram of the methanolic extract of *M. quadrangula* obtained by maceration.

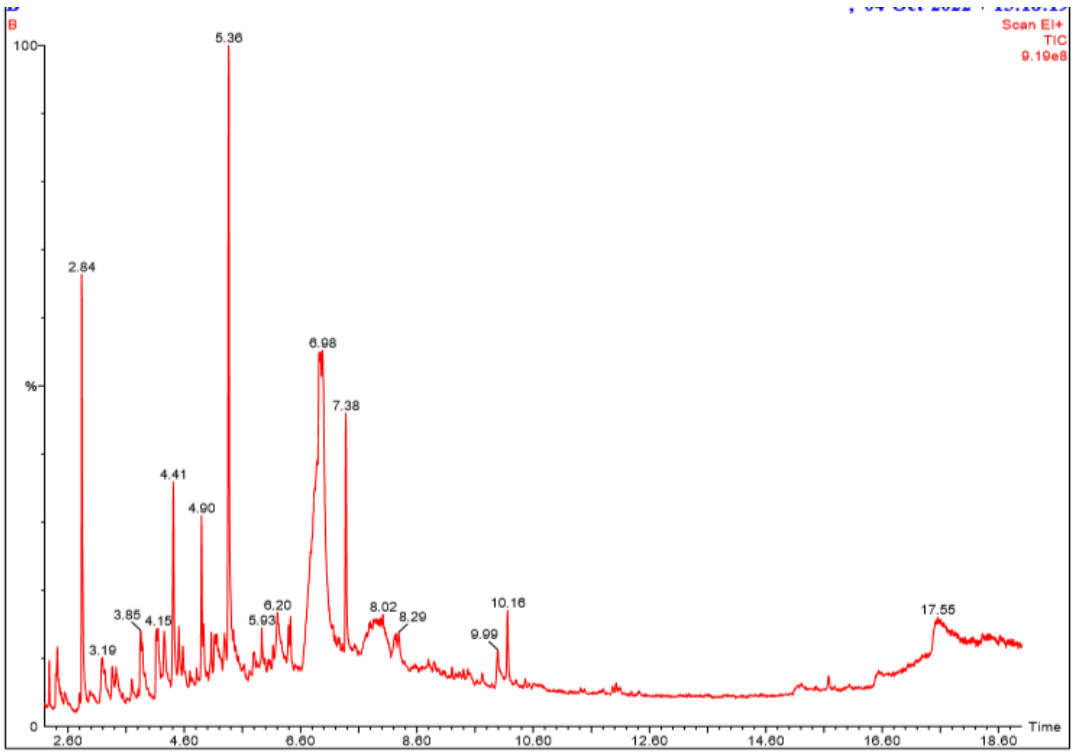


Figure 3. GC–MS chromatogram of the methanolic extract of *M. quadrangula* obtained by Ultrasound-assisted.

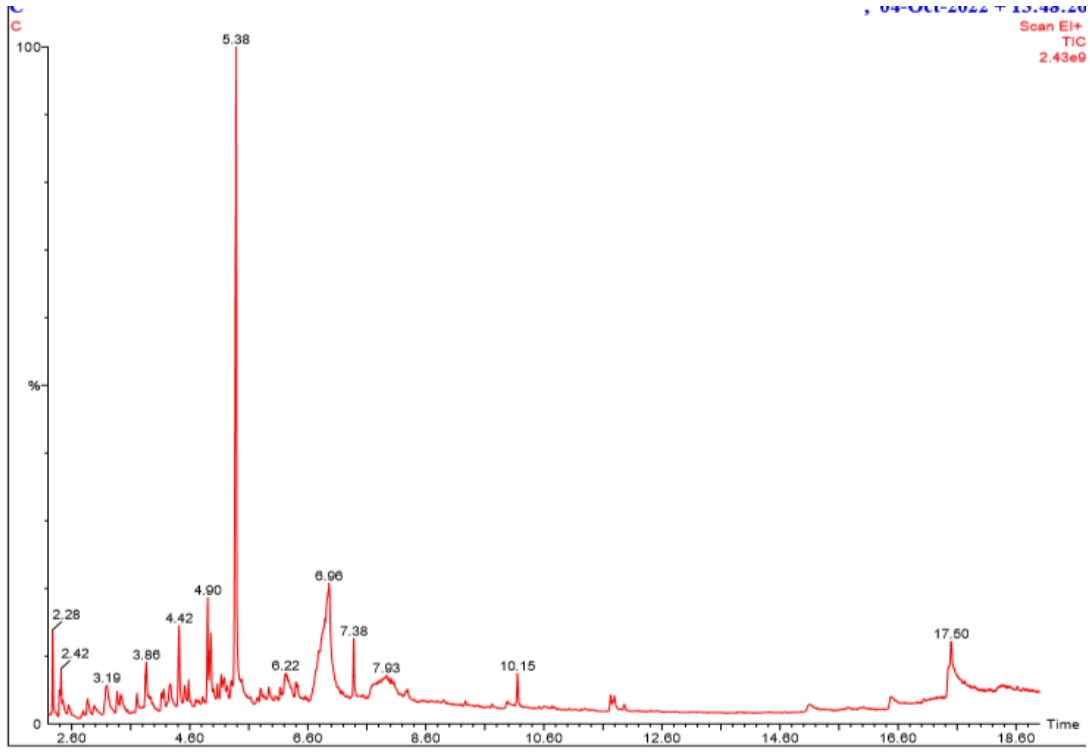


Figure 4. GC–MS chromatogram of the methanolic extract of *M. quadrangula* obtained by Soxhlet.

GC–MS analysis was performed to compare the chemical profiles of the methanolic extracts of *Monolluma quadrangula* obtained by ultrasound-assisted extraction, maceration, and Soxhlet extraction. As no internal standard or compound-specific calibration curves were

used, peak-area percentages were interpreted as semi-quantitative indicators of the relative abundance of the detected constituents rather than as absolute concentrations.

A total of 11, 7, and 6 tentatively identified compounds were detected in the ultrasound-assisted, maceration, and Soxhlet methanolic extracts, respectively. The tentatively identified compounds in the ultrasound-assisted extract accounted for 96.98% of the total chromatographic peak area, whereas those reported for the maceration and Soxhlet extracts represented 100% of their respective chromatographic peak areas. In all three extracts, 5-hydroxymethylfurfural was the predominant constituent. Its relative abundance was 39.99% in the UAE extract, increased to 81.50% in the maceration extract, and reached 72.05% in the Soxhlet extract. In the ultrasound-assisted extract, the second most abundant constituent was 3,5-dimethoxyacetophenone (13.87%), followed by formic acid, 2-propenyl ester (11.49%) and 3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one (7.45%). The maceration extract showed a less diverse chromatographic profile, with 5-hydroxymethylfurfural representing the major proportion of the total peak area. The other detected constituents were present at much lower relative abundances, including 3,5-dihydroxy-6-methyl-2,3-dihydropyran-4-one (4.59%), 3,5-dimethoxyacetophenone (4.32%), furfural (3.15%), and 5-methylfurfural (2.74%).Similarly, the Soxhlet extract was dominated by 5-hydroxymethylfurfural (72.05%). Among the remaining tentatively identified constituents, the compound assigned as methylfurfural represented 11.12% of the total peak area, followed by 3,5-dihydroxy-6-methyl-2,3-dihydropyran-4-one (7.10%) and palmitic acid (4.34%). Overall, 5-hydroxymethylfurfural and 3,5-dihydroxy-6-methyl-2,3-dihydropyran-4-one were detected in the three methanolic extracts, whereas differences were observed in the number of detected constituents and in their relative abundances according to the extraction method. The detected constituents were tentatively identified by matching the acquired mass spectra with the NIST MS 2011 library, and the corresponding reverse match factor (R.Match) values are reported in Table 6,7,8.

Table 6. Tentatively identified constituents in methanolic extracts of *M. quadrangula* obtained by maceration as determined by GC–MS.

N°	Compound	TR (Min)	Molecular Formula	Masse Mol. (g/mol)	Peak Area (%)	R.Match
1	Furfural	2,859	$C_5H_4O_2$	96.08	3,147	953
2	1,2-cyclopentanedione	3,429	$C_5H_6O_2$	98.10	2,629	888
3	5-Methylfurfural	3.709	$C_6H_6O_2$	110.11	2,738	890
4	3,5-Dihydroxy-6-methyl-2,3-dihydropyran-4-one	4.909	$C_6H_8O_4$	144.13	4,592	923
5	5-Hydroxymethylfurfural	5.405	$C_6H_6O_3$	126.11	81,5	906
6	2-Methoxy-4-vinylphenol	5.945	$C_9H_{10}O_2$	150.17	1,07	826
7	3,5-Dimethoxyacetophenone	7.390	$C_{10}H_{12}O_3$	180.20	4,324	775

Compounds were tentatively identified by comparison of the acquired mass spectra with the NIST MS 2011 library. RT: retention time; Area (%): relative peak-area percentage; R.Match: reverse match factor. Peak-area percentages represent semi-quantitative relative abundances and not absolute concentrations.

Table 7. Tentatively identified constituents in methanolic extracts of *M. quadrangula* obtained by ultrasound-assisted extraction as determined by GC–MS.

N°	Compound[a]	TR (Min)	Molecular Formula	Masse Mol. (g/mol)	Peak Area (%)	R.Match
1	Furfuryl alcohol	2.984	$C_5H_6O_2$	98.10	2,8198	748
2	1,2-cyclopentanedione	3.429	$C_5H_6O_2$	98.10	5,0881	896
3	5-Methylfurfural	3.699	$C_6H_6O_2$	110.11	2,2442	870
4	Formic acid, 2-propenyl ester (allyl formate)	3.849	$C_4H_6O_2$	86.09	11,4898	895
5	3,5-Dihydroxy-6-methyl-2,3-dihydropyran-4-one	4.899	$C_6H_8O_4$	144.13	7,4466	926
6	Benzoic acid	4.934	$C_7H_6O_2$	122.12	3,7953	890
7	(S)-(+)-2',3'-Dideoxyribonolactone	5,150	$C_5H_8O_3$	116.12	5,4711	925
8	5-Hydroxymethylfurfural	5.365	$C_6H_6O_3$	126.11	39,986	887
9	2-Methoxy-4-Vinylphenol	5.935	$C_9H_{10}O_2$	150.17	3,5709	800
10	3′,5′Dimethoxyacetophenone	7.380	$C_{10}H_{12}O_3$	180.20	13,8705	787
11	Palmitic Acid	10.162	$C_{16}H_{32}O_2$	256.42	1,1948	826

Compounds were tentatively identified by comparison of the acquired mass spectra with the NIST MS 2011 library. RT: retention time; Area (%): relative peak-area percentage; R.Match: reverse match factor. Peak-area percentages represent semi-quantitative relative abundances and not absolute concentrations.

Table 8. Tentatively identified constituents in methanolic extracts of *M. quadrangula* obtained by Soxhlet extraction, as determined by GC–MS.

N°	Compound	TR (Min)	Molecular Formula	Masse Mol. (g/mol)	Peak Area (%)	R.Match
1	3-Furaldehyde	2.874	$C_5H_4O_2$	96.08	2,972	940
2	2-Methylidenecyclopropane-1-carboxylic acid	2.984	$C_5H_6O_2$	98.10	2,419	875
3	2-Hydroxy-gamma-butyrolactone	3.864	$C_4H_6O_3$	102.09	11,116	877
4	3,5-Dihydroxy-6-methyl-2,3-dihydropyran-4-one	4.904	$C_6H_8O_4$	144.12	7,1	932
5	5-Hydroxymethylfurfural	5.385	$C_6H_6O_3$	126.11	72,049	908
6	Palmitic Acid	10.152	$C_{16}H_{32}O_2$	256.42	4,344	822

Compounds were tentatively identified by comparison of the acquired mass spectra with the NIST MS 2011 library. RT: retention time; Area (%): relative peak-area percentage; R.Match: reverse match factor. Peak-area percentages represent semi-quantitative relative abundances and not absolute concentrations.

4. Discussion

The preliminary phytochemical screening conducted in the present study indicated the presence of alkaloids, terpenoids, and saponins in the investigated *M. quadrangula* extracts. The semi-quantitative scores (+, ++, and +++) were assigned according to the intensity of the colour reactions and/or the amount of precipitate formed and were used only

as relative indicators of the apparent abundance of these phytochemical groups in each extract [31]. The negative carotenoid screening result suggests that carotenoids were either absent, present below the detection limit of the assay, or insufficiently extracted under the applied extraction conditions. Therefore, this result should be interpreted cautiously, particularly because qualitative colour-based screening methods may have limited sensitivity and specificity.Overall, the detected phytochemical classes suggest that *M. quadrangula* contains bioactive secondary metabolites that may contribute to its biological potential. Alkaloids comprise a diverse class of secondary metabolites that have been associated with antioxidant, antibacterial, and antifungal activities. Similarly, terpenoids have been reported to possess antioxidant and antidiabetic potential, partly through their possible effects on glucose metabolism and oxidative-stress-related pathways. Saponins are natural surfactants with emulsifying properties and have also been associated with antioxidant, antimicrobial, anti-inflammatory, and antidiabetic activities [32,33]. Thus, the presence of alkaloids, terpenoids, and saponins may partly contribute to the antioxidant and antibacterial activities observed in the present study. However, the contribution of individual compounds cannot be confirmed from preliminary phytochemical screening alone and requires further targeted phytochemical characterization and bioactivity-guided investigations.

Soxhlet extraction of *M. quadrangula* produced the highest extraction yield among the investigated methods, indicating enhanced recovery of solvent-soluble constituents under the applied conditions. The higher yield obtained with Soxhlet extraction may be attributed to the continuous recirculation of heated solvent through the plant matrix, which can improve solvent penetration, mass transfer, and the diffusion of intracellular constituents into the extraction solvent. Similarly, the higher yield obtained by maceration compared with ultrasound-assisted extraction may be related to the longer solvent plant contact time, which may allow a more exhaustive recovery of extractable constituents [34]. In contrast, ultrasound-assisted extraction can accelerate mass transfer through acoustic cavitation and disruption of plant cell structures; however, the shorter extraction time applied in the present study may have limited the overall recovery of some solvent-soluble constituents. Previous studies have shown that extraction yield is influenced by several factors, including extraction temperature, duration, solvent properties, solvent-to-solid ratio, and the characteristics of the plant matrix. In general, increased temperature and prolonged extraction time may enhance the release of extractable constituents from plant materials [35]. Therefore, the higher yields observed for Soxhlet extraction and maceration may be associated with extended extraction periods and improved solvent–plant interactions under the experimental conditions. Nevertheless, extraction yield alone should not be interpreted as a direct indicator of phytochemical richness or biological activity. Prolonged heating during Soxhlet extraction may also affect heat-sensitive constituents, whereas different extraction methods may preferentially recover different classes of compounds. Therefore, the extraction-yield results should be interpreted together with the phytochemical, antioxidant, antibacterial, and GC–MS findings.

The results revealed significant differences in phytochemical contents among the extraction methods employed. The Soxhlet extract exhibited the highest total phenolic

content, whereas the ultrasound-assisted extract showed the highest total flavonoid content. The greater phenolic recovery obtained with Soxhlet extraction may be associated with the combined effects of elevated temperature and continuous solvent recycling, which can improve solvent penetration into plant tissues and facilitate the release of extractable phenolic constituents. Alara *et al*. (2021) attributed the enhanced recovery of phenolic compounds by Soxhlet extraction to the effects of heat and continuous solvent circulation. Similarly, previous studies have reported that extraction efficiency is influenced by extraction temperature, duration, solvent characteristics, and solvent–matrix interactions [36]. In contrast, the higher flavonoid content observed in the ultrasound-assisted extract may be related to acoustic cavitation, which can disrupt plant cell walls, increase solvent penetration, and enhance mass transfer.Under appropriate extraction conditions, ultrasound-assisted extraction may facilitate the release of flavonoid compounds while reducing prolonged exposure to elevated temperature. These observations suggest that the extraction methods used in the present study may selectively influence the recovery of different phytochemical classes [37].

The phytochemical richness observed in the *M. quadrangula* extracts is consistent with previous reports describing *Caralluma* species as sources of phenolic compounds, flavonoids, and other bioactive secondary metabolites [38]. Because phenolic compounds are widely associated with radical-scavenging activity and the inhibition of oxidative processes, the high total phenolic content observed in the present study may partly contribute to the antioxidant activity of the extracts. However, the contribution of individual compounds to the observed antioxidant activity cannot be determined from total phenolic content alone. Differences between the present findings and previous reports may be related to geographical origin, environmental conditions, harvesting season, developmental stage, plant part, solvent system, and extraction method. Overall, these findings support further investigation *of M. quadrangula* as a potential source of antioxidant phytochemicals for pharmaceutical, nutraceutical, and food-related applications.Interestingly, tannins were not detected in any of the investigated *M. quadrangula* extracts and were therefore recorded as ND in Table 3. This finding indicates that tannins were not detected under the conditions of the qualitative phytochemical screening assay; it does not establish their complete absence from the plant material. The result may reflect the specific phytochemical profile of *M. quadrangula*, the concentration of tannins being below the detection limit of the method, or limited extraction under the applied conditions. Previous phytochemical investigations of *M. quadrangula* have mainly reported flavonoids, pregnane glycosides, sterols, and other bioactive constituents, whereas tannins have not generally been described as major phytochemical components of this species [11]. Variations in extraction procedures, solvent systems, geographical origin, environmental conditions, and plant developmental stage may also contribute to differences in phytochemical profiles among studies.

The concentration-dependent increase in radical-scavenging activity observed for the three extracts indicates that their antioxidant response was influenced by extract concentration. In addition, the antioxidant profiles differed according to the extraction

method employed. Based on the IC_{50} values, the ultrasound-assisted extract exhibited the greatest radical-scavenging potency, as it required the lowest concentration to achieve 50% inhibition. These differences may be related to variations in the extraction efficiency and in the composition and relative abundance of phenolic and flavonoid compounds recovered under the different extraction conditions. Phenolic compounds and flavonoids can act as hydrogen- or electron-donating agents and may therefore contribute to free-radical neutralisation and protection against oxidative damage [39]. The antioxidant capacity of the extracts was further evaluated using the ferric reducing power assay, which assesses the ability of antioxidants to reduce ferric ions $Fe^{3+}$to ferrous ions Fe^{2+}. To our knowledge, limited information is available regarding the ferric reducing power activity of *M. quadrangula*. Therefore, the present results provide preliminary data that may contribute to the phytochemical and pharmacological characterisation of this species. Although the magnitude of the response varied among the extraction methods, all extracts exhibited measurable ferric reducing power. The ultrasound-assisted extract showed the highest antioxidant response in the DPPH, ABTS, and ferric reducing power assays. This enhanced activity may be associated with acoustic cavitation during ultrasound-assisted extraction, which can disrupt plant cell walls, improve solvent penetration and mass transfer, and reduce prolonged exposure to elevated temperatures. Consequently, ultrasound-assisted extraction may represent a useful approach for recovering antioxidant phytochemicals from *M. quadrangula* under the conditions used in the present study.

The markedly higher IC_{50} values obtained in the ABTS assay than in the DPPH assay indicate that the radical-scavenging response of *M. quadrangula* extracts was assay-dependent. Although both assays are based on radical reduction, DPPH- and ABTS- + are chemically distinct radical species and may differ in their reactivity toward the constituents of crude plant extracts. Instead, differences in radical specificity, reaction kinetics, steric accessibility, concentration range, and the relative sensitivity of the two assays may have contributed to the lower DPPH IC_{50} values compared with the ABTS IC_{50} values. Accordingly, the results of the two assays should be considered complementary, assay-specific measures of antioxidant activity rather than directly interchangeable estimates of the overall antioxidant capacity of the extracts [40].

Overall, the antioxidant activity observed for *M. quadrangula* is consistent with the antioxidant potential reported for other *Caralluma* species. However, the magnitude of the observed activity varied across studies, and direct numerical comparison should be interpreted cautiously because of differences in species, geographical origin, plant part, extraction method, solvent system, assay conditions, and result-reporting units. For example, the methanolic extract of *C. russeliana* showed concentration-dependent antioxidant activity, with IC_{50} values of 119.17±5.97,155.71±9.59, and 203.48±5.41µg/mL in the DPPH, ABTS, and ferric reducing power assays, respectively [41]. In addition, *C. hexagona* has been reported to exhibit antioxidant activity in several assays, with results expressed as Trolox-equivalent antioxidant capacity, illustrating how differences in assay design and reporting units may limit direct numerical comparison among studies [42]. Antioxidant activity has also been

reported for *C. arabica*, with the measured response varying according to extract type and solvent system [43]. Overall, variations in antioxidant activity among *Caralluma* species may be related to differences in phytochemical composition, geographical origin, plant part, extraction solvent, extraction method, and assay conditions. Therefore, the antioxidant activity observed for *M. quadrangula* should be interpreted within this methodological and biological context rather than through direct numerical comparison alone.

Comparable variability in antioxidant activity has also been reported among other medicinal plants. A recent comparative study of *Caralluma arabica*, *Ocimum basilicum*, *Teucrium polium*, and *Cleome amblyocarpa*, using DPPH, FRAP, and Trolox-equivalent antioxidant capacity (TEAC) assays, demonstrated that antioxidant responses may vary according to plant species, extraction solvent, and assay method [43]. These findings further highlight the influence of plant species, phytochemical composition, and extraction conditions on the antioxidant activity of plant extracts.

According to the in vitro antibacterial assays, all methanolic extracts of *M. quadrangula* exhibited inhibitory activity against the four tested bacterial strains. However, antibacterial potency varied among the extraction methods. The Soxhlet and ultrasound-assisted extracts showed lower minimum inhibitory concentration (MIC) values than the maceration extract, indicating comparatively stronger antibacterial activity under the tested conditions. The MIC values of the crude extracts ranged from 15.625 to 250 mg/mL, indicating modest in vitro antibacterial potency. However, antibacterial activity of crude plant extracts is commonly detected at mg/mL concentrations because these preparations contain complex mixtures of phytochemicals rather than a single purified active constituent. For example, Jatropha gossypiifolia floral extracts showed MIC values ranging from 12.5 to 200 mg/mL [44], while other medicinal plant extracts have been reported to exhibit MIC values ranging from 1.95 to 250 mg/mL against bacterial isolates [45]. Therefore, the MIC range observed in the present study is within the range reported for crude plant extracts, although direct comparisons should be interpreted cautiously because MIC values are influenced by plant species, plant part, extraction solvent, extraction procedure, bacterial strain, inoculum density, and assay conditions [46].

However, direct comparison of the antibacterial potency of the present extracts with conventional antibiotics was not possible because a standard antibiotic positive control was not included in the assay .This variation may partly reflect differences in the extraction efficiency of bioactive constituents among the applied methods. The comparatively higher total phenolic content observed in the Soxhlet and ultrasound-assisted extracts may also have contributed to their antibacterial activity [47]. However, this relationship should be interpreted cautiously because individual phenolic compounds were not identified and a statistically robust correlation analysis could not be performed with only three extract groups. At the molecular level, phenolic compounds and flavonoids may interact with the bacterial cell wall and cytoplasmic membrane, leading to membrane destabilization, increased permeability, and leakage of intracellular components. They may also interfere with membrane-associated functions and essential enzymes involved in energy metabolism and macromolecular

biosynthesis. In addition, some phenolic compounds may promote oxidative stress through the generation of reactive oxygen species (ROS), potentially causing lipid peroxidation, protein damage, and DNA injury. Other proposed targets include DNA replication, protein synthesis, quorum-sensing signaling, and biofilm formation [48]. However, as these mechanisms were not directly investigated in the present study, they should be considered plausible mechanisms associated with phytochemical constituents potentially present in the extracts rather than confirmed mechanisms of action of *M. quadrangula*.

The present findings indicate that the extraction method affects the in vitro antibacterial potential of *M. quadrangula* extracts, probably by altering the type and/or concentration of extracted phytochemicals.The differential susceptibility observed among the tested bacterial species may also be related to differences in bacterial cell-envelope structure. Bacillus subtilis, as a Gram-positive bacterium, lacks the outer membrane found in Gram-negative bacteria, whereas *Escherichia coli* and *Pseudomonas aeruginosa* possess an outer membrane that may restrict the penetration of certain phytochemicals. In addition, UAE and Soxhlet extraction may yield extracts with different phytochemical profiles because of their distinct extraction conditions, which may contribute to the strain-dependent antibacterial responses observed in the present study. However, chemical characterization of the extracts is required to identify the constituents responsible for these differences [49]. The antibacterial activity observed in this study is generally consistent with previous reports describing antimicrobial effects of *M. quadrangula* extracts against selected bacterial species, including *Pseudomonas aeruginosa*, *Escherichia coli*, and other microorganisms. A comparison with other Yemeni *Caralluma* species provides useful regional context. Antibacterial activity has been reported for *Caralluma deflersiana* from Yemen against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* . In addition, antibacterial activity has been reported for the Yemeni species *Caralluma wissmannii* against Gram-positive and Gram-negative bacteria using an agar-diffusion assay [50]. However, direct comparison with the present results should be interpreted cautiously because of differences in plant species, plant part, extraction solvent, phytochemical composition, bacterial strains, and antimicrobial assay procedures. These reports nevertheless suggest that antibacterial activity may occur among Yemeni *Caralluma* species, while its magnitude and spectrum can vary according to phytochemical and methodological factors.

Collectively, these results support the potential of *M. quadrangula* as a source of bioactive compounds for further antibacterial investigation. Nevertheless, isolation and characterization of the active constituents, testing against a broader panel of clinically relevant and resistant bacterial isolates, and evaluation of cytotoxicity and in vivo efficacy are needed before therapeutic applications can be considered [51,52] .

The GC–MS analysis of the methanolic extracts of *M. quadrangula* obtained by ultrasound-assisted extraction, maceration, and Soxhlet extraction revealed differences in both the number and relative abundance of the tentatively identified constituents. Since methanol was used as the extraction solvent in all three procedures, the observed variation in chromatographic profiles is more likely associated with differences in extraction conditions,

including extraction time, temperature exposure, solvent–sample contact, and mass-transfer efficiency. These factors may affect the extraction and relative recovery of individual constituents, leading to differences in the chromatographic composition of the extracts.The ultrasound-assisted extract showed the greatest chemical diversity, with 11 tentatively identified constituents, compared with seven and six constituents in the maceration and Soxhlet extracts, respectively. In contrast, the maceration and Soxhlet extracts displayed chromatographic profiles strongly dominated by a single constituent, namely 5-hydroxymethylfurfural (5-HMF). The peak-area percentage of 5-HMF was 39.99% in the UAE extract, 81.50% in the maceration extract, and 72.05% in the Soxhlet extract. These values represent relative abundances rather than absolute concentrations because no internal standard or compound-specific calibration curves were used. Therefore, the differences in peak-area percentages should not be interpreted as definitive quantitative differences in the concentrations of the corresponding compounds.

5-Hydroxymethylfurfural is a furan derivative that may occur in plant-derived matrices but can also be formed through the dehydration of hexose-derived constituents during thermal processing. Its high relative abundance should consequently be interpreted with caution, particularly in the Soxhlet extract, which was obtained under heating conditions. The presence of 5-HMF in the present extracts may reflect the composition of the plant material and/or the transformation of carbohydrate-derived constituents during extraction, solvent evaporation, sample preparation, or GC–MS analysis [53]. Accordingly, the present results do not demonstrate that 5-HMF is a definitive native major metabolite *of M. quadrangula*. Confirmation using an authentic standard, retention-index comparison, and targeted quantitative analysis would be required to establish its identity and concentration conclusively. Thermal treatment is widely recognized as a factor that can promote the formation of 5-HMF in carbohydrate-containing matrices.

Although 5-HMF has been associated in previous reports with antioxidant and antimicrobial effects, its apparent predominance in the chromatograms cannot be used to assign it sole responsibility for the biological activities of the extracts. The antioxidant and antibacterial properties of plant extracts generally result from the combined effects of multiple constituents. Furthermore, a substantial proportion of polar, non-volatile, and thermolabile metabolites may not be efficiently detected under conventional GC–MS conditions. Therefore, the biological activities observed for the methanolic extracts of *M. quadrangula* may involve both the volatile and semi-volatile compounds detected in this analysis and additional constituents not characterized by the current GC–MS method. Another constituent detected in all three extracts was 3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one, also known as DDMP. Its relative peak-area percentage ranged from 4.59% in the maceration extract to 7.45% in the UAE extract. Previous studies have shown that DDMP can exhibit free-radical-scavenging activity, and its hydroxyl groups are considered important contributors to its antioxidant behavior [54]. The repeated detection of DDMP in the three extracts suggests that this compound was consistently recovered and detected under the applied conditions. Nevertheless, its contribution to the antioxidant activity of *M.*

quadrangula extracts remains tentative and should be verified by targeted quantification and biological testing of the isolated or authentic compound.

The ultrasound-assisted extract also contained relatively higher proportions of 3,5-dimethoxyacetophenone (13.87%) and formic acid, 2-propenyl ester (11.49%), which contributed to its more diverse GC–MS profile. However, these compounds were tentatively assigned based on library matching, and their individual biological relevance cannot be confirmed without further analytical verification and compound-specific bioassays. In addition, 2-methoxy-4-vinylphenol was detected in the ultrasound-assisted and maceration extracts. This phenolic derivative has been associated with antioxidant and antibacterial effects in previous experimental studies; nevertheless, its low relative abundance in the present extracts and the tentative nature of the assignment do not justify attributing the observed activities mainly to this compound.

Palmitic acid was detected at low relative abundances in the ultrasound-assisted (1.19%) and Soxhlet (4.34%) extracts. Its occurrence may indicate the extraction of lipid-associated constituents, particularly in the Soxhlet extract, where prolonged extraction and heat exposure may facilitate the recovery of relatively non-polar compounds. However, palmitic acid was not among the major constituents in the analyzed extracts and should not be considered a principal contributor to the observed antioxidant or antibacterial activities.

Previous phytochemical investigations of *M. quadrangula* have reported polyoxypregnane glycosides as important constituents of this species. These compounds are generally polar, relatively high in molecular weight, and often non-volatile or thermolabile; consequently, they may not be adequately detected using the present GC–MS method. The current analysis should therefore be considered a preliminary characterization of the volatile and semi-volatile constituents detected in the methanolic extracts rather than a comprehensive profile of all phytochemicals present in *M. quadrangula*. Complementary LC–MS or UPLC–ESI–Q–TOF-MS analysis would be particularly valuable for detecting and characterizing pregnane glycosides and other polar metabolites of this species [8].

Finally, all compound assignments in the present study should be interpreted as tentative because they were obtained through comparison of the acquired mass spectra with the NIST MS 2011 library and the corresponding R.Match values. The absence of co-injection with authentic standards and experimentally determined retention indices prevents definitive structural confirmation. In addition, peak-area percentages are semi-quantitative and do not represent absolute concentrations. Future investigations should confirm the major constituents using authentic standards, retention-index comparison, and compound-specific calibration curves, while LC–MS-based analysis should be considered to obtain a more comprehensive characterization of the polar and non-volatile phytochemical constituents of *M. quadrangula*. Unequivocal GC–MS identification normally requires agreement with an authentic standard in both mass spectrum and retention behavior under comparable analytical conditions.

4.1. Study Limitations and Future Perspectives

This study has several limitations that should be considered when interpreting the findings.First, each extraction method was conducted using one independent extraction batch.because the plant material was available in a limited quantity and additional material was difficult to obtain from the country of origin.; therefore, extraction yields were presented as single observed percentages and were not subjected to statistical comparison. In addition, only three extract groups were available, which was insufficient for a statistically robust correlation analysis between total phenolic content, total flavonoid content, and antioxidant or antibacterial activities.

Second, the GC–MS data provide a tentative chemical profile of the methanolic extract. The reported compound assignments were based on comparison with the NIST MS 2011 library and were supported by the corresponding reverse match factors. However, retention indices were not determined using an n-alkane series, and authentic reference standards were not used for confirmation. In addition, the peak-area percentages represent relative semi-quantitative abundances rather than absolute concentrations because no internal standard or compound-specific calibration curves were used GCMS

Third, the antibacterial evaluation was limited to MIC determination using a resazurin-based broth microdilution assay. No standard antibiotic positive control was included; consequently, the results permit comparison of the relative antibacterial activity among the tested extracts under the same experimental conditions but do not allow direct benchmarking against conventional antibiotics. In addition, MBC determination and time-kill kinetic assays were not performed; thus, the bactericidal activity and temporal dynamics of bacterial inhibition could not be established

Future studies should include independently prepared extraction batches, a wider range of extraction conditions, standardized antibiotic controls, MBC determination, and time-kill kinetic analysis of the most active extracts. Detailed phytochemical characterization and testing of isolated compounds, including possible synergistic interactions, are also needed to clarify the mechanisms underlying the antioxidant and antibacterial activities. Finally, cytotoxicity, formulation, standardization, and in vivo efficacy studies are required to evaluate the safety and therapeutic relevance of *M. quadrangula* before any potential clinical application can be considered.

5. Conclusions

This study demonstrates that the extraction method substantially influences the phytochemical characteristics and biological activities of *Monolluma quadrangula*. Among the investigated approaches, Soxhlet extraction produced the highest total phenolic content and showed the strongest antibacterial activity, particularly against *Pseudomonas aeruginosa* and *Escherichia coli*. In contrast, ultrasound-assisted extraction recovered higher flavonoid content and exhibited the greatest antioxidant activity in the DPPH, ABTS, and ferric-

reducing assays. GC–MS analysis further indicated qualitative differences in the chemical constituents detected among the extracts, which may contribute to their distinct biological responses.

Collectively, these findings support the traditional medicinal relevance of *M. quadrangula* and identify it as a potential source of natural antioxidant and antibacterial bioactive compounds. The differential antibacterial responses observed among the tested bacteria, including the greater activity of the UAE extract against the Gram-positive *Bacillus subtilis* and the stronger activity of the Soxhlet extract against the Gram-negative *P. aeruginosa* and *E. coli*, suggest that extraction-dependent differences in phytochemical composition may influence antibacterial selectivity..

The findings also provide a basis for further investigation of *M. quadrangula* as a source of bioactive molecules relevant to microbial research and natural-product drug discovery. Future studies should focus on the isolation and structural characterization of the major bioactive constituents, followed by evaluation of their individual and synergistic biological activities. Mechanistic investigations are particularly warranted to elucidate the molecular interactions underlying the antibacterial effects, including their potential effects on relevant bacterial cellular targets. In addition, in vivo studies are needed to establish efficacy, pharmacological relevance, and safety before any therapeutic application can be considered. Appropriate formulation and standardization strategies should also be explored to facilitate the development of potential plant-derived antimicrobial and antioxidant products.

Further research should include independent extraction batches, detailed phytochemical profiling, fractionation, isolation, and structural characterization of the active constituents. The antibacterial potential of the most active extracts should also be further evaluated through the inclusion of standard antibiotic controls, determination of minimum bactericidal concentrations, and time-kill kinetic studies. In addition, cytotoxicity, formulation, standardization, pharmacokinetic, and in vivo efficacy studies are required to establish safety, biological relevance, and therapeutic feasibility before any clinical application can be considered.

By integrating extraction-method comparison, phytochemical characterization, antioxidant assessment, and antibacterial screening, this work expands current knowledge of medicinal plants from the Arabian Peninsula. It also provides a basis for future microbial and molecular investigations of plant-derived bioactive compounds.

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References

1. Riaz M, Khalid R, Afzal M, *et al*. Phytobioactive compounds as therapeutic agents for human diseases: A review. Food Science & Nutrition 2023; 11(9): 5095–5125. doi: 10.1002/fsn3.3308.

2. Eze CN, Aduba CC, Ezema BO, *et al*. The role of antioxidants in food safety and preservation: Mechanisms, applications, and challenges. Cogent Food & Agriculture 2025; 11(1): 2591436. doi: 10.1080/23311932.2025.2591436.

3. Intharuksa A, *et al*. Flavonoids and Other Polyphenols: Bioactive Molecules from Traditional Medicine Recipes/Medicinal Plants and Their Potential for Phytopharmaceutical and Medical Application. Molecules 2024; 29(23): 5760. doi: 10.3390/molecules29235760.

4. Jomova K, Raptova R, Alomar SY, *et al*. Reactive oxygen species, toxicity, oxidative stress, and antioxidants: Chronic diseases and aging. Archives of Toxicology 2023; 97(10): 2499–2574. doi: 10.1007/s00204-023-03562-9.

5. A Fitri ZA, Ahmadi F, Islam MA, *et al*. A systematic review of extraction methods, phytochemicals, and food applications of Moringa oleifera leaves using PRISMA methodology. Food Science & Nutrition 2025; 13(4): e70138. doi: 10.1002/fsn3.70138.

6. Alara OR, Abdurahman NH, Ukaegbu CI. Extraction of phenolic compounds: A review. Current Research in Food Science 2021; 4: 200–214. doi: 10.1016/j.crfs.2021.03.011.

7. Yusoff IM, Taher ZM, Rahmat Z, *et al*. A review of ultrasound-assisted extraction for plant bioactive compounds: Phenolics, flavonoids, thymols, saponins and proteins. Food Research International 2022; 157: 111268. doi: 10.1016/j.foodres.2022.111268.

8. Hamed AI, Ben Said R, Ben Aissa MA, *et al*. Comprehensive polyoxypregnane glycosides report in Caralluma quadrangula using UPLC–ESI–Q–TOF and their antioxidant effects in human plasma. Biomedicine & Pharmacotherapy 2022; 150: 112954. doi: 10.1016/j.biopha.2022.112954.

9. Abdel-Sattar E, Sabry MM, Shalabi AA, *et al*. Genus Caralluma in Yemen: A comprehensive review of taxonomy, ethnomedicine, phytochemistry, and biological activities. Chemistry & Biodiversity 2025; 22(11): e01112. doi: 10.1002/cbdv.202501112.

10. Linh NTT, Huong DTL, Ni HTN, *et al*. The genus Caralluma: Traditional use, phytochemistry, nutritional value, pharmacology, nanoformulation, and toxicology. Fitoterapia 2025; 188: 106961. doi: 10.1016/j.fitote.2025.106961.

11. Ismail GA, Mostafa ME, Mubarak AY, *et al*. Quadrangulosides A–F: New pregnane glycosides from Caralluma quadrangula. RSC Advances 2023; 13: 11800–11806. doi: 10.1039/d3ra01103h.

12. Bin-Jumah MN. Antidiabetic effect of Monolluma quadrangula is mediated via modulation of glucose metabolizing enzymes, antioxidant defenses, and adiponectin in type 2 diabetic rats. Oxidative Medicine and Cellular Longevity 2019; 2019: 6290143. doi: 10.1155/2019/6290143.

13. El-Shiekh RA, Hassan M, Hashem RA, *et al*. Bioguided isolation of antibiofilm and antibacterial pregnane glycosides from Caralluma quadrangula: Disarming multidrug-resistant pathogens. Antibiotics 2021; 10(7): 811. doi: 10.3390/antibiotics10070811.

14. Chemat F, Rombaut N, Sicaire AG, *et al*. Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. Ultrason Sonochem 2017; 34: 540–560. doi: 10.1016/j.ultsonch.2016.06.035.

15. Ouassor I, Aqil Y, Belmaghraoui W, *et al*. Characterization of two Moroccan watermelon seed oil varieties by three different extraction methods. Oilseeds and Fats Crops and Lipids 2020; 27: 13. doi: 10.1051/ocl/2020010.

16. Naima R, Oumam M, Hannache H, *et al*. Comparison of the impact of different extraction methods on polyphenols yields and tannins extracted from Moroccan Acacia mollissima barks. Industrial Crops and Products 2015; 70: 245–252. doi: 10.1016/j.indcrop.2015.03.016.

17. Senrayan J, Venkatachalam S. Optimization of ultrasound-assisted solvent extraction (UASE) based on oil yield, antioxidant activity and evaluation of fatty acid composition and thermal stability of Coriandrum sativum L. seed oil. Food Science and Biotechnology 2019; 28(2): 377–386. doi: 10.1007/s10068-018-0467-1.

18. Muthee KB, Maitho TE, Kanja LW, *et al*. Evaluation of uterotonic activity, acute oral toxicity, and phytochemical composition of Uvariodendron anisatum Verdc. root extracts. Evidence-Based Complementary and Alternative Medicine 2022; 2022: 7393537. doi: 10.1155/2022/7393537.

19. Oladeji OS, Odelade KA, Oloke JK. Phytochemical screening and antimicrobial investigation of Moringa oleifera leaf extracts. African Journal of Science, Technology, Innovation and Development 2020; 12(1): 79–84. doi: 10.1080/20421338.2019.1589082.

20. Nortjie E, Basitere M, Moyo D, *et al*. Extraction methods, quantitative and qualitative phytochemical screening of medicinal plants for antimicrobial textiles: a review. Plants 2022; 11: 2011. doi: 10.3390/plants11152011.

21. Bouslamti M, Metouekel A, Chelouati T, *et al*. Solanum elaeagnifolium var. obtusifolium (Dunal) Dunal: antioxidant, antibacterial, and antifungal activities of polyphenol-rich extracts chemically characterized by use of in vitro and in silico approaches. Molecules 2022; 27: 8688. doi: 10.3390/molecules27248688.

22. Aboalhaija NH, Syaj H, Afifi F, *et al*. Chemical evaluation, in vitro and in vivo anticancer activity of Lavandula angustifolia grown in Jordan. Molecules 2022; 27(18): 5910. doi: 10.3390/molecules27185910.

23. Aqil Y, El Hajjaji S, Belmaghraoui W, *et al*. Phenolic profile, antioxidant, antidiabetic, and antigout potential of stem extracts of four sweet cherry cultivars. Evidence-Based Complementary and Alternative Medicine 2023; 2023: 8535139. doi: 10.1155/2023/8535139.

24. Bouhlal F, Aqil Y, Chamkhi I, *et al*. GC-MS analysis, phenolic compounds quantification, antioxidant, and antibacterial activities of the hydro-alcoholic extract of spent coffee grounds. Journal of Biologically Active Products from Nature 2020; 10(4): 325–337. doi: 10.1080/22311866.2020.1802333.

25. Nurdin SU, Nurdjanah S, Triyandi R, *et al*. Antioxidant activity, glycemic response, and functional properties of rice cooked with red palm oil. Journal of Nutrition and Metabolism 2024; 2024: 3483292. doi: 10.1155/2024/3483292.

26. Elveren M. Physiological and biochemical responses of wheat (Triticum aestivum L.) and barley (Hordeum vulgare L.) to combined exposure of pharmaceuticals, personal care products (PPCPs), and artificial sweeteners (ASs). BMC Plant Biology 2025; 25(1): 1662. doi: 10.1186/s12870-025-07730-0.

27. El- Sawy YNA, Abdel-Salam AB, Abd-Elhady HM, *et al*. Elimination of detached Listeria monocytogenes from the biofilm on stainless steel surfaces during milk and cheese processing using natural plant extracts. Scientific Reports 2024; 14: 2288. doi: 10.1038/s41598-024-52394-9.

28. Gulluce M, Sahin F, Sokmen M, *et al*. Antimicrobial and antioxidant properties of the essential oils and methanol extract from Mentha longifolia L. ssp. Longifolia. Food Chemistry 2007 ;103(4) :1449–1456. doi: 10.1016/j.foodchem.2006.10.061.

29. Chaari S, El Arnabi FZ, Benali A, *et al*. Chemical diversity and chemotype classification of Artemisia herba-alba essential oils from western and southern Morocco. Biochemical Systematics and Ecology 2025; 123: 105106. doi: 10.1016/j.bse.2025.105106.

30. Stein SE, Mirokhin YA, Chekhovskoi DV, *et al*. The NIST Mass Spectral Search Program for the NIST/EPA/NIH Mass Spectral Library, version 2.0. Gaithersburg, MD: National Institute of Standards and Technology; 2011.

31. Maharaj A, Naidoo Y, Dewir YH, *et al*. Phytochemical screening, and antibacterial and antioxidant activities of Mangifera indica L. leaves. Horticulturae 2022; 8: 909. doi: 10.3390/horticulturae8100909.

32. Mohammadi-Cheraghabadi M, Hazrati S. Terpenoids, steroids, and phenolic compounds of medicinal plants. In: Arora C, Verma DK, Aslam J, Mahish PK, editors. Phytochemicals in Medicinal Plants: Biodiversity, Bioactivity and Drug Discovery. Berlin, Boston: De Gruyter; 2023. p. 105–130. doi: 10.1515/9783110791891-005.

33. Singh S, Bansal A, Singh V, *et al*. Flavonoids, alkaloids and terpenoids: a new hope for the treatment of diabetes mellitus. Journal of Diabetes & Metabolic Disorders 2022; 21(1): 941–950. doi: 10.1007/s40200-021-00943-8.

34. Núñez S, Marta Sofía Valero, Ahmed M Mustafa, *et al*. Ultrasound-assisted extraction versus traditional Soxhlet apparatus for the obtention of polyphenols, carotenoids and tocopherols from Tagetes erecta L. flowers. Journal of the Science of Food and Agriculture 2025; 105(6): 3453–3462. doi: 10.1002/jsfa.14105.

35. El Amri N, Chabir R, Kadda S, *et al*. Optimization of Phenolic Compounds Extraction from Capparis spinosa Flowers Using Response Surface Methodology. Moroccan Journal of Chemistry 2025; 13(4): 1798–1817. https://revues.imist.ma/index.php/morjchem/index.

36. Kumar K, Srivastav S, Sharanagat VS. Ultrasound-assisted extraction (UAE) of bioactive compounds from fruit and vegetable processing by-products: a review. Ultrasonics Sonochemistry 2021; 70: 105325. doi: 10.1016/j.ultsonch.2020.105325.

37. Shen L, Pang S, Zhong M, *et al*. A comprehensive review of ultrasonic assisted extraction (UAE) for bioactive components: principles, advantages, equipment, and combined technologies. Ultrasonics Sonochemistry 2023; 97: 106646. doi: 10.1016/j.ultsonch.2023.106646.

38. Doukkali Z, El Jemli M, Bouyahya A, *et al*. Radical-scavenging effect, ferric reducing ability and phytochemical analysis of Urtica urens (L.) and Mercurialis annua (L.). Progress in Microbes and Molecular Biology 2023; 6(1): a0000335. doi: 10.36877/pmmb.a0000335.

39. Indrianingsih AW, Styaningrum P, Suratno, *et al*. The effect of extraction method on biological activity and phytochemical content of Artocarpus heterophyllus (jackfruit) leaves extract concurrent with its principal component analysis. Process Biochemistry 2024; 143: 135–147. doi: 10.1016/j.procbio.2024.04.034.

40. Platzer M, Kiese S, Herfellner T, *et al*. Common trends and differences in antioxidant activity analysis of phenolic substances using single electron transfer based assays. Molecules 2021; 26(5): 1244. doi: 10.3390/molecules26051244.

41. Alsenani NI. Alkaloid, phenolic profiling and biological activities of Caralluma russeliana methanol extracts from Al Baha region, Saudi Arabia. Oriental Journal of Chemistry 2023; 39(2): 378–386. doi: 10.13005/ojc/390217.

42. Choucry MA, Shalabi AA, El Halawany AM, *et al*. New pregnane glycosides isolated from Caralluma hexagona Lavranos as inhibitors of α-glucosidase, pancreatic lipase, and advanced glycation end products formation. ACS Omega 2021; 6(30): 19424–19434. doi: 10.1021/acsomega.1c02056.

43. Juma Al-Mutaani, Tahani Zorgui, Abdalla A Mohamed, *et al*. Comparative antioxidant activity of four Omani medicinal plants: Ocimum basilicum, Teucrium polium, Caralluma arabica, and Cleome amblyocarpa. Open Veterinary Journal 2025; 15(9): 4671–4686. doi: 10.5455/OVJ.2025.v15.i9.71.

44. Khalifa SM, Harras MF, Badawy MSEM. Antibacterial, antibiofilm activities of Jatropha gossypiifolia L. floral extracts and molecular docking study of the major compound. Natural Product Research 2025; 1–6. doi: 10.1080/14786419.2025.2487693.

45. Taye B, Giday M, Animut A, *et al*. Antibacterial activities of selected medicinal plants in traditional treatment of human wounds in Ethiopia. Asian Pacific Journal of Tropical Biomedicine 2011; 1(5): 370–375. doi: 10.1016/S2221-1691(11)60082-8.

46. Battah B, Rajab A, Shbibe L, *et al*. Evaluation of antibiofilm activity of Thymus syriacus essential oil against clinically isolated MDR bacteria. Progress in Microbes and Molecular Biology 2022; 5(1). doi: 10.36877/pmmb.a0000284.

47. Fatima F, Bhat SH, Ullah MF, *et al*. In vitro antimicrobial activity of herbal extracts from Tabuk region (Kingdom of Saudi Arabia) against nosocomial pathogens: a preliminary study. Global Journal of Health Science 2018; 10(3): 83–91. doi: 10.5539/gjhs.v10n3p83.

48. da Cunha MSB, Miguel CMT, Lima EMF, *et al*. Phenolic compounds: from traditional uses to innovative applications and everything in between. Molecular Nutrition & Food Research 2026; 70(9): e70478. doi: 10.1002/mnfr.70478.

49. Sun S, Yu Y, Jo Y, *et al*. Impact of extraction techniques on phytochemical composition and bioactivity of natural product mixtures. Frontiers in Pharmacology 2025; 16: 1615338. doi: 10.3389/fphar.2025.1615338.

50. Al-attab BM, Almaqtari MA, Mubarak AY. Antioxidant and antimicrobial properties of three extracts of Caralluma deflersiana Laver. Sana'a University Journal of Applied Sciences and Technology 2024; 2(2): 154–157. doi: 10.59628/jast.v2i2.862.

51. El-Shiekh RA, Hassan M, Hashem RA, *et al*. Bioguided isolation of antibiofilm and antibacterial pregnane glycosides from Caralluma quadrangula: disarming multidrug-resistant pathogens. Antibiotics 2021; 10(7): 811. doi: 10.3390/antibiotics10070811.

52. Mondal S, Das M, Debnath S, *et al*. An overview of extraction, isolation and characterization techniques of phytocompounds from medicinal plants. Natural Product Research 2026; 40(7): 1939–1961. doi: 10.1080/14786419.2024.2426059.

53. Martins FCO L, Alcantara GMRN, Silva AFS, *et al*. The role of 5-hydroxymethylfurfural in food and recent advances in analytical methods. Food Chemistry 2022; 395: 133539. doi: 10.1016/j.foodchem.2022.133539.

54. Chen Z, Liu Q, Zhao Z, *et al*. Effect of hydroxyl on antioxidant properties of 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one to scavenge free radicals. RSC Advances 2021; 11(55): 34456–34461. doi: 10.1039/D1RA06317K.

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