scientific reports

Check for updates

OPEN

Comparative analysis of solvent and advanced extraction techniques for optimizing phytochemical yield and bioactivity of *Matthiola ovatifolia* (Boiss.) Aerial parts

Majid Sharifi-Rad[1]✉, Ippolito Camele[2], Pawel Pohl[3], Yugal Kishore Mohanta[4,5] & Hazem S. Elshafie[2]

This study investigated the influence of different solvents (ethanol, water, DMSO, and acetone) and extraction methods, conventional solvent extraction (CSE), ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and ultrasound-microwave-assisted extraction (UMAE), on the phytochemical composition and biological activities of the extracts prepared from the aerial parts of *Matthiola ovatifolia* Boiss. Total phenolics, flavonoids, tannins, alkaloids and saponins were spectrophotometrically measured. In addition, antioxidant, antibacterial, and cytotoxic activities against human hepatoma (Hep-G2) and human breast (MCF-7) cancer cells, antidiabetic properties, and the anti-inflammatory activity were also investigated. The results showed significant differences in total phenolics, flavonoids, tannins, alkaloids and saponins concentrations among different extraction methods. The ethanolic extract prepared using the MAE contained the highest total phenolics (69.6 ± 0.3 mg gallic acid equivalent (GAE)/g dry weight), total flavonoids (44.5 ± 0.1 mg quercetin equivalent (QE)/g dry weight), total tannins (45.3 ± 0.5 mg catechin/g dry weight), total alkaloids (71.6 ± 0.2 mg atropine equivalent (AE)/g dry weight) and total saponins (285.6 ± 0.1 mg escin equivalent (EE)/g dry weight) as compared to the extracts obtained with the aid of other methods. The highest antioxidant, antibacterial, cytotoxic, antidiabetic, and anti-inflammatory activities were also found for the same extract. According to the obtained results, the MAE method was much more appropriate for the extraction of phytochemicals from the *M. ovatifolia*.

Keywords Medicinal plant, Bioactive compounds, Phytochemicals, Bio-pharmaceutical properties, Therapeutic potential, Antibacterial activity

The *Matthiola* genus, belonging to the Brassicaceae family, includes several species known for their medicinal, biochemical, and ornamental significance[1]. These plants are rich in bioactive compounds, making them valuable in pharmacological and industrial applications. *Matthiola* species contain a wide range of secondary metabolites, including flavonoids, alkaloids, glucosinolates, tannins, and essential oils[2]. These phytochemicals have a major impact on the human health and are essential for the plant defence. Among them, phenolic compounds and flavonoids have potent antioxidant properties that scavenge free radicals and reduce the oxidative stress, which

[1]Department of Range and Watershed Management, Faculty of Water and Soil, University of Zabol, Zabol 98613-35856, Iran. [2]Department of Agricultural, Forestry, Food and Environmental Sciences, University of Basilicata, Via dell'Ateneo Lucano, Potenza 10 - 85100, Italy. [3]Department of Analytical Chemistry and Chemical Metallurgy, Faculty of Chemistry, Wroclaw University of Science and Technology, Wyspianskiego 27, Wroclaw 50-370, Poland. [4]Nano-biotechnology and Translational Knowledge Laboratory, Department of Applied Biology, University of Science and Technology Meghalaya, 9th Mile, Baridua, Ri-Bhoi-793101, Techno City, Meghalaya, India. [5]Centre for Herbal Pharmacology and Environmental Sustainability, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education, Kelambakkam 603103, Tamil Nadu, India. ✉email: majidsharifirad@uoz.ac.ir

is connected to a number of chronic diseases[1,3]. Additionally, *Matthiola* species extracts demonstrated a broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria, indicating their potential for the development of plant-based antibiotics and food preservatives[1,4,5]. Some *Matthiola* species also exhibit cytotoxic effects on the cancer cell lines, possibly as a result of glucosinolates and their hydrolysis byproducts, such as isothiocyanates, which are investigated for their anticancer properties[6,7]. Regarding the environmental and agricultural importance, as a member of the Brassicaceae family, *Matthiola* contributes to the health and biodiversity of soil[8,9]. Some species were found to have potential applications in the phytoremediation[10]. The choice of the extraction method plays a crucial role in determining the yield, quality, and bioactivity of the plant-derived compounds[11]. Different techniques influence the efficiency of the phytochemical recovery, stability, and biological potency, making the selection of an appropriate method essential for research and industrial applications. In particular, different solvents and techniques (e.g., conventional solvent extraction, ultrasound-assisted, microwave-assisted) vary in their ability to dissolve and release bioactive compounds[12]. Modern techniques like the microwave-assisted extraction (MAE) and the ultrasound-assisted extraction (UAE) gained in popularity because of their effectiveness, environmental friendliness, and capacity to preserve the thermolabile substances. By reducing the temperature and duration of extraction, these techniques increase the yields of the bioactive compounds with their minimum degradation. For example, the MAE was shown to reduce the processing time and the solvent consumption while producing higher concentrations of the bioactive compounds from a variety of plant materials than traditional methods. Similar to this, the UAE improves the extraction process by creating cavitation bubbles that break down the plant cell walls, allowing the intracellular compounds to be released and protecting heat-sensitive materials[13,14]. The extraction method directly impacts the antioxidant, antimicrobial, and anti-inflammatory properties of the extract[15].

The primary objective of this study was to systematically evaluate and compare different extraction methodologies to optimize the recovery of bioactive compounds from the aerial parts of *Matthiola ovatifolia* (Boiss.). The purpose of this study was to evaluate the effect of different extraction methods and solvents on the biological activities of the resulting extracts as well as the yield and composition of the phytochemicals in these extracts. Our investigation focused on comparing the extraction efficiency of different solvents (ethanol, acetone, water, and dimethyl sulfoxide [DMSO]) and extraction techniques in obtaining the extracts rich in the secondary metabolites with the enhanced therapeutic potential. The study specifically quantifies the key bioactive compounds, including total phenolics, flavonoids, tannins, alkaloids, and saponins. Furthermore, the biological activities of the extracts were evaluated, focusing on their antioxidant, antibacterial, cytotoxic, antidiabetic, and anti-inflammatory properties, in order to determine which extraction conditions will maximize the therapeutic efficacy of the resulting extracts.

Materials and methods

Chemicals and reagents

All chemicals and reagents used in this study were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Plant materials

The aerial parts of *M. ovatifolia* Boiss. were collected from natural rangelands in Kerman Province, Iran (29°56′ N, 56°16′ E). The plant material was taxonomically identified by Dr. Majid Sharifi-Rad, and a voucher specimen (ZAB, No. 79364) was deposited in the herbarium of the Department of Rangeland and Watershed Management, University of Zabol, Zabol, Iran. The fresh samples were thoroughly rinsed with tap water and shade-dried at room temperature for 24–48 h to decrease surface moisture. After drying, the samples were chopped into small pieces and frozen at − 20 °C for 24 h. The frozen samples were lyophilized for 48 h at − 50 °C and 0.05 mbar of pressure using a laboratory freeze-dryer (Model FD-5005-BT, Sanat Pardaz Dena Co., Iran). Using an electric grinder (Pars Khazar, Tehran, Iran), the lyophilized plant materials were ground into a fine powder and kept in airtight containers at − 20 °C until it was needed.

Methods for extraction of phytochemical compounds

The plant extracts were prepared from the above-prepared plant material and using various extraction methods, as outlined by Cheng et al., (2023)[16]. The conventional solvent extraction (CSE), the microwave-assisted extraction (MAE), the ultrasound-assisted extraction (UAE), and the ultrasound-microwave-assisted extraction (UMAE) were carried out using 4 different solvents: ethanol, acetone, water, and DMSO. Each extraction was conducted at a temperature of 25 °C with a material-to-liquid ratio of 1:30 (g/mL). The specific factors for each extraction technique, including temperature, duration, and power, were selected according to well-established literature precedents to guarantee a common ground for the comparative analysis.

CSE method

The CSE method based on Bochi et al. (2014)[17] with slight modifications. In brief, 1 g of the lyophilized *M. ovatifolia* powder was combined with 30 mL of 4 different solvents: ethanol, acetone, water, and DMSO. The mixtures were subjected to the magnetic stirring in dark for 1 h to facilitate the extraction process. Following this, the supernatants were separated by the centrifugation at 10,000×g for 10 min at 4 °C. The collected supernatants were then concentrated at 40 °C using a rotary evaporator (R-215, Buchi Labortechnik AG, Switzerland) and stored at − 18 °C for the subsequent analysis.

UAE method

The UAE method was performed using a commercial UAE instrument (CW-2000, XTrust Instruments, Shanghai, China) following the modified procedure reported by Jiang et al. (2017)[18]. In brief, 1 g of the lyophilized *M. ovatifolia* powder was mixed with 4 different solvents (as in the Subsection 2.3.1.) at a material-to-liquid ratio

of 1:30 (g/mL). After vortexing, the resulting mixtures were sonicated for 15 min at an ultrasonic power of 250 W. The resulting extracts were then centrifuged at 10,000×g for 10 min at 4 °C to remove all solid particulates. The collected supernatants were concentrated at 40 °C using the rotary evaporator and stored at − 18 °C for the further analysis.

MAE method

The MAE method was carried out following the modified methods described by Dahmoune et al. (2015)[19] and Castro-Lopez et al. (2017)[20]. Briefly, 1 g of the lyophilized *M. ovatifolia* powder was mixed with 4 different solvents (as in the Subsection 2.3.1.) at a material-to-liquid ratio of 1:30 (g/mL). The extraction was performed for 165 s at a microwave power level of 550 W using the microwave-assisted extraction instrument. This method effectively ruptures the plant cell walls by creating the internal pressure through the rapid volumetric heating. The resulting mixtures were then centrifuged at 10,000×g for 10 min at 4 °C. The collected supernatants were concentrated at 40 °C using the rotary evaporator and stored at − 18 °C for the further analysis.

UMAE method

An ultrasound-microwave-assisted extraction (UMAE) instrument was used. Briefly, 1 g of the lyophilized *M. ovatifolia* powder was mixed separately with 4 different solvents (as in the Subsection 2.3.1.) at a material-to-liquid ratio of 1:30 (g/mL). The extraction was performed at a 250 W ultrasound power and a 550 W microwave power for 165 s. The UMAE process disrupts the plant matrix in a synergistic way by combining the mechanical effects of the ultrasound cavitation with the volumetric heating of the microwave energy. The resulting mixtures were centrifuged at 10,000×g at 4 °C, after which the supernatants were collected, concentrated at 40 °C using the rotary evaporator, and stored at − 18 °C for the further analysis[16].

Phytochemical analysis

Total phenolics content

The total phenolic content (TPC) in various extracts of the aerial parts of *M. ovatifolia* was quantified using a spectrophotometric method adapted from Sharifi-Rad et al. (2021)[21]. For consistency, all extracts were prepared at a concentration of 300 µg/mL prior to the analysis. In brief, 0.5 mL of each extract was combined with 2.5 mL of a 10% Folin–Ciocalteu reagent solution and 2.5 mL of a 7.5% sodium bicarbonate ($NaHCO_3$) solution. The resulting reaction mixtures were incubated in dark at room temperature for 45 min. The absorbance readings were subsequently taken at 765 nm using a UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). A calibration curve was constructed using gallic acid as the standard, with concentrations of the working standard solutions ranging from 10 to 100 µg/mL. The strong linear relationship was approved by a coefficient of determination (R^2 of 0.997). The TPC in the samples was expressed as the gallic acid equivalent (GAE), i.e., in mg of GAE/g dry weight of a sample. The spectrophotometer was zeroed using a respective procedural blank solution.

Total flavonoids content

The total flavonoid content (TFC) in various extracts of the aerial parts of *M. ovatifolia* was determined using a colorimetric assay based on the method described by Sharifi-Rad et al. (2020)[22]. All extracts were prepared at a uniform concentration of 300 µg/mL for the analysis. Briefly, 0.5 mL of each extract was mixed with 0.1 mL of a 10% aluminum chloride ($AlCl_3$) solution, 0.1 mL of a 1 mol/L potassium acetate solution, and 4.3 mL of distilled water. The reaction mixtures were then incubated at room temperature for 30 min. The absorbance of the resulting solutions was measured at 510 nm using the UV-1800 spectrophotometer. A calibration curve was prepared using quercetin as the standard, with concentrations of the working standard solutions ranging from 10 to 100 µg/mL. The high degree of linearity was approved by a coefficient of determination (R^2 of 0.994). The TFC in the samples was expressed as the quercetin equivalents (QE), i.e., in mg of QE/g dry weight of a sample. The spectrophotometer was zeroed using a respective procedural blank solution.

Total tannins content

The total tannin content (TTC) in various extracts of the aerial parts of the *M. ovatifolia* was determined using the method adapted from Sun et al. (1998)[23]. Briefly, 2 mL of a 4% vanillin solution in methanol and 1.5 mL of concentrated hydrochloric acid (HCl) were added to 50 µL of the plant extracts (solutions containing 300 µg/mL of the extract). The reaction mixtures were allowed to stand for 25 min, after which their absorbances were measured at 500 nm using the UV-Vis spectrophotometer. Catechin served as the standard for the calibration curve, which was prepared using the working standard solutions containing from 10 to 100 µg/mL of catechin. The strong linear relationship was approved by a coefficient of determination (R^2 of 0.994). The TTC in the samples was expressed as milligrams of the (+)-catechin equivalents per gram of dry weight of a sample (mg of CE/g dry weight). The spectrophotometer was zeroed using a respective procedural blank solution.

Total alkaloids content

The total alkaloid content (TAC) in various extracts of the aerial parts of *M. ovatifolia* was determined using a colorimetric method reported by Ajanal et al. (2012)[24]. Briefly, the extracts, prepared at a concentration of 300 µg/mL, were dissolved in dimethyl sulfoxide (DMSO) and mixed with 1 mL of a 2 mol/L HCl solution. The resulting mixtures were then filtered and transferred to separating funnels. Subsequently, 5 mL of a phosphate buffer and 5 mL of a bromocresol green solution were added to each funnel. Such mixtures were vigorously shaken with chloroform, and the chloroform layers were collected into 10 mL volumetric flasks. The absorbance of the resulting chloroformic solutions was measured at 470 nm using the UV-1800 spectrophotometer. A standard calibration curve was prepared using atropine as the standard, where the working standard solutions had

concentrations of atropine ranging from 10 to 100 µg/mL. The excellent linearity was approved by a coefficient of determination (R^2 of 0.9927). The TAC in the samples was expressed as the atropine equivalents (mg of AE/g dry weight). The spectrophotometer was zeroed using a respective procedural blank solution.

Total saponins content

The total saponin content (TSC) in various extracts of the aerial parts of *M. ovatifolia* was determined using a modified method described by Vuong et al. (2013)[25]. Briefly, 0.5 mL of each extract (prepared at a concentration of 300 µg/mL) was mixed with 0.5 mL of a 8% vanillin solution, followed by the addition of 5 mL of a 72% sulfuric acid (H_2SO_4) solution. The resulting mixtures were thoroughly mixed and cooled on ice. Subsequently, the mixtures were incubated in a water bath at 60 °C for 15 min. After incubation, the mixtures were cooled again on ice, and their absorbances were measured at 560 nm using the UV-1800 spectrophotometer. A calibration curve was prepared using escin as the standard, with concentrations of the working standard solutions ranging from 10 to 100 µg/mL. A strong linear relationship was confirmed by a coefficient of determination (R^2 of 0.9901). The total saponin content in the samples was expressed as escin equivalents (mg of EE/g dry weight). The spectrophotometer was zeroed using a respective procedural blank solution.

Antioxidant properties

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH radical scavenging activity of the extracts prepared from the aerial parts of *M. ovatifolia* was assessed according to the methodology outlined by Sharifi-Rad et al. (2015)[26]. In brief, 1 mL of each extract (300 µg/mL) was combined with 3 mL of a 0.1 mM DPPH solution. The reaction mixtures were then incubated for 30 min at room temperature in the absence of light. Next, the absorbances of the resulting solutions were measured at 517 nm using the UV–visible spectrophotometer. Ascorbic acid at an equivalent concentration served as the standard, while methanol was used as the negative control. The DPPH radical scavenging activity (%) was calculated using the following formula:

$$DPPH\ scavenging\ activity\ (\%) = \frac{\text{Absorbance } control\ -\ \text{Absorbance } sample}{\text{Absorbance } control} \times\ 100$$

Ferric ion reducing antioxidant power (FRAP) assay

The FRAP assay was performed based on the method described by Benzie and Strain (1996)[27] with slight modifications. The FRAP reagent was made by mixing a 20 mM iron (III) chloride ($FeCl_3$) solution (1 mL) and a 10 mM TPTZ (2,4,6-tripyridyl-S-triazine) solution in 40 mM HCl (1 mL) with a 300 mM sodium acetate buffer (pH 3.6, 10 mL). All these solutions were then incubated for 10 min at 37 °C in a water bath. The samples of the prepared plant extracts (20 µL, 300 µg/mL) were mixed with 150 µL of the FRAP reagent solution and allowed to react for 5 min in dark. Then, the absorbances of the so-prepared samples were acquired at 593 nm. Ascorbic acid at an equivalent concentration served as the positive control, while methanol was used as the negative control (blank). The FRAP value (%) was calculated using the following formula:

$$\text{FARP value}\ (\%)\ =\ [(A_s - A_b)/(A_c - A_b)]\ \times\ 2$$

where A_s represents the absorbance of the sample, A_b represents the absorbance of the blank and A_c represents the absorbance of the positive control.

ABTS radical scavenging activity

The ABTS radical scavenging activity of various extracts of the aerial parts of *M. ovatifolia* was expressed as the percentage inhibition of the ABTS radicals and was determined using the assay described by Ko et al. (2020)[28]. The ABTS radical working solution was prepared by adjusting its initial absorbance to 0.70 ± 0.01 at 734 nm. For the assay, 300 µL of each extract (at a concentration of 300 µg/mL) was mixed with 3 mL of the ABTS radical working solution in a micro cuvette. After 30 min, the absorbances of the resulting mixtures were measured at 734 nm using the spectrophotometer. Ascorbic acid at an equivalent concentration was used as the standard, while the ABTS radical working solution served as the control. The ABTS radical scavenging activity (%) was calculated using the following formula:

$$DPPH\ scavenging\ activity\ (\%) = \frac{\text{Absorbance } control\ -\ \text{Absorbance } sample}{\text{Absorbance } control} \times 100$$

Antibacterial activity

The antibacterial activity of various extracts derived from the *M. ovatifolia* plant material (aerial parts) was evaluated against both Gram-positive and Gram-negative bacterial strains. The Gram-positive strains included *Bacillus cereus* (ATCC 8035) and *Staphylococcus aureus* (ATCC 25923), while the Gram-negative strains comprised *Shigella flexneri* (ATCC 12022) and *Escherichia coli* (ATCC 25922). All bacterial strains were obtained from the Iranian Research Organization for Science and Technology (IROST).

Minimum inhibitory concentration

The micro-broth dilution assay was applied to estimate the minimum inhibitory concentration (MIC) for the different extracts of the *M. ovatifolia* plant material, when using them on the studied pathogenic microorganisms as suggested by the Clinical and Laboratory Standards Institute (2012)[29]. The concentrations of the studied extracts were varied from 3.12 to 400 µg mL^{-1}. The test was performed using polystyrene *96*-well plates.

Accordingly, 50 µL of the different extracts and 50 µL of the Müller–Hinton broth were poured into each well. Then, 50 µL of a given 0.5 McFarland bacteria suspension was added to the wells. The plates were incubated at 37 °C for 24 h. The pure medium and the medium including the bacteria were considered as the negative and positive controls, respectively. The lowest concentration of the studied extracts that showed no observable growth of the tested bacteria were intended as the MIC.

Minimum bactericidal concentration

The minimum bactericidal concentration (MBC) was determined following the assay outlined by the Clinical and Laboratory Standards Institute (2012)[29]. Briefly, 50 µL from each well of the broth microdilution test that showed no visible bacterial growth was subcultured onto the Mueller-Hinton Agar (MHA) plates. These plates were then incubated at 37 °C for 24 h. The lowest concentrations of the various extracts from the *M. ovatifolia* plant material that resulted in no bacterial growth on the agar plates were identified as the MBC. The concentration that showed the pathogen re-viability after reculturing was considered to have a bacteriostatic effect (MIC), while the concentration that resulted in no viability was regarded as having a bactericidal effect (MBC).

Cytotoxic activity

The human breast adenocarcinoma (MCF-7, ATCC® HTB-22™) and human hepatocellular carcinoma (Hep-G2, ATCC® HB-8065™) cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). The cells were cultured in the Dulbecco's Modified Eagle's Medium (DMEM) supplemented with a 10% fetal bovine serum (FBS) and a 1% antibiotic solution, and maintained at 37 °C in a humidified incubator with 5% CO_2. The cytotoxicity of the *M. ovatifolia* extracts was evaluated using the standard crystal violet staining method[30]. Briefly, MCF-7 and Hep-G2 cells were seeded separately in the 96-well plates. Once the cells reached a 70% confluence, the medium was discarded, and the solutions containing various extracts (300 µg/mL) were added. The plates were then incubated at 37 °C for 48 h. To the assess cell viability, a 2% (v/v) crystal violet solution in methanol was added to each well, followed by 200 µL of a glacial acetic acid solution. The mixture was thoroughly mixed, and absorbance was measured at 595 nm using a microplate reader (BioTek EL 800, USA). Vinblastine sulfate served as the standard anticancer drug.

Anti-diabetic activity

α-amylase inhibitory assay

The pancreatic porcine α-amylase inhibition assay was conducted following the methods described by Sudha et al. (2011)[31] and Parmar and Rupasinghe (2015)[32]. The studied plant extracts, the enzyme, and soluble starch were dissolved in a 20 mM sodium phosphate buffer containing 6 mM NaCl (pH 6.9). In a test tube, 250 µL of pancreatic porcine α-amylase (1 U/mL, dissolved in the buffer at pH 6.9) and 100 µL of each extract (at a concentration of 300 µg/mL) were combined. The mixture was pre-incubated at 37 °C for 15 min, after which 250 µL of a 0.5% starch solution was added. The mixture was vortexed and incubated again at 37 °C for 15 min. One mL of the dinitrosalicylic acid (DNS) color reagent was added to stop the process. After 5 min in a boiling water bath, the tubes were allowed to cool to room temperature before being diluted. Two hundred microliters of each reaction mixture were transferred to a 96-well clear plate, and the absorbance was measured at 540 nm using a FLUOstar OPTIMA plate reader. The control (α-amylase at 1 U/mL, no inhibitor) represented the 100% enzyme activity. The extract controls excluded the enzyme to correct for the color interference. Acarbose, a known α-amylase inhibitor, served as the positive control. Inhibition percentages were calculated using the standard formula.

$$Inhibition\ (\%) = 100 \times (A_C - A_S) / A_C$$

Where A_S is the absorbance of the sample and A_C is the absorbance of the control.

α-glucosidase inhibitory assay

The α-glucosidase inhibitory assay was adapted from Li et al. (2010)[33] with modifications. Briefly, various plant extracts were prepared in a 10 mM potassium phosphate buffer (pH 6.8). In a *96*-well microplate, a reaction mixture containing 20 µL of each extract (300 µg/mL), 20 µL of a α-glucosidase solution (0.5 U/mL), and 60 µL of the 10 mM potassium phosphate buffer (pH 6.8) was pre-incubated at 37 °C for 15 min. Then, 20 µL of a 5 mM *p*-nitrophenol-α-D-glucopyranoside substrate solution was added, and the mixture was incubated at 37 °C. After 15 min, 80 µL of a "stop" solution (200 mM sodium carbonate) was added, and the absorbance was acquired at 405 nm using a microplate reader. The positive control contained the enzyme and the substrate without the inhibitor. The inhibition percentages were calculated similarly to the α-amylase assay, with acarbose as the standard inhibitor for comparison.

Anti-inflammatory activity

Human red blood cell membrane assay

To evaluate the anti-inflammatory activity of the extracts of the *M. ovatifolia* plant material, the Human Red Blood Cell (HRBC) membrane stabilization assay was performed following a standard protocol[34]. Blood samples were collected from healthy, random volunteers and mixed with the Alsever's solution.The mixture was then centrifuged at 4000 rpm for 15 min. The resulting pellet was washed with a 0.85% iso-saline solution, and a 10% cell suspension was prepared using iso-saline as the working blood solution. Next, 1 mL of each extract (300 µg/mL) was taken and treated with 2 mL of a 0.36% hyposaline solution, 1 mL of a phosphate buffer (0.15 M), and finally, 0.5 mL of the working blood solution was added. Diclofenac sodium (as a standard drug) at the same concentration was used as the positive control. In the control setup, 2 mL of distilled water was used instead

of saline. The prepared mixtures were incubated at 37 °C for 30 min and then centrifuged at 4000 rpm for 15 min. The supernatants were discarded, and the hemoglobin concentration was measured at 560 nm using a UV-visible spectrophotometer. The percentage of the membrane stabilization (protection) was calculated using the following equation:

$$\text{ABTS radical scavenging activity } (\%) = \frac{\text{Absorbance } control - \text{Absorbance } sample}{\text{Absorbance } control} \times 100$$

Statistical analysis

The experiments were performed at least 3 times to ensure the accuracy and the reliability of the results. The statistical analysis was carried out by the SPSS software (version 11.5, IBM Corporation, Armonk, NY, USA). The one-way analysis of variance (ANOVA), then the *post-hoc* comparisons were conducted by the Duncan multiple range test to evaluate the statistically significant variations among the groups. The results were expressed as mean values ± standard deviations (SD), and the significant differences were presented at $p < 0.05$.

Results and discussion

Phytochemical analysis

The results on the phytochemical analysis (TPC, TFC, TTC, TAC, TSC) of various extracts of *M. ovatifolia*, as prepared from the aerial parts of this plant, were given in Table 1. They showed that there were significant differences ($p < 0.05$) in the TPC, the TFC, the TTC, the TAC and the TSC achieved among various extracts of *M. ovatifolia*. The ethanolic extract achieved using the MAE method consistently yielded the highest content for all investigated phytochemicals, with the values of 69.6 mg GAE/g DW for phenolics, 44.5 mg QE/g DW for flavonoids, 45.3 mg CE/g DW for tannins, 71.6 mg AE/g DW for alkaloids, and 285.6 mg EE/g DW for saponins. Conversely, the water extract obtained using the CSE method generally represented the lowest values among these categories, specifically: 26.3 mg GAE/g DW (phenolics), 24.5 mg QE/g DW (flavonoids), 22.6 mg CE/g DW (tannins), 35.4 mg AE/g DW (alkaloids), and 224.1 mg EE/g DW (saponins).

Various letters indicate statistically significant differences ($p < 0.05$).

These results highlighted the critical interaction between the solvent polarity, the extraction mechanism, and the chemical nature of target compounds in determining the extraction efficiency. The observed differences in recovery of the studied phytochemicals were mainly attributed to two interrelated factors: the polarity of the extraction solvent and the mechanism of the extraction method.

First, the polarity of the solvent plays a very important role in its ability to dissolve and extract special compounds from the plant matrix. The plant secondary metabolites usually show an extended range of polarities[35]. The phenolic compounds (including many tannins and flavonoids) and alkaloids, although their exact structures are diverse, often have moderate polarities due to their aromatic rings, nitrogen atoms, and hydroxyl groups[36]. Saponins, which are characterized by non-polar aglycone (sapogenin) and polar sugar chains, also have varying degrees of polarity due to their type and number of sugar moieties[37,38]. The results of Do et al. (2014), who highlighted ethanol versatility in extracting various phytochemicals due to its ability to effectively interact with compounds of diverse polarities, also support the idea that ethanol, as an organic solvent with moderate polarity at 25 °C, has a favorable balance that leads to the effective dissolution of a wide range of compounds, from relatively polar phenolics to less polar saponins and alkaloids[39]. Conversely, water is a highly polar solvent at 25 °C that is very effective on highly polar compounds, but less effective for the compounds with significant lipophilic properties[36]. These reasons explain why the water extract obtained using the CSE method

		TPC (mg GAE/g DW)	TFC(mg QE/g DW)	TTC (mg catechin/g DW)	TAC (mg atropine/g DW)	TSC (mg escin/g DW)
CSE	Ethanol	44.2 ± 0.1^{j}	34.6 ± 0.3^{h}	37.4 ± 0.1^{f}	54.3 ± 0.4^{g}	251.5 ± 0.4^{j}
	Water	26.3 ± 0.4^{o}	24.5 ± 0.3^{m}	22.6 ± 0.3^{l}	35.4 ± 0.2^{m}	224.1 ± 0.3^{n}
	DMSO	32.4 ± 0.3^{n}	30.3 ± 0.5^{k}	28.3 ± 0.2^{k}	41.3 ± 0.1^{l}	232.5 ± 0.2^{m}
	Acetone	37.6 ± 0.2^{m}	32.2 ± 0.4^{j}	31.2 ± 0.1^{j}	48.3 ± 0.2^{j}	246.2 ± 0.1^{l}
UAE	Ethanol	57.3 ± 0.2^{f}	39.5 ± 0.2^{d}	40.2 ± 0.2^{d}	62.6 ± 0.3^{d}	264.6 ± 0.5^{g}
	Water	32.5 ± 0.4^{n}	28.7 ± 0.5^{l}	32.8 ± 0.6^{i}	44.5 ± 0.4^{k}	232.6 ± 0.2^{m}
	DMSO	45.3 ± 0.1^{i}	34.2 ± 0.3^{h}	36.3 ± 0.2^{g}	49.3 ± 0.2^{i}	248.3 ± 0.1^{k}
	Acetone	51.2 ± 0.3^{h}	38.6 ± 0.1^{e}	38.7 ± 0.5^{e}	56.8 ± 0.1^{f}	257.3 ± 0.4^{h}
MAE	Ethanol	69.6 ± 0.3^{a}	44.5 ± 0.1^{a}	45.3 ± 0.5^{a}	71.6 ± 0.2^{a}	285.6 ± 0.1^{a}
	Water	42.3 ± 0.2^{k}	35.3 ± 0.4^{g}	37.4 ± 0.1^{f}	56.3 ± 0.3^{f}	267.2 ± 0.3^{f}
	DMSO	58.6 ± 0.4^{e}	38.4 ± 0.3^{e}	39.7 ± 0.3^{d}	61.2 ± 0.4^{e}	275.4 ± 0.2^{d}
	Acetone	67.2 ± 0.2^{b}	42.1 ± 0.2^{b}	43.5 ± 0.4^{b}	67.4 ± 0.2^{c}	280.7 ± 0.5^{b}
UMAE	Ethanol	65.2 ± 0.1^{c}	42.6 ± 0.2^{b}	43.6 ± 0.2^{b}	68.4 ± 0.6^{b}	279.6 ± 0.3^{c}
	Water	38.4 ± 0.3^{l}	33.2 ± 0.3^{i}	35.6 ± 0.3^{h}	50.2 ± 0.5^{h}	253.2 ± 0.2^{i}
	DMSO	52.5 ± 0.2^{g}	36.7 ± 0.1^{f}	38.2 ± 0.4^{e}	56.5 ± 0.2^{f}	264.3 ± 0.1^{g}
	Acetone	61.6 ± 0.4^{d}	40.2 ± 0.5^{c}	41.4 ± 0.1^{c}	62.3 ± 0.4^{d}	271.4 ± 0.4^{e}

Table 1. The total phenols (TPC), total flavonoids (TFC), total tannins (TTC), total alkaloids (TAC), and total saponins (TSC) concentrations in the extracts prepared from the aerial parts of *M. ovatifolia*.

showed the lowest concentrations of all phytochemicals examined in this work, as many of these compounds in the *M. ovatifolia* plant material were probably not optimally soluble in water alone.

Secondly, the extraction method significantly affects the transfer efficiency of compounds from the plant material to the solvent. The CSE method is primarily concerned with the simple diffusion and solubility, and often requires higher temperatures and longer extraction times to obtain the adequate extraction yields. This process is limited by the slow diffusion of the compounds through the intact plant cell walls[36,40]. In contrast, the MAE method provides the significant benefits that specifically address these drawbacks. By using the microwave energy, the MAE method directly interacts with the polar molecules in the plant matrix and solvent. A rapid volumetric heating brought on by this interaction normally causes the internal pressure to build up inside the plant cells. The quick heating breaks down the cell walls, which makes it easier for the intracellular components to be released and speeds up their diffusion into the solvent[37]. Despite possibly having similar extraction times, the ethanolic extract obtained with the aid of the MAE method produced noticeably higher concentrations of all target phytochemicals than the water extract obtained with the CSE method, which could be explained by the improved mass transfer kinetics and the increased cell permeability under the microwave irradiation. An ideal environment for the maximum recovery of different groups of compounds from the *M. ovatifolia* plant material was achieved by the disruptive, accelerated extraction mechanism of the MAE method in conjunction with the suitable polarity of ethanol. In conclusion, the synergistic effect of solvent polarity and the extraction method was directly responsible for the notable variations in the extraction yields of phytochemicals. The disruptive and kinetic benefits of the microwave assistance, combined with a wide range of secondary metabolites willingly soluble in ethanol, made it incredibly effective. These results highlighted how crucial it is to maximize the recovery of the valuable bioactive compounds from the plant materials for a possible use in cosmetics, nutraceuticals, and pharmaceuticals by optimizing the extraction parameters.

The colorimetric assays used in this investigation have inherent limitations, even though they offered a thorough summary of the overall phytochemical content and biological activities. These techniques measure large classes of compounds (like total phenolics, and flavonoids) rather than single, targeted compounds, so they are semi-quantitative and non-specific. As a result, the correlations drawn between the chemical compounds and the measured biological activities, like antibacterial and antioxidant effects, should be considered as general trends rather than precise, compound-specific relationships. Future research should concentrate on separating and describing the distinct bioactive compounds to improve the scientific knowledge. This can be obtained by using more specific and advanced analytical techniques like high-performance liquid chromatography (HPLC) along with nuclear magnetic resonance (NMR) spectroscopy or mass spectrometry (MS). These techniques would make it possible to precisely measure the active components and establish a stronger link between them and their special biological activities.

Antioxidant properties

DPPH radical scavenging activity

Figure 1 provides a summary of the DPPH radical scavenging activity of the *M. ovatifolia* extracts, emphasizing the impact of the solvent type and the extraction method on the antioxidant potential of these extracts. The highest radical scavenging activity was consistently produced by the MAE method out of all extraction methods used, especially when ethanol was applied as the extraction solvent (71.8% inhibition). This was most likely caused by the quick and even heating that the microwave energy produces, which presumably promoted the efficient cell disruption and increased the release of the intracellular antioxidant substances like phenolic acids and flavonoids[37,38]. Additionally, the extracts obtained with the UMAE method showed a significant antioxidant activity, particularly with ethanol (66.3%) and acetone (58.2%), ranking as the second one only to the MAE method. This suggested that the ultrasound-induced cavitation and the microwave energy together facilitated the effective release of the phytochemicals. An important factor in determining the extraction efficiency was the solvent polarity. In every method, ethanol performed better than other solvents, with acetone, DMSO, and water coming in the second and the third, respectively. Ethanol under the use of the MAE method reached a 71.8% score, while the water-based extracts had the lowest DPPH scavenging values, reaching a maximum of 51.3%. This pattern illustrated how well ethanol extracts the medium-polarity antioxidant molecules[41]. Regardless of the solvent type, the extracts produced using the CSE method showed the lowest antioxidant activities. For example, in the absence of the physical support (such as ultrasounds or microwaves), the ethanolic extract prepared with the aid of the CSE method only demonstrated a 56.5% inhibition, suggesting a rather limited cell wall disruption and compound diffusion. Considering the scavenging level, the ethanolic extracts obtained with the MAE and UMAE methods were relatively close to the positive control, i.e., ascorbic acid (83.6%, but none of the extracts were able to match it. These results implied that *M. ovatifolia* may be a powerful natural source of the antioxidant agents when extracted under the optimal conditions. The general pattern, as illustrated in Fig. 1, makes it evident that the advanced extraction methods, in particular the MAE method and the UMAE method, when paired with ethanol as the solvent, can produce noticeably greater antioxidant capacities than the CSE method and less efficient solvents.

Ferric reducing antioxidant power (FRAP) assay

The ability of antioxidants to donate electrons or convert the Fe^{3+} (ferric) ions into the Fe^{2+} (ferrous) ions was measured by the Ferric Reducing Antioxidant Power (FRAP) assay. The FRAP assay is a useful indicator of the total reducing power rather than the free radical scavenging alone because it is primarily governed by the single electron transfer (SET) mechanisms, as opposed to the hydrogen atom transfer (HAT)-based DPPH assay[27,42]. Figure 2 illustrates that extracts produced by the MAE method had the highest FRAP values, especially when ethanol (57.4%) and acetone (55.3%) were utilized. This was likely because the MAE method could increase the release of the reducing agents like tannins and phenolic acids by disrupting the cell wall and heating the interior

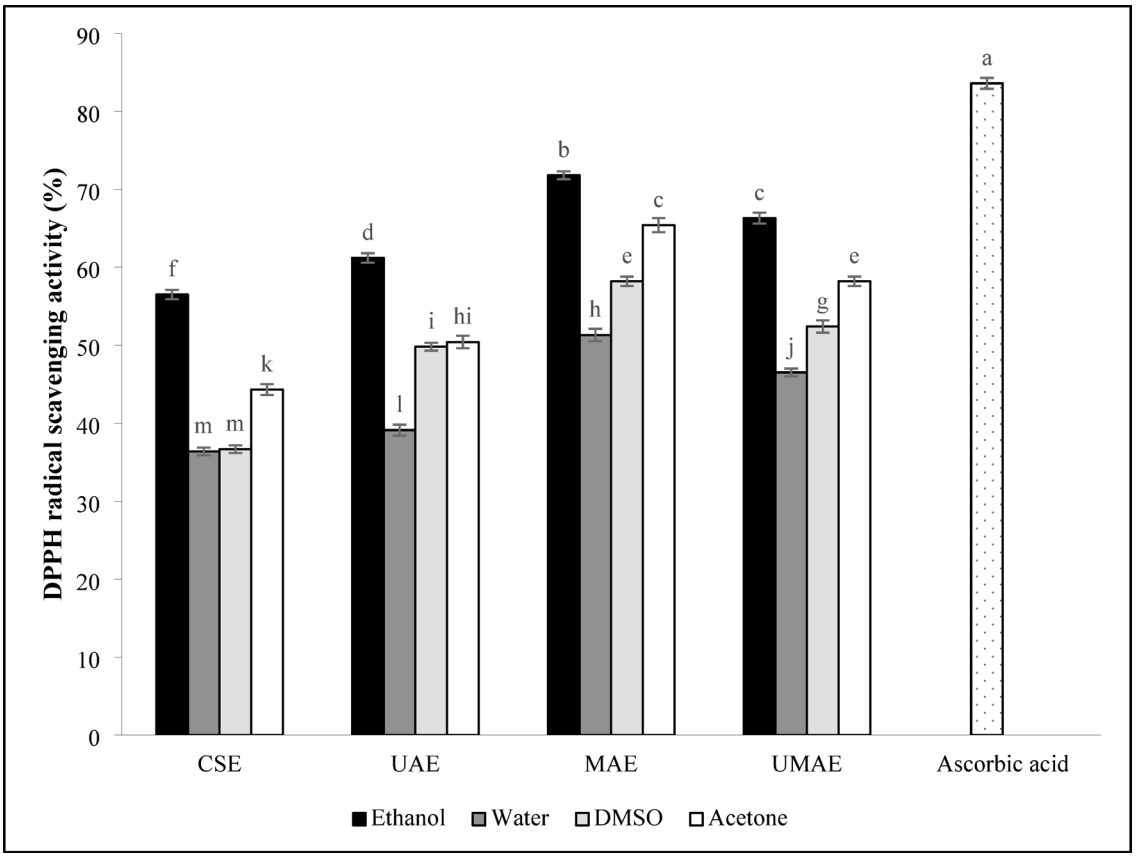

Fig. 1. The DPPH radical scavenging activity ($n = 3$) of various extracts prepared from the aerial parts of *M. ovatifolia*. The extract concentration is 300 µg/mL. Various letters indicate statistically significant differences based on the Duncan's test ($p < 0.05$).

quickly[37]. These substances were most likely the primary contributors to the FRAP values because they are efficient electron donors[43]. The results achieved for the extracts obtained with the UMAE method were similar to those achieved with the MAE method, with ethanol (55.8%) and acetone (52.7%), once more demonstrating the strong reducing power. According to Chemat et al. (2017) this implied that the extraction of the redox-active compounds could benefit from the synergistic effects of the microwave heating and the ultrasound-induced cavitation[44]. It is interesting to note that the DMSO-based extracts performed noticeably better in the FRAP assay than in the DPPH assay. According to Apak et al. (2016), DMSO could be especially useful for extracting some polar and high molecular weight antioxidants with the electron-donating properties, such as complex flavonoids and hydrolyzable tannins, that are more responsive to FRAP than to DPPH[42]. Among all the extraction methods, water extracts produced the lowest FRAP values; in particular, those obtained with the CSE method produced the FRAP values of only 28.7%. The limited solubility of many powerful reducing agents in water, including flavonoids and polyphenols, particularly those with a moderate to low polarity, might be the cause of this. According to earlier research, polyphenolic compounds are typically more soluble and extractable in organic solvents with the intermediate polarity than in water[45,46]. As a positive control, ascorbic acid was used and it showed a FRAP value of 73.6%, which is a standard for the highest level of the antioxidant capacity. Although none of the extracts were able to achieve this level, the ethanol-based MAE and UMAE extracts came very close, indicating that, when extracted under ideal circumstances, *M. ovatifolia* has the potential to be a source of the antioxidants that donate the electrons. These results demonstrated that compounds with the high redox potential, such as phenolic acids, flavonols, and condensed tannins, are especially sensitive to FRAP. This also showed that the MAE method improves the extraction efficiency of these compounds.

ABTS radical scavenging activity

The ABTS assay is a flexible technique for evaluating both the hydrophilic and lipophilic antioxidant compounds. It assesses the capacity of antioxidants to quench the $ABTS^{\bullet+}$ radical cation and functions *via* both the electron transfer (ET) and the hydrogen atom transfer (HAT) mechanisms. This allows the ABTS assay to better detect the antioxidant activity than the more lipophilic DPPH assay or the FRAP assay, which is exclusively based on the ET[42,47].

According to Fig. 3, ethanol-based extracts had the highest ABTS scavenging activity of any solvents applied when using the MAE method, with the highest value being 71.6%. This was most likely because the MAE method could extract a wide variety of antioxidants, including polar and non-polar compounds, many of which have the

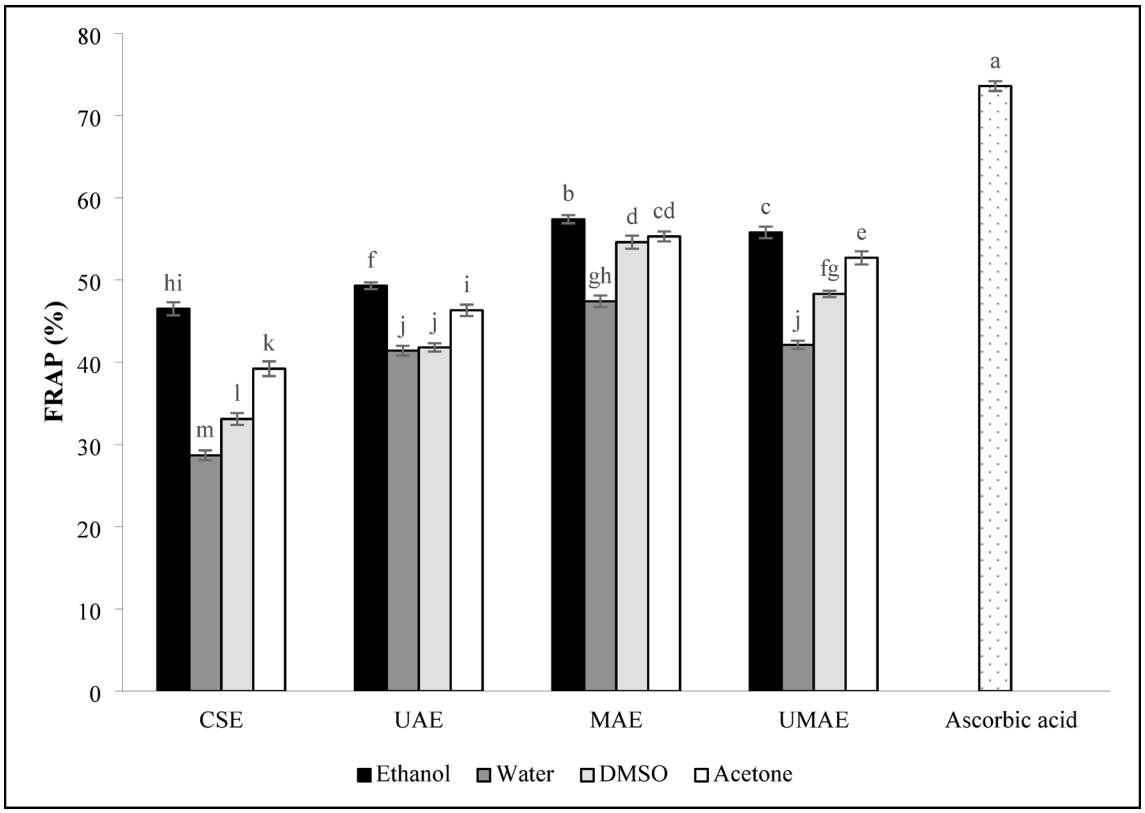

Fig. 2. Ferric reducing antioxidant power (FRAP) ($n = 3$) of various extracts prepared from the aerial parts of *M. ovatifolia*. The extract concentration is 300 µg/mL. Various letters indicate statistically significant differences based on the Duncan's test ($p < 0.05$).

capacity to interact with the $ABTS^{\bullet+}$ radical through dual mechanisms[43]. Additionally, the extract prepared using the UMAE method showed a strong activity, especially with ethanol (67.3%) and acetone (62.5%), confirming the synergistic effect of the microwave heating and the ultrasonic cavitation in enhancing the mass transfer and compound release from the plant matrix. Curiously, DMSO extracts achieved under the use of the MAE method yielded 67.8%, which was significantly higher than the results in the FRAP and DPPH tests. This showed that some mid-polar bioactives that were more sensitive to the $ABTS^{\bullet+}$ scavenging but less detectable by DPPH or FRAP, like glycosylated flavonoids and sulfur-containing compounds, might be dissolved by DMSO as well[46]. The ABTS activity of water-based extracts, which performed poorly in the FRAP and DPPH tests, was relatively improved; for instance, under the use of the MAE method and the UMAE method, the water extracts scored 57.3 and 56.9%, respectively. This suggested that ABTS was more sensitive to the hydrophilic antioxidants, such as phenolic acids, ascorbic acid analogs, and specific glycosides, many of which are soluble in aqueous solutions and active against the $ABTS^{\bullet+}$ radical[48]. As it was expected, ascorbic acid had the highest ABTS value (90.6%), indicating that the method was sensitive to both the fat- and the water-soluble antioxidants. The ethanolic extract obtained with the MAE method was the plant extract that came closest to this reference, indicating that it could be a source of the multifunctional antioxidants.

Antibacterial activity

The antibacterial activity of extracts from the aerial part of *M. ovatifolia* was examined against 4 pathogenic bacteria: two Gram-positive (*Bacillus cereus*, *Staphylococcus aureus*) and two Gram-negative (*Escherichia coli*, *Shigella flexneri*) strains. Table 2 summarizes the MIC and the MBC values for the extracts made with various solvents and the extraction methods. The antibacterial activity of the extracts obtained using the MAE method was greatly increased, especially when ethanol and acetone were used as solvents. The strong inhibitory capacity against the Gram-positive bacteria was demonstrated by the lowest MIC values of 3.12 µg/mL for the ethanolic extracts obtained *via* the MAE method against *S. aureus* and 6.25 µg/mL against *B. cereus*. Using the same solvents, these MIC values were roughly 4–8 times lower than those obtained using the UAE method and the CSE method, suggesting that the MAE method significantly increased the extraction efficiency of the bioactive phytoconstituents. For the Gram-positive strains, the corresponding MBC values for the ethanolic extracts obtained using the MAE method varied from 6.25 to 12.5 µg/mL, indicating the strong bactericidal activity at relatively low concentrations. In contrast to the Gram-positive strains, the Gram-negative bacteria showed the noticeably higher MIC and MBC values. In particular, when exposed to the ethanolic extracts made by the MAE method, *E. Coli* and *S. flexneri* displayed the MICs between 12.5 and 25 µg/mL and the MBCs between 25 and 50 µg/mL. The presence of an outer membrane in the Gram-negative bacteria, which acts as a strong

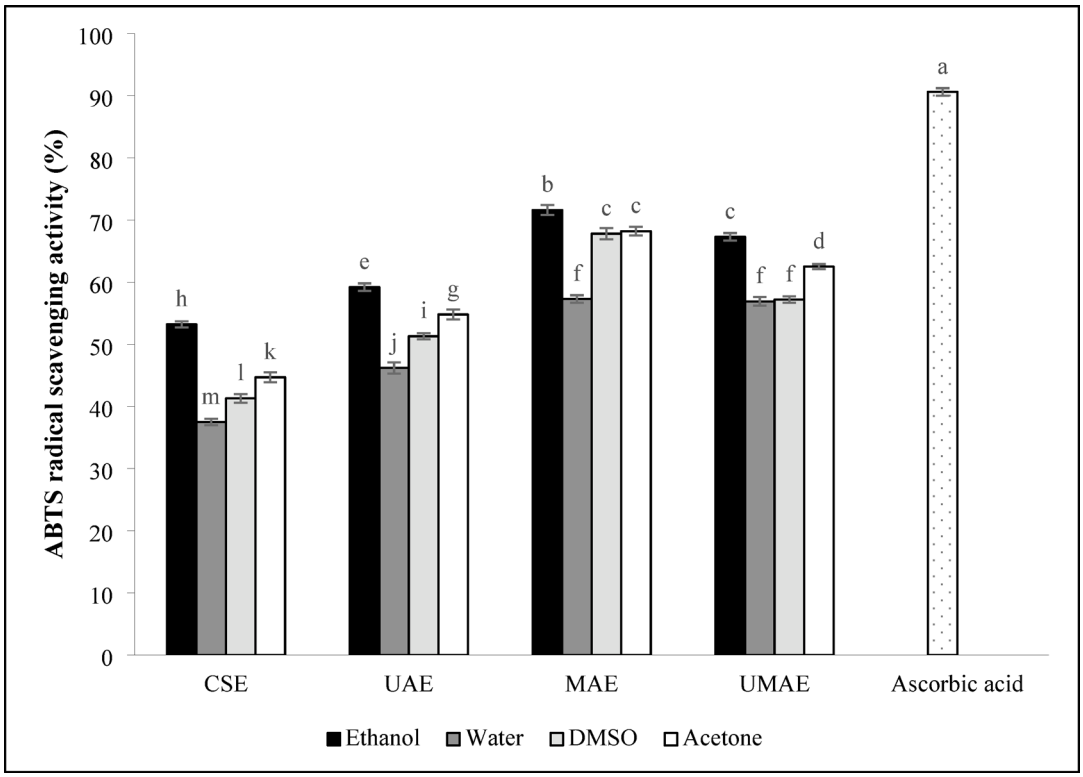

Fig. 3. The ABTS radical scavenging activity ($n = 3$) of various extracts prepared from the aerial parts of *M. ovatifolia*. The extract concentration is 300 µg/mL. Various letters indicate statistically significant differences based on the Duncan's test ($p < 0.05$).

		Gram -ve bacteria				Gram + ve bacteria			
		Escherichia coli		*Shigella flexneri*		*Bacillus cereus*		*Staphylococcus aureus*	
		MIC (µg/mL)	MBC(µg/mL)	MIC (µg/mL)	MBC(µg/mL)	MIC (µg/mL)	MBC(µg/mL)	MIC (µg/mL)	MBC(µg/mL)
CSE	Ethanol	100	200	50	100	25	50	12.5	25
	Acetone	100	200	50	100	25	50	12.5	25
	DMSO	200	400	100	200	50	100	25	50
	Water	200	400	100	200	50	100	25	50
UAE	Ethanol	100	200	50	100	25	50	12.5	25
	Acetone	100	200	50	100	25	50	12.5	25
	DMSO	200	400	100	200	50	100	25	50
	Water	200	400	100	200	50	100	25	50
MAE	Ethanol	25	50	12.5	25	6.25	12.5	3.12	6.25
	Acetone	25	50	12.5	25	6.25	12.5	3.12	6.25
	DMSO	50	100	25	50	12.5	25	6.25	12.5
	Water	50	100	25	50	12.5	25	6.25	12.5
UMAE	Ethanol	50	100	25	50	12.5	25	6.25	12.5
	Acetone	50	100	25	50	12.5	25	6.25	12.5
	DMSO	100	200	50	100	25	50	12.5	25
	Water	100	200	50	100	25	50	12.5	25

Table 2. The antibacterial activity of the *M. ovatifolia* extracts.

permeability barrier that restricts the penetration of many antimicrobial compounds, especially those with the hydrophobic properties, is consistent with this decreased susceptibility[49]. Furthermore, the plant extracts made with DMSO, and water showed a relatively low antibacterial efficacy in comparison to all other extraction solvents. This was probably because important non-polar bioactive components like flavonoids and alkaloids are less soluble in these solvents[50]. The antibacterial activity of the extracts obtained with the UMAE method was moderate, with the MIC and MBC values typically falling between those achieved for the extracts prepared

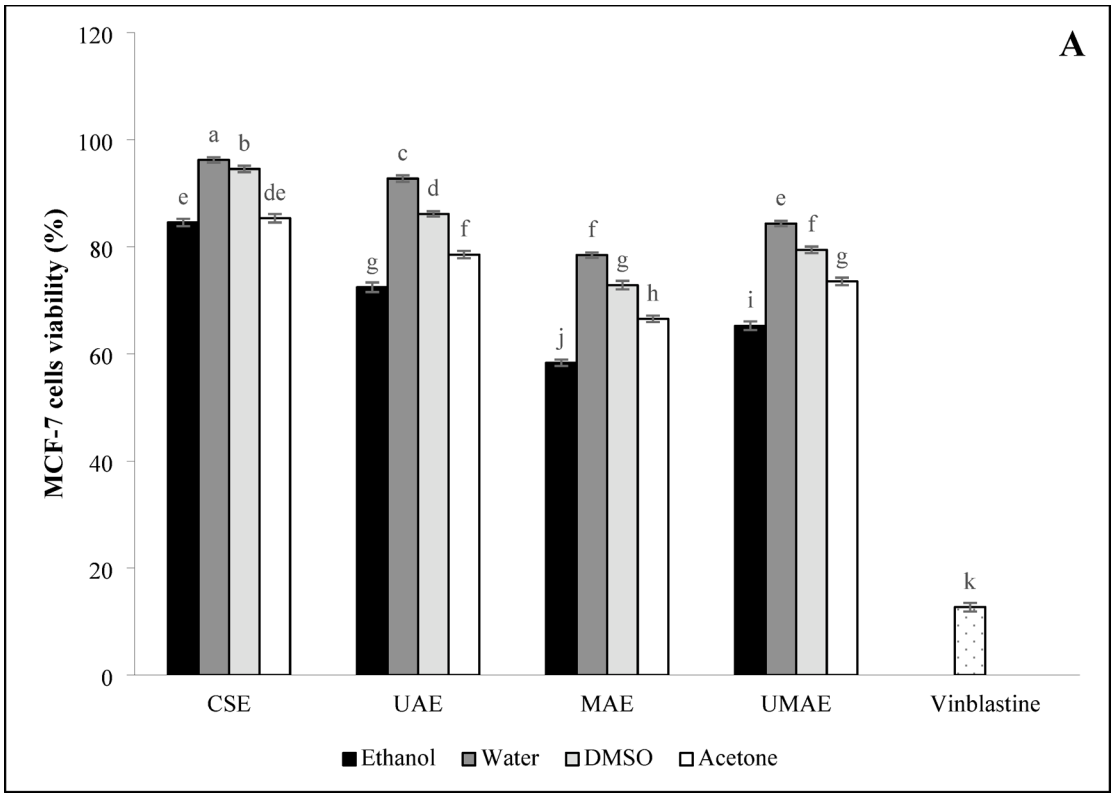

Fig. 4. The cytotoxic activity of the *M. ovatifolia* extracts toward the human cancer cell lines. Various letters indicate statistically significant differences based on the Duncan's test ($p < 0.05$).

using the UAE method and the MAE method. Although marginally less effective than in the case of the extracts obtained with the MAE method, the extracts obtained with the UMAE method demonstrated the encouraging antibacterial properties. Even though the UAE method the CSE method were simpler and easier to use, their lower effectiveness in extracting the strong antibacterial compounds from the *M. ovatifolia* plant material was demonstrated by the higher MIC values achieved for the extracts obtained with these extraction methods, which ranged from 12.5 to 25 µg/mL for the Gram-positive bacteria and up to 100 µg/mL for the Gram-negative bacteria. As noted in the Sect. 3.1, the higher content of the phytochemicals in the extracts obtained using the MAE method, particularly of phenolics, flavonoids, and alkaloids, well correlated with their superior antibacterial potency. The ability of these classes of compounds to cause the oxidative stress in the microbial cells, disrupt the bacterial membranes, and inhibit the vital enzymes is well-established[51,52]. According to Daglia (2012), the phenolic hydroxyl groups have the ability to create hydrogen bonds with the membrane proteins and bacterial enzymes, jeopardizing both the structural integrity and the metabolic functions[53]. In conclusion, it is evident from the results that the type of the solvent and the extraction method are important factors in terms of the antibacterial activity of the resulting plant extracts. The MAE with ethanol produced the extracts with the highest amount of the bioactive compounds and the strongest antibacterial effects among the extracts obtained with other tested methods, particularly against the significant Gram-positive bacteria like *Staphylococcus aureus*. These results were consistent with the recent studies that also showed that the MAE method is dependable and effective in extracting the plant compounds with a potent biological activity[54,55].

Cytotoxic activity

As shown in Fig. 4A and B, the type of the solvent and the extraction method significantly affected the cytotoxic response when the extracts prepared from the aerial parts of *M. ovatifolia* were tested against the MCF-7 (human breast adenocarcinoma) and HepG2 (human liver carcinoma) cell lines using the MTT assay. The most significant cytotoxic effect of all treatments was demonstrated by the MAE method with ethanol, which decreased the viability of MCF-7 and HepG2 cells to 58.3% and 68.2%, respectively. Acetone extracts prepared by the MAE method came in the second, with the 69.1% viability in HepG2 and 66.5% in MCF-7. These effects were significantly greater than those of other extraction methods ($p < 0.05$), despite being moderate when compared to vinblastine (positive control, 12.7% in MCF-7, and 14.1% in HepG2). A higher cell viability (often above 85–90%) was demonstrated by the extracts made using the CSE and UAE methods, particularly when water or DMSO were taken as solvents. These solvents probably extracted fewer hydrophobic bioactive molecules with known pro-apoptotic and anti-proliferative properties, like alkaloids and flavonoids[56]. The decreased cytotoxic activity of water extracts may be explained by the fact that water is less effective at solubilizing these compounds because of its high polarity[57]. The improved performance of the extracts prepared by the MAE methods aligned

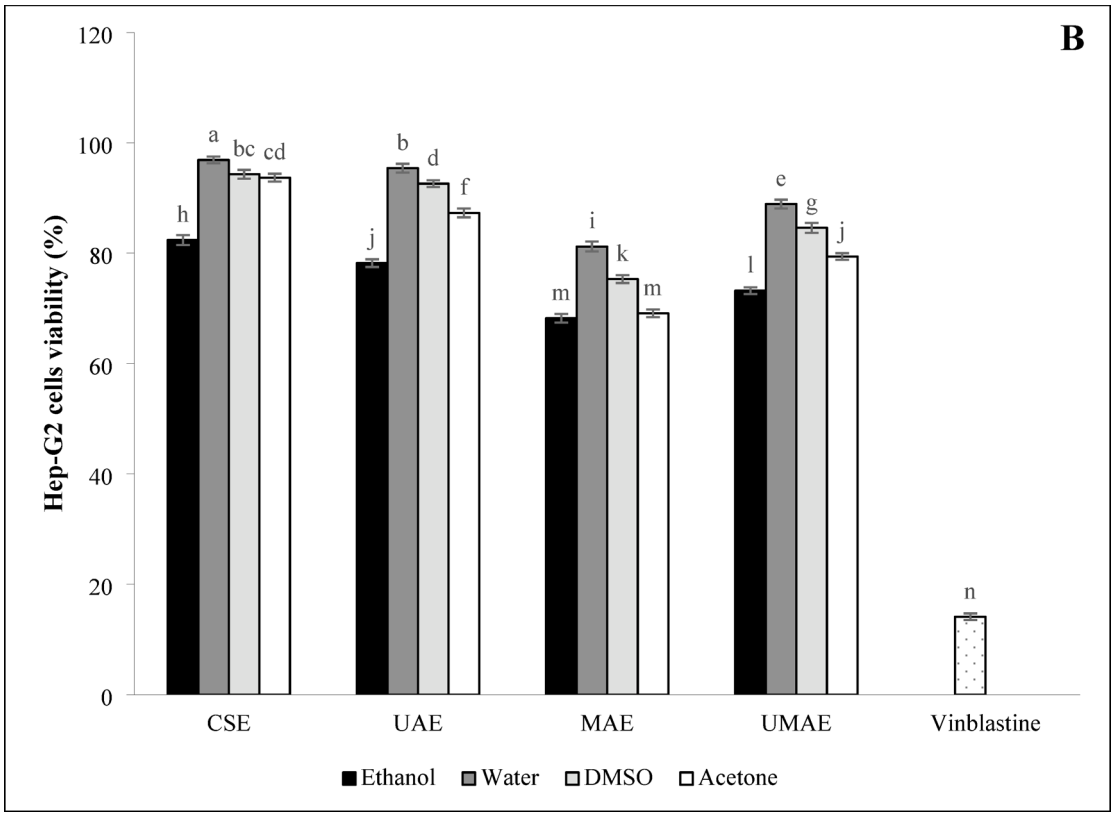

Fig. 4. (continued)

in agreement with the earlier research showing that the breakdown of the plant cell walls and the faster solvent penetration through the MAE enhance the release of various phytochemicals[37,58]. Additionally, the hypothesis that *M. ovatifolia* contains active secondary metabolites with the potential anticancer activity was supported by the moderate cytotoxicity seen here, though more fractionation and mechanism-based researches are required.

Anti-diabetic activity

The antidiabetic potential of the studied extracts prepared from the aerial part of *M. ovatifolia* was assessed by evaluating their inhibitory activities against the enzymes α-amylase and α-glucosidase. Both enzymes are crucial for the digestion of carbohydrates and the control of postprandial hyperglycemia, which makes them targets for the type 2 diabetes treatment[59,60].

α-amylase inhibitory assay

Among all the extracts, the ethanolic extract obtained through the MAE method had the highest α-amylase inhibitory activity (75.4%), with the ethanolic extract obtained using the UMAE method came in the second (72.5%). These values indicate a significant inhibition even though they are below the reference standard acarbose (97.5%). Water-based extracts, on the other hand, consistently displayed a lower inhibition across all the extraction methods; the CSE-water extract, for example, only showed a 44.2% inhibition (Fig. 5). This result was consistent with the previous research demonstrating that ethanol efficiently extracts the flavonoid and phenolic compounds that function as the α-amylase inhibitors[61]. Higher concentrations of the bioactive components likely resulted from the enhanced cell wall disruption and improved compound release when the MAE method was used[62].

α-glucosidase inhibitory assay

The α-glucosidase inhibition assay showed a similar pattern to the α-amylase inhibitory assay (Fig. 6). The ethanolic extract obtained using the MAE method showed the highest inhibition (68.3%); other sophisticated extraction methods (UMAE and UAE) also demonstrated a significant activity. Acarbose, a common medication, showed an 85.4% inhibition. Because of its intermediate polarity, which allows it to solubilize both the hydrophilic and moderately lipophilic phytochemicals, ethanol once again demonstrated its superiority as a solvent[63]. These results demonstrated how the phenolic-rich plant extracts can be used as the natural supplements or substitutes for traditional antidiabetic medications because they can inhibit the key enzymes involved in the digestion of carbohydrates[64].

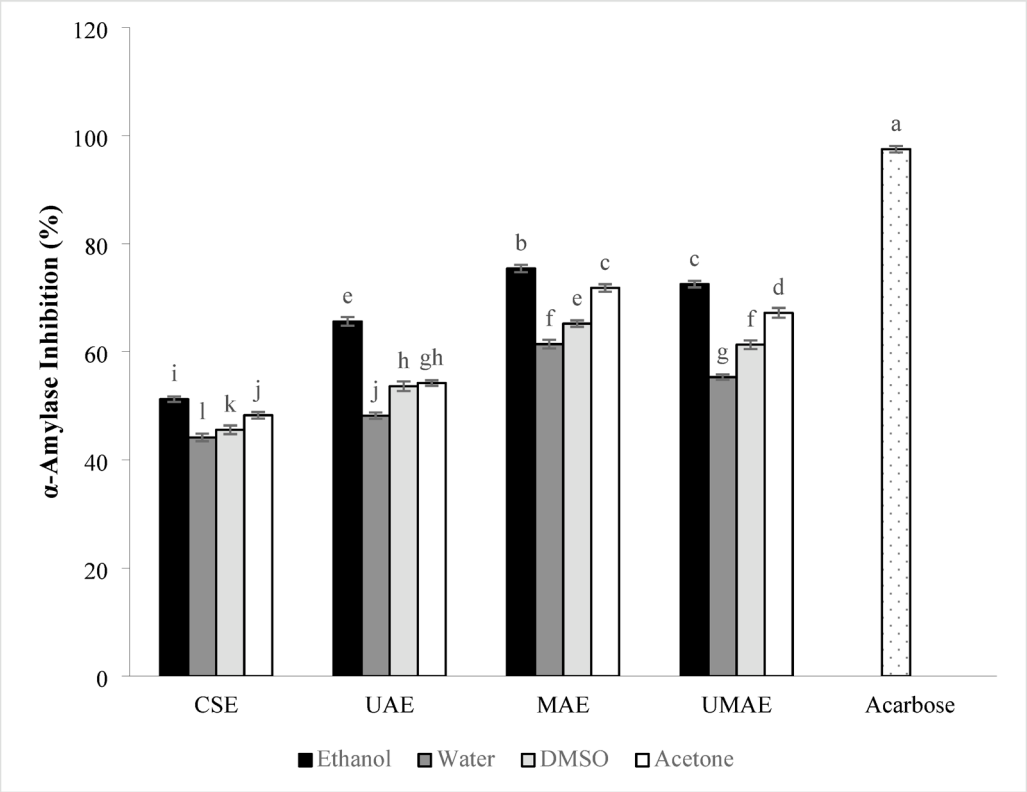

Fig. 5. The in vitro anti-diabetic (α-Amylase Inhibitory Assay) evaluation of various extracts of *M. ovatifolia*. Various letters indicate statistically significant differences based on the Duncan's test ($p < 0.05$).

Anti-inflammatory activity

Human red blood cell membrane assay

The Human Red Blood Cell (HRBC) membrane stabilization method, a highly regarded in vitro model for assessing the capacity of compounds to protect erythrocyte membranes from the hemolysis under the hypotonic stress, was used to evaluate the anti-inflammatory potential of extracts from the aerial parts of *M. ovatifolia*. The idea behind this model is that the substances that stabilize the lysosomal membranes can also stabilize red blood cell membranes, making it an indirect indicator of the anti-inflammatory activity[65,66]. The findings showed that the capacity of the membrane stabilization varied significantly amongst the extraction methods and solvents applied (Fig. 7). The highest anti-inflammatory activity was shown by the ethanolic extracts, especially those made using the MAE method, which inhibited the hemolysis by 65.3%. The ethanolic extract prepared using the UMAE method (63.4%) came in the second. Overall, water-based extracts demonstrated the least rate of activity; the CSE-water extract only showed a 30.4% inhibition. The reference anti-inflammatory drug, diclofenac sodium, had the greatest membrane stabilization effect, at 87.2%. A number of the ethanol-based extracts showed a significant activity, indicating the presence of bioactive compounds with anti-inflammatory properties, even though none of them were able to match the level of inhibition shown by diclofenac sodium. A high concentration of flavonoids, phenolic compounds, and other secondary metabolites with anti-inflammatory properties in ethanolic extracts is probably what gives them their superior performance[67]. These substances can decrease the cell lysis by stabilizing the cell membranes and preventing the release of the inflammatory mediators[68,69]. The bioactivity of the plant extracts was further increased by advanced extraction methods like the MAE and the UMAE, which likely increase the mass transfer and break down the plant cell walls, leading to a higher yield of the active compounds[62]. These results concured with the earlier research showing that the MAE and the UAE greatly improve the bioactivity and the extraction efficiency of the extracts from the medicinal plants[70]. According to these results, *M. ovatifolia* has the encouraging anti-inflammatory properties, particularly when ethanol is used as the solvent and such sophisticated methods like MAE are applied. The human red blood cell (HRBC) membrane stabilization assay is a non-specific and comparatively indirect technique, despite offering a helpful initial indication of the anti-inflammatory potential of the extracts. This test primarily assesses the ability of compounds to stabilize the erythrocyte membranes, which is a broad indicator of the anti-inflammatory activities, but it does not provide the insight into the specific inflammatory pathways or mechanisms involved. Future research should use more specific in vitro models, such as the inhibition of pro-inflammatory cytokines, the COX/LOX activity, or the nitric oxide production in macrophages, to supplement this assay and draw more reliable and mechanistic conclusions.

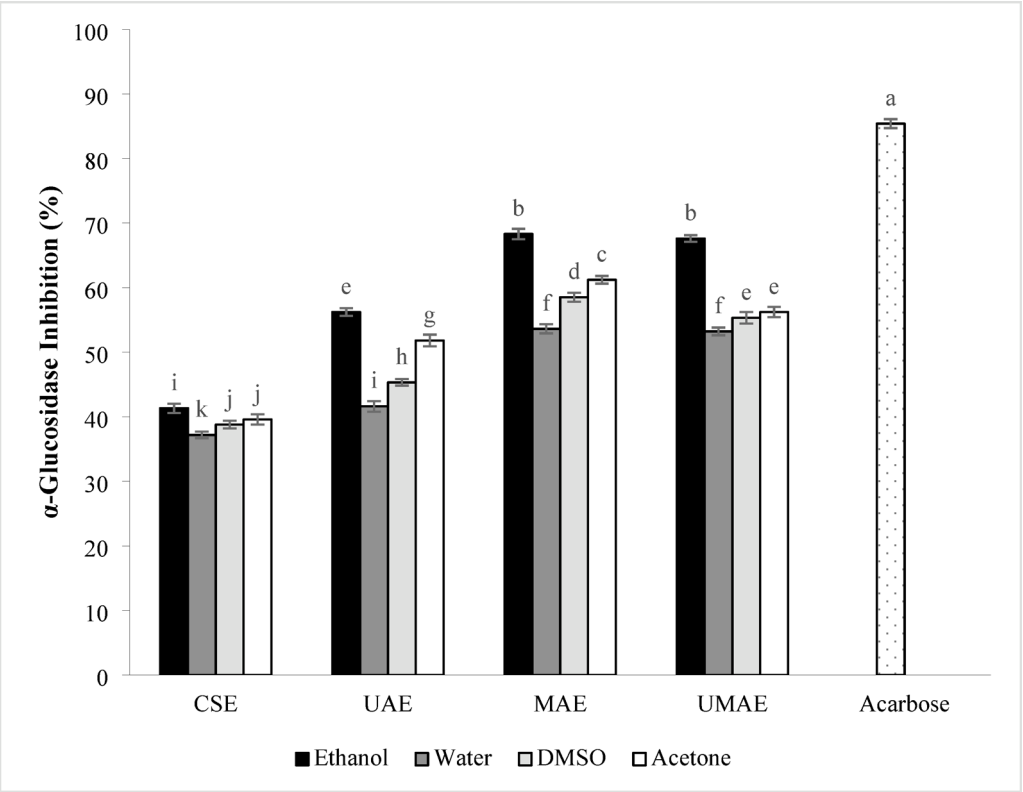

Fig. 6. The in vitro anti-diabetic (α-Glucosidase Inhibitory Assay) evaluation of various extracts of *M. ovatifolia*. Various letters indicate statistically significant differences based on the Duncan's test ($p < 0.05$).

Conclusions

The present study demonstrated that the choice of the solvent and the extraction method significantly influences the phytochemical composition and biological activities of the extracts prepared from the aerial part of *M. ovatifolia*. Among the tested methods, the microwave-assisted extraction (MAE) using ethanol yielded the highest concentrations of total phenolics, flavonoids, tannins, alkaloids, and saponins, correlating with the superior antioxidant, antibacterial, cytotoxic, antidiabetic, and anti-inflammatory activities. The ethanolic extract prepared by using the MAE method exhibited the strongest DPPH and ABTS radical scavenging capacity, the highest ferric ion reducing power, and notable inhibitory effects against α-amylase and α-glucosidase, suggesting its potential antidiabetic applications. Additionally, its significant antibacterial activity, particularly against Gram-positive bacteria, and the potent cytotoxicity against Hep-G2 and MCF-7 cancer cell lines highlight its therapeutic potential. The anti-inflammatory properties, evidenced by the RBC membrane stabilization, further support its medicinal value. These findings suggest that the MAE method with ethanol is the most efficient one for extracting various bioactive compounds from *M. ovatifolia*, making it a promising candidate for pharmaceutical and nutraceutical applications. Future research should focus on isolating and characterizing the active constituents responsible for these effects and evaluating their mechanisms of action in vivo.

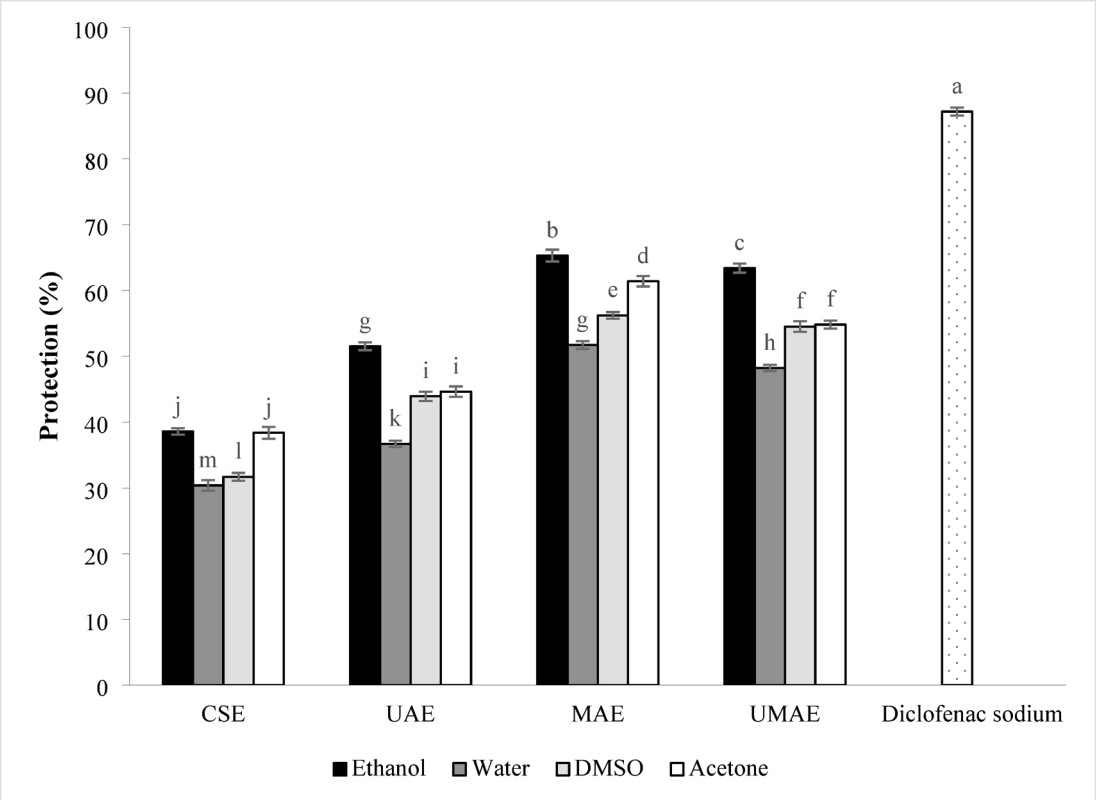

Fig. 7. The anti-inflammatory activity of various extracts of *M. ovatifolia*. Various letters indicate statistically significant differences based on the Duncan's test ($p < 0.05$).

Data availability

All data generated or analysed during this study are included in this published article.

Received: 24 June 2025; Accepted: 8 October 2025
Published online: 14 November 2025

References

1. Miceli, N. et al. Phytochemical characterization and biological activities of a hydroalcoholic extract obtained from the aerial parts of *Matthiola Incana* (L.) R. Br. subsp. *Incana* (Brassicaceae) growing wild in Sicily (Italy). *Chem. Biodivers.* **16**, e1800677. https://doi.org/10.1002/cbdv.201800677 (2019).
2. Mohammed, E. et al. Anti-fibrotic potential of a *Matthiola Arabica* isothiocyanates-rich fraction: impact on oxidative stress, inflammatory and fibrosis markers. *Int. J. Phytomed.* **72**, 614–624. https://doi.org/10.1691/ph.2017.7590 (2017).
3. Zhang, H., Zhang, H. & Yu, S. -f. Phenolic compounds and antioxidant activities in ethanolic extracts from seven cultivars of Chinese jujube. *Food Sci. Hum. Wellness.* **3**, 183–190. https://doi.org/10.1016/j.fshw.2014.12.005 (2014).
4. Canli, K. et al. Chemical composition and in vitro antimicrobial activity of *Matthiola tricuspidata* ethanol extract. *Fresenius Environ. Bull.* **29**, 8863–8868 (2020).
5. El-Amier, Y., Zaghloul, N. & Abd-ElGawad, A. Bioactive chemical constituents of *Matthiola longipetala* extract showed antioxidant, antibacterial, and cytotoxic potency. *Separations* **10**, 53. https://doi.org/10.3390/separations10010053 (2023).
6. Rasool, N. et al. Evaluation of antioxidant activity, cytotoxic studies and GC-MS profiling of *Matthiola Incana* (stock flower). *Legume Res.* **36**, 21–32 (2013).
7. Taviano, M. F. et al. Cytotoxic, antioxidant, and enzyme inhibitory properties of the traditional medicinal plant *Matthiola Incana* (L.) R. Br. *Biology (Basel).* **9**, 163. https://doi.org/10.3390/biology9070163 (2020).
8. Wortman, S. E., Francis, C. & Lindquist, J. L. Cover crop mixtures for the Western corn belt: opportunities for increased productivity and stability. *Agron. J.* **104**, 699–705. https://doi.org/10.2134/agronj2011.0422 (2012).
9. Rajagopal, V., Duff, J. & Hall, J. Brassica biofumigants for improved soil health. *Proceedings* **36** 77 https://doi.org/10.3390/proceedings2019036077 (2020).
10. Mohtadi, A., Ghaderian, S. M. & Schat, H. The effect of EDDS and citrate on the uptake of lead in hydroponically grown *Matthiola flavida*. *Chemosphere* **93**, 986–989. https://doi.org/10.1016/j.chemosphere.2013.05.066 (2013).
11. Chakanaka, P. M., King'ondu, C. K., Sigauke, P. & Obadele, B. A. Conventional and modern techniques for bioactive compounds recovery from plants: review. *Sci. Afr.* **27**, e02509. https://doi.org/10.1016/j.sciaf.2024.e02509 (2025).
12. Chibuye, B., Sen, I. S., Chimuka, L. & Kakoma, M. K. A review of modern and conventional extraction techniques and their applications for extracting phytochemicals from plants. *Sci. Afr.* **19**, e01585. https://doi.org/10.1016/j.sciaf.2023.e01585 (2023).
13. Bhadange, Y. A., Carpenter, J. & Saharan, V. K. A comprehensive review on advanced extraction techniques for retrieving bioactive components from natural sources. *ACS Omega*. **9**, 31274–31297. https://doi.org/10.1021/acsomega.4c02718 (2024).
14. Roobab, U., Aadil, R. M., Kurup, S. S. & Maqsood, S. Comparative evaluation of ultrasound-assisted extraction with other green extraction methods for sustainable recycling and processing of date palm bioresources and by-products: a review of recent research. *Ultrason. Sonochem* https://doi.org/10.1016/j.ultsonch.2025.107252 (2025).

15. El-Hadary, A. A. R., Abulmakarm, A. M., Sulieman, A. M., El-Shorbagy, G. A. & Mohamed, A. S. Impact of the unconventional extraction on antioxidants and antimicrobial characteristics of hibiscus and olive leaves extracts. *Heliyon* https://doi.org/10.1016/j.heliyon.2025.e41792 (2025).
16. Cheng, M. et al. Comparison of microwave, ultrasound and ultrasound–microwave assisted solvent extraction methods on phenolic profile and antioxidant activity of extracts from jackfruit (*Artocarpus heterophyllus* Lam.) pulp. *LWT* **173**, 114395. https://doi.org/10.1016/j.lwt.2022.114395 (2023).
17. Bochi, V. C. et al. Polyphenol extraction optimization from Ceylon gooseberry (*Dovyalis hebecarpa*) pulp. *Food Chem.* **164**, 347–354. https://doi.org/10.1016/j.foodchem.2014.05.031 (2014).
18. Jiang, H., Yang, J. & Shi, Y. Optimization of ultrasonic cell grinder extraction of anthocyanins from blueberry using response surface methodology. *Ultrason. Sonochem.* **34**, 325–331. https://doi.org/10.1016/j.ultsonch.2016.06.003 (2017).
19. Dahmoune, F., Nayak, B., Moussi, K., Remini, H. & Madani, K. Optimization of microwave-assisted extraction of polyphenols from *Myrtus communis* L. leaves. *Food Chem.* **166**, 585–595. https://doi.org/10.1016/j.foodchem.2014.06.066 (2015).
20. Castro-Lopez, C. et al. Impact of extraction techniques on antioxidant capacities and phytochemical composition of polyphenol-rich extracts. *Food Chem.* **237**, 1139–1148. https://doi.org/10.1016/j.foodchem.2017.06.032 (2017).
21. Sharifi-Rad, M., Pohl, P. & Epifano, F. Phytofabrication of silver nanoparticles (AgNPs) with pharmaceutical capabilities using *Otostegia persica* (Burm.) Boiss. Leaf extract. *Nanomaterials* **11**, 1045. https://doi.org/10.3390/nano11041045 (2021).
22. Sharifi-Rad, M., Pohl, P., Epifano, F. & Álvarez-Suarez, J. M. Green synthesis of silver nanoparticles using *Astragalus tribuloides* delile root extract: characterization, antioxidant, antibacterial, and anti-inflammatory activities. *Nanomaterials* **10**, 2383. https://doi.org/10.3390/nano10122383 (2020).
23. Sun, B., Richardo-Da-Silvia, J. M. & Spranger, I. Critical factors of Vanillin assay for catechins and proanthocyanidins. *J. Agric. Food Chem.* **46**, 4267–4274. https://doi.org/10.1021/jf980366j (1998).
24. Ajanal, M., Gundkalle, M. B. & Nayak, S. U. Estimation of total alkaloid in Chitrakadivati by UV-Spectrophotometer. *Anc. Sci. Life.* **31**, 198. https://doi.org/10.4103/0257-7941.107361 (2012).
25. Vuong, Q. V. et al. Effect of extraction conditions on total phenolic compounds and antioxidant activities of *Carica Papaya* leaf aqueous extracts. *J. Herb. Med.* **3**, 104–111. https://doi.org/10.1016/j.hermed.2013.04.004 (2013).
26. Sharifi-Rad, J., Hoseini-Alfatemi, S. M., Sharifi-Rad, M. & Teixeira da Silva, J. A. Antibacterial, antioxidant, antifungal and anti-inflammatory activities of crude extract from *Nitraria schoberi* fruits. *3 Biotech* **5**, 677–684. https://doi.org/10.1007/s13205-014-0266-1 (2015).
27. Benzie, I. F. & Strain, J. J. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. *Anal. Biochem.* **239**, 70–76. https://doi.org/10.1006/abio.1996.0292 (1996).
28. Ko, M. J., Nam, H. H. & Chung, M. S. Subcritical water extraction of bioactive compounds from *Orostachys japonicus* A. Berger (Crassulaceae). *Sci. Rep.* **10**, 1–10. https://doi.org/10.1038/s41598-020-67508-2 (2020).
29. CLSI. *Reference Method for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow aerobically; Approved Standard M7-A6* (National Committee for Clinical Laboratory Standards, 2012).
30. Nayak, D., Ashe, S., Rauta, P. R. & Nayak, B. Assessment of antioxidant, antimicrobial and anti-osteosarcoma potential of four traditionally used Indian medicinal plants. *J. Appl. Biomed.* **15**, 119–132. https://doi.org/10.1016/j.jab.2016.10.005 (2017).
31. Sudha, P., Zinjarde, S. S., Bhargava, S. Y. & Kumar, A. R. Potent α-amylase inhibitory activity of Indian ayurvedic medicinal plants. *BMC Complement. Altern. Med.* **11**, 5. https://doi.org/10.1186/1472-6882-11-5 (2011).
32. Parmar, I. & Rupasinghe, H. Antioxidant capacity and anti-diabetic activity of wild berry stem infusions. *Eur. J. Med. Plants.* **8**, 11–28. https://doi.org/10.9734/EJMP/2015/17115 (2015).
33. Li, D. Q., Qian, Z. M. & Li, S. P. Inhibition of three selected beverage extracts on α-glucosidase and rapid identification of their active compounds using HPLC-DAD-MS/MS and biochemical detection. *J. Agric. Food Chem.* **58**, 6608–6613. https://doi.org/10.1021/jf100853c (2010).
34. Vane, J. R. & Botting, R. M. Anti-inflammatory drugs and their mechanism of action. *Inflamm. Res.* **47**, 78–S87. https://doi.org/10.1007/s000110050284 (1998).
35. Gil-Martín, E. et al. Influence of the extraction method on the recovery of bioactive phenolic compounds from food industry by-products. *Food Chem.* **378**, 131918. https://doi.org/10.1016/j.foodchem.2021.131918 (2022).
36. Azmir, J. et al. Techniques for extraction of bioactive compounds from plant materials: a review. *J. Food Eng.* **117**, 426–436. https://doi.org/10.1016/j.jfoodeng.2013.01.014 (2013).
37. Routray, W. & Orsat, V. Microwave-assisted extraction of flavonoids: a review. *Food Bioprocess. Technol.* **5**, 409–424. https://doi.org/10.1007/s11947-011-0573-z (2012).
38. Mahmoudpour, M., Mohtadinia, J., Mousavi, M. M., Ansarin, M. & Nemati, M. Application of the microwave-assisted extraction and dispersive liquid–liquid Microextraction for the analysis of PAHs in smoked rice. *Food Anal. Methods.* **10**, 277–286. https://doi.org/10.1007/s12161-016-0579-2 (2017).
39. Do, Q. D. et al. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. *J. Food Drug Anal.* **22**, 296–302. https://doi.org/10.1016/j.jfda.2013.11.001 (2014).
40. Ameer, K., Shahbaz, H. M. & Kwon, J. H. Green extraction methods for polyphenols from plant matrices and their byproducts: a review. *Compr. Rev. Food Sci. Food Saf.* **16**, 295–315. https://doi.org/10.1111/1541-4337.12253 (2017).
41. Tiwari, P., Kumar, B., Kaur, M., Kaur, G. & Kaur, H. Phytochemical screening and extraction: a review. *Int. Pharm. Sciencia.* **1**, 7 (2011).
42. Apak, R., Özyürek, M., Güçlü, K. & Çapanoglu, E. Antioxidant activity/capacity measurement. 1. Classification, physicochemical principles, mechanisms, and electron transfer (ET)-based assays. *J. Agric. Food Chem.* **64**, 997–1027. https://doi.org/10.1021/acs.jafc.5b04739 (2016).
43. Dai, J. & Mumper, R. J. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules* **15**, 7313–7352. https://doi.org/10.3390/molecules15107313 (2010).
44. Chemat, F. et al. Review of green food processing techniques. Preservation, transformation, and extraction. *Innov. Food Sci. Emerg. Technol.* **41**, 357–377. https://doi.org/10.1016/j.ifset.2017.04.016 (2017).
45. Iloki-Assanga, S. B. et al. Solvent effects on phytochemical constituent profiles and antioxidant activities, using four different extraction formulations for analysis of *Bucida buceras* L. and *Phoradendron Californicum*. *BMC Res. Notes.* **8**, 1. https://doi.org/10.1186/s13104-015-1388-1 (2015).
46. Xiang, Z. et al. Solvent effects on the phenolic compounds and antioxidant activity associated with *Camellia polyodonta* flower extracts. *ACS Omega.* **9**, 27192–27203. https://doi.org/10.1021/acsomega.4c01321 (2024).
47. Re, R. et al. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol. Med.* **26**, 1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3 (1999).
48. Kim, D. O., Jeong, S. W. & Lee, C. Y. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chem.* **81**, 321–326. https://doi.org/10.1016/S0308-8146(02)00423-5 (2003).
49. Nikaido, H. Molecular basis of bacterial outer membrane permeability revisited. *Microbiol. Mol. Biol. Rev.* **67**, 593–656. https://doi.org/10.1128/mmbr.67.4.593-656.2003 (2003).
50. Muhamad, N., Muhmed, S. A., Yusoff, M. M. & Gimbun, J. Influence of solvent Polarity and conditions on extraction of antioxidant, flavonoids and phenolic content from *Averrhoa bilimbi*. *J. Food Sci. Eng.* **4**, 255–260. https://doi.org/10.17265/2159-5828/2014.05.006 (2014).

51. Cowan, M. M. Plant products as antimicrobial agents. *Clin. Microbiol. Rev.* **12**, 564–582. https://doi.org/10.1128/cmr.12.4.564 (1999).
52. Cushnie, T. T. & Lamb, A. J. Recent advances in Understanding the antibacterial properties of flavonoids. *Int. J. Antimicrob. Agents.* **38**, 99–107. https://doi.org/10.1016/j.ijantimicag.2011.02.014 (2011).
53. Daglia, M. Polyphenols as antimicrobial agents. *Curr. Opin. Biotechnol.* **23**, 174–181. https://doi.org/10.1016/j.copbio.2011.08.007 (2012).
54. Boeira, C. et al. Ultrasonic assisted extraction to obtain bioactive, antioxidant and antimicrobial compounds from marcela. *Ciênc Rural* **48**, e20170722. https://doi.org/10.1590/0103-8478cr20170772 (2018).
55. Horablaga, N. M. et al. Influence of sample preparation/extraction method on the phytochemical profile and antimicrobial activities of 12 commonly consumed medicinal plants in Romania. *Appl. Sci.* **13**, 2530. https://doi.org/10.3390/app13042530 (2023).
56. Mali, S. B. Cancer treatment: role of natural products. Time to have a serious rethink. *Oral Oncol. Rep.* **6**, 100040. https://doi.org/10.1016/j.oor.2023.100040 (2023).
57. Mikucka, W., Zielinska, M., Bulkowska, K. & Witonska, I. Subcritical water extraction of bioactive phenolic compounds from distillery stillage. *J. Environ. Manage.* **318**, 115548. https://doi.org/10.1016/j.jenvman.2022.115548 (2022).
58. Yusoff, I. M., Taher, Z. M., Rahmat, Z. & Chua, L. S. A review of ultrasound-assisted extraction for plant bioactive compounds: phenolics, flavonoids, thymols, saponins and proteins. *Food Res. Int.* **157**, 111268. https://doi.org/10.1016/j.foodres.2022.111268 (2022).
59. Teng, H. & Chen, L. α-Glucosidase and α-amylase inhibitors from seed oil: a review of liposoluble substances to treat diabetes. *Crit. Rev. Food Sci. Nutr.* **57**, 3438–3448. https://doi.org/10.1080/10408398.2015.1129309 (2017).
60. Kashtoh, H. & Baek, K. H. New insights into the latest advancement in α-amylase inhibitors of plant origin with anti-diabetic effects. *Plants* **12**, 2944. https://doi.org/10.3390/plants12162944 (2023).
61. Ćorković, I., Gašo-Sokač, D., Pichler, A., Šimunović, J. & Kopjar, M. Dietary polyphenols as natural inhibitors of α-amylase and α-glucosidase. *Life* **12**, 1692. https://doi.org/10.3390/life12111692 (2022).
62. Mandal, V., Mohan, Y. & Hemalatha, S. Microwave assisted extraction—an innovative and promising extraction tool for medicinal plant research. *Pharmacogn Rev.* **1**, 7 (2007).
63. Tourabi, M. et al. Optimization of extraction process and solvent polarities to enhance the recovery of phytochemical compounds, nutritional content, and biofunctional properties of *Mentha longifolia* L. extracts. *Bioresour Bioprocess.* **12**, 24. https://doi.org/10.1186/s40643-025-00859-8 (2025).
64. Marghich, M. et al. Antioxidant activity and Inhibition of carbohydrate digestive enzymes activities of *Artemisia Campestris* L. *Front. Biosci.* **14**, 25. https://doi.org/10.31083/j.fbs1404025 (2022).
65. Yesmin, S. et al. Membrane stabilization as a mechanism of the anti-inflammatory activity of ethanolic root extract of Choi (*Piper chaba*). *Clin. Phytosci.* **6**, 1. https://doi.org/10.1186/s40816-020-00207-7 (2020).
66. Sharifi-Rad, M. et al. *Teucrium polium* (L.): phytochemical screening and biological activities at different phenological stages. *Molecules* **27**, 1561. https://doi.org/10.3390/molecules27051561 (2022).
67. Fiorito, S. et al. Auraptene and umbelliprenin: a review on their latest literature acquisitions. *Phytochem Rev.* https://doi.org/10.1007/s11101-020-09713-5 (2022).
68. Kumar, S., Bajwa, B. S., Kuldeep, S. & Kalia, A. N. Anti-inflammatory activity of herbal plants: a review. *Int. J. Adv. Pharm. Biol. Chem.* **2**, 272–281 (2013).
69. Kim, J. H. et al. Antioxidant and anti-inflammatory effects of *Oxalis corniculata* hot water extract. *Korean J. Med. Crop Sci.* **30**, 419–429 (2022).
70. Dhanani, T., Shah, S., Gajbhiye, N. A. & Kumar, S. Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of *Withania somnifera*. *Arab. J. Chem.* **10**, S1193–S1199. https://doi.org/10.1016/j.arabjc.2013.02.015 (2017).

Acknowledgements

Ethics declarationsField studies involving Matthiola ovatifolia (Boiss.) were conducted in accordance with institutional, national, and international guidelines. Although the species is not listed by the IUCN or CITES, only minimal, non-destructive sampling was carried out to avoid any ecological disturbance.

Author contributions

Majid Sharifi-Rad: Writing—review & editing, Writing—original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisi tion, Formal analysis, Data curation, Conceptualization. Ippolito Camele: Writing—review & editing, Writing—original draft. Pawel Pohl: Writing—review & editing, Investigation, Writing—original draft. Yugal Kishore Mohanta: Writing—review & editing, Writing—original draft. Hazem S. Elshafie: Writing—review & editing, Writing—original.

Funding

This research was funded by the University of Zabol, Zabol, Iran (grant number: IR-UOZ-GR-9186).

Declarations

Competing interests

The authors declare no competing interests.

Ethics

Field studies involving *Matthiola ovatifolia* (Boiss.) were conducted in accordance with institutional, national, and international guidelines. Although the species is not listed by the IUCN or CITES, only minimal, non-destructive sampling was carried out to avoid any ecological disturbance.

Additional information

Correspondence and requests for materials should be addressed to M.S.-R.