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Phytochemical analysis and in vitro biological activity assessment of extracts from *Micromeria myrtifolia*

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ABSTRACT

In this study, the antioxidant capacity, the effect on anti-acetylcholinesterase and anti-urease enzyme inhibition, and kidney stone-reducing effects of different extracts from *M.myrtifolia*. In addition, the phytochemical content of the bioactive methanol extract was analyzed by HPLC-DAD. The methanol extract showed strong DPPH (IC_{50}:0.036 mg/mL) radical scavenging, Cu (II) ion (6.535 mmTroloxE) and Fe^{3+} (6.476 $FeSO_4$) ion reducing antioxidant activity compared to other extracts. The methanol extract contained the highest amount of phenolic (400 mgGAE) and flavonoid (2.519 QuE) compounds. Moreover, the methanol extract had significant anticholinesterase (78.935%), anti-urease (71.014%) and calcium oxalate anti-crystallization (18.22%, 37.427%) activities. The findings show that all extracts exhibited varying amounts of antibacterial activity against *Staphylococcus aureus*. The bioactive methanol extract contained rosmarinic acid (31.35 µg), quercetin (12.99 µg) and chlorogenic acid (3.55 µg). The analyzed compounds are thought to contribute significantly to the pharmacological effect of the methanol extract.

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Micromeria myrtifolia; biological activity; HPLC-DAD

Introduction

The genus *Micromeria* Benth. has a diverse distribution, ranging from the Macaronesian-Mediterranean region to southeast Africa, India, and China. Its taxa species are adaptable and can thrive at altitudes ranging from sea level to 4500 meters. Within the Flora of Turkey, there are a total of 14 *Micromeria* species, encompassing 22 distinct taxa. Notably, 12 of these species are exclusive to the region, making them endemic (Davis 1970). *Micromeria* has a rich history in traditional medicine, where it has been used for generations to alleviate inflammation, fever, asthma, skin maladies, heart problems, and digestive system irregularities (Said et al. 2002). In addition to their traditional uses in folk medicine, studies conducted on *Micromeria* species have revealed various pharmacological effects, including antimicrobial, antifungal, antioxidant, anticholinesterase, anti-inflammatory, gastroprotective, hepatoprotective, and cytotoxic activities. *Micromeria* species are known to contain flavonoids, saponins, tannins, and anthraquinone

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derivatives among their chemical constituents. Furthermore, they serve as a source of essential oils with a highly rich composition (Akkol et al. 2019).

M. myrtifolia is commonly found in Israel, Lebanon, and Turkey (Sarikurkcu et al. 2019). *M. myrtifolia* is a strongly aromatic herb that grows in Turkey. It is known by various local names such as "Kayakekiği," "Altınbaşçayı," "Haydarotu," and "Topukçayı." In the areas of Kahramanmaraş, Gaziantep, and Antakya, as well as the surrounding villages, it is a popular choice for making herbal tea (Tabanca et al. 2001). In traditional folk medicine, infusions made from the aerial parts of the *M. myrtifolia;* this plant also used for the treatment of common colds, flu, skin disorders, gastrointestinal issues, and gallstone therapy. Additionally, infusions obtained from the aerial parts are consumed by the public due to their relaxant and sedative properties, at a rate of 1–2 tea cups per day (Tabanca et al. 2001; Akkol et al. 2019). Akkol et al., have determined the effects of n-hexane, ethanol, and methanol extracts obtained from the aerial parts of *M. myrtifolia* species on depression using in vitro and in vivo depression models. They identified the most active extract as the methanol extract. They believe that the major components responsible for this activity are rosmarinic acid, myricetin, apigenin, and naringenin (Akkol et al. 2019). In another study, the water extract was found to have the highest antioxidant activity among the extracts prepared with water, methanol and ethyl acetate, which was attributed to its high total phenolic and flavonoid content. After the water extract, the methanol and ethyl acetate extracts showed relatively low antioxidant activity. The methanol extract showed the strongest inhibition in terms of anti-alpha-amylase and anti-tyrosinase activities (Sarikurkcu et al. 2020). In another study conducted by Sarikurkcu et al. (2020), the essential oil of *M. myrtifolia* species was analyzed. It was found that the essential oil exhibited high antioxidant capacity and showed strong inhibition of both anti-alpha-amylase and anti-tyrosinase activities (Tabanca et al. 2001). In another study conducted on the essential oil of *M. myrtifolia* plant, it was found that the essential oil exhibited strong radical scavenging activity, had a reducing capacity for Fe (III) ions, and contained high levels of phenolic and flavonoid compounds (Formisano et al. 2014). In other biological studies, the species has been found to exhibit antifungal, antiviral, antimicrobial, and cytotoxic activities (Kremer et al. 2014).

In the literature review, it has been observed that activity studies of extracts obtained using various solvents are quite limited. In this study, the antioxidant capacity, the effect on anti-acetylcholinesterase and anti-urease enzyme inhibition, and kidney stone-reducing effects of petroleum ether, chloroform and methanol extracts obtained by sequential maceration method were investigated. In addition, the phytochemical content of the bioactive methanol extract was analyzed by HPLC-DAD.

Materials and methods

Plant material

M. myrtifolia plant was collected from Adıyaman province (north of Gürlevik water supply, from rocky-stony slopes), on June 2022. Plant sample was identified by Dr. Ömer Kılıç, who works at Adıyaman University, Faculty of Pharmacy, Department of Pharmaceutical Botany. Herbarium number of plant sample is: 58 and plant species were dried in the shade at 25°C.

Preparation of M. myrtifolia extracts

The aerial parts of *M. myrtifolia* were subjected to a desiccation process under ambient environmental conditions, resulting in their complete drying. Following this, the desiccated plant material was finely pulverized utilizing an herb grinder known as the RENAS RBT1250. The maceration method was selected as the preferred technique for the extraction of bioactive compounds. The maceration process was conducted as follows: A total of 45 grams of the plant powder was treated with an appropriate volume of three distinct solvents, namely petroleum ether, chloroform, and

methanol. These solvent-plant mixtures were allowed to interact for 72 hours at room temperature. Subsequently, upon completion of the extraction process, the resultant extracts were subjected to a concentration procedure employing a rotary evaporator under low pressure and controlled temperature conditions. The concentrated extracts, now enriched with bioactive compounds, were carefully preserved, and stored within a refrigeration unit, maintaining a constant temperature of 4°C. These stored extracts were reserved for subsequent biological activity experiments, ensuring their integrity and efficacy for future analyses and investigations.

Antioxidant activity of M. myrtifolia extracts

The assessment of antioxidant capacities within extracts derived from the aerial components of *M. myrtifolia* plant specimens was undertaken using the sequential maceration technique employing petroleum ether, ethyl acetate, and methanol solvents. A comparative analysis was conducted employing the CUPRAC, FRAP, and DPPH methodologies. Additionally, the determination of the overall phenolic and flavonoid contents within the extracts was achieved via employment of the Folin-Ciocalteu reagent and $AlCl_3$ colorimetric methods, enabling the exploration of the relationship between the antioxidant capacity exhibited by these extracts and the concentrations of phenolic compounds therein.

DPPH radical scavenging activity

Briefly, 240 µL of 0.1 mm DPPH solution was added to 10 µl of plant extract, and the solution was vigorously mixed for one minute before being incubated at 25°C for 30 min. The absorbance levels of the mixtures were determined at 517 nm. Determination of the absorbance of the control sample was carried out under identical conditions, replacing the extract with 10 µL of methanol. DPPH radical scavenging experiments were also conducted using ascorbic acid as a standard, with solutions prepared at different concentrations (0.5, 0.4, 0.2, 0.1, 0.05 mg/mL). The resultant data from this inquiry are presented in the form of IC_{50} = mg/mL. The experimental procedures were conducted in triplicate, and after these repetitions, the mean values and standard deviations of the results were computed (Padmanabhan and Jangle 2012).

Determination of copper (II) ion reducing antioxidant capacity (CUPRAC)

The determination of antioxidant capacity, specifically the reduction of copper (II) ions, in the extracts was carried out in accordance with the methodology established by (Apak et al. 2004). Succinctly, a mixture comprising 60 µL of Cu(II).2 h_2O, 60 µL of neocuproine, and 60 µL of 1 M NH_4Ac was prepared, followed by the incorporation of 60 µL of the extracts and the addition of 10 µL of ethanol to the mixture. After an incubation period of 60 minutes, the absorbance values of the resultant mixtures were determined via spectrophotometric analysis at 450 nm, relative to a reference solution prepared by substituting the plant extracts with ethanol. The CUPRAC values for the extracts were reported in terms of milligrams of Trolox per gram of extract (Apak et al. 2004).

Determination of Ferric (III) reducing antioxidant power (FRAP)

In this study, the Ferric ion reducing antioxidant potential test (FRAP) was conducted in accordance with the method established by Benzie and Strain in 1996. This test is a widely used approach for assessing the antioxidant capacity of an extract. The FRAP reagent is composed of a carefully prepared mixture, which includes 2.5 mL of 300 mm acetate buffer (pH 3.6), 2.5 mL of TPTZ (2,4,6-tripyridyl-s-triazine) solution, and 2.5 mL of 20 mm $FeCl_3$. 6 h_2O. This mixture is kept at a specific temperature, namely 37°C, for 30 minutes. Adherence to these precise conditions is essential to ensure the accuracy of the results. In the subsequent stages of the test, 190 µL of the FRAP reagent is mixed with 10 µL of the extract, and the absorbance of the mixture is measured at 593 nm after 4 minutes. This

measurement yields a value that reflects the antioxidant potential of the extract. Consequently, the antioxidant potential of the extracts is expressed as milligrams of Fe^{2+} per milligram of extract (Benzie and Strain 1996).

Determination of total phenolic content (TPC)

Briefly, 0.025 mL of the diluted plant extracts and 0.1 mL of the Folin-Ciocalteu reagent were combined, and then 0.075 mL of sodium carbonate solution (2%) was added. Then continuous shaking of the medium was performed for one minute. After two hours at room temperature, the absorbance at 765 nm was measured using a microplate reader (AMR 100-Allsheng, China). Total phenolic content was calculated as mg gallic acid equivalents (GAE) per gram of plant extract (Oke et al. 2009).

Determination of total flavonoids contents

Total flavonoid content (TFC) in extracts was analyzed with aluminum chloride colorimetric assay. 50 µL of plant extract was placed in a 96-well plate containing 10 µL of 10% aluminum chloride, 130 µL of 96% ethanol, and 10 µL of 1 M sodium acetate. The mixtures were incubated for 40 minutes at room temperature in the dark. Absorbance at 415 nm was measured using a microplate reader. TFC was expressed as mg quercetin equivalents per gram of extract (mg QE/g DW) via a calibration curve with quercetin. All samples were run in triplicate (Oke et al. 2009).

Determination of anti-acetylcholinesterase activity

The inhibition activities of Acetylcholinesterase (AchE) in the provided study were assessed using a microplate reader, specifically the AMR-100 Allsheng model. To perform this analysis, 20 µL of AChE and 20 µL of various concentrations of the test extracts were combined within a phosphate buffer solution (pH 8, 0.1 M, 40 µL). This resulting mixture was subsequently incubated at a controlled temperature of 25°C for 10 minutes. Following the incubation period, 100 µL of DTNB (5,5'-dithiobis (2-nitrobenzoic acid)) and 20 µL of AcI (acetylthiocholine iodide) as the substrate were introduced into the reaction mixture. It's noteworthy that this identical procedure was implemented for galantamine, serving as the standard compound for comparative purposes. The progress of the reaction was monitored by spectrophotometrically measuring the absorbance of 5-thio-2-nitrobenzoic acid at a wavelength of 412 nm. This analytical approach allowed for the evaluation of the acetylcholinesterase inhibition activities of the tested extracts, as well as the standard compound, within a controlled experimental setting (Ellman et al. 1961).

Determination of anti-urease activity

Plant extracts (100 µL) and an enzyme solution (500 µL) were mixed together and kept at 37°C for 30 minutes in an incubator. Then, 1.100 µL of urea was added to this mixture, and it was left to sit in an incubator at 37°C for 30 minutes. The mixture was removed from the incubator, mixed with the reagents R_1 (1% phenol, 0.005% sodium nitroprusside), and R_2 (0.5% NaOH, 0.1% sodium hypochlorite), and then the resulting mixture was incubated for two hours at 37°C. The mixture's absorbance (635 nm) was measured against a reference solution created by switching out the urease enzyme solution for a buffer solution (Ghous et al. 2010).

Determination of calcium oxalate anti-crystallization

Nucleus Assays

To create calcium oxalate crystals, solutions of sodium oxalate and calcium chloride were made in a Tris HCl (0.05 M, pH 6.5) buffer that contained 0.15 M NaCl. One milliliter of extract was added

to three milliliters of each prepared solution, and the mixture was vortexed for 30 seconds. After that, the mixture was incubated at 37 ± 0.1°C, or the temperature of a human body, for 30 minutes. At 620 nm, the samples' absorbance was measured (Bawari et al. 2018).

Aggregation Assays

Plant extracts at varying concentrations were added to the crystal we made to stop the calcium oxalate monohydrate crystal from growing. The absorbance of the mixtures was then measured spectrophotometrically at 620 nm, in accordance with the relevant literature (Bawari et al. 2018).

Antimicrobial effectiveness test

The Antimicrobial activity potential of plant extracts was determined primarily by the agar well diffusion method. Minimal inhibitory concentration (MIC) was determined for extracts showing antimicrobial activity in the agar well diffusion method. Agar well diffusion test: As bacteria; *Pseudomonas aeruginosa* ATCC 27,853, *Staphylococcus aureus* ATCC 43,300, *S. aureus* ATCC 29,213, *S. epidermidis* ATCC 11,228, *Klebsiella pneumonia* ATCC 4352, *Proteus mirabilis* ATCC 14,153, *Salmonella typhimurium* ATCC 14,028, *Acinetobacter baumannii* ATCC 19,606, *Enterococcus faecalis* ATCC 29,212, *Escherichia coli* ATCC 25,922 and as yeasts *Candida albicans* ATCC 90,028, *C. tropicaliskuen* 1021, *C. glabrata* ATCC 90,030 strains were used. Bacteria were inoculated on tryptic soy agar and yeasts on sabouraud dextrose agar (SDA), incubated at 37°C for 24 hours. Microorganism suspensions were prepared from colonies in 0.85% NaCl physiological saline solution (PSS). Bacterial suspensions were adjusted to 108 cfu/ml and yeast suspensions to a concentration of 106 cfu/mL. The microorganism suspensions were spread over the surface of the Mueller Hinton agar for bacteria and SDA for yeasts by sterile swabs under aseptic conditions and then 5 mm diameter wells were made the surface of the medium with a sterile punch. The wells were filled with 50 µL (50 mg/mL) of the extracts dissolved in appropriate solvents. In addition, meropenem (10 µg/well) for bacteria and amphotericin B (100 µg/well) for yeasts were used as positive controls. Solvent (DMSO) and PSS were used as negative controls. Inoculated petri dishes were incubated at 37°C for 18–24 hours for bacteria, at 35°C for 24–48 hours for yeasts, and at the end of incubation time, inhibition zones were measured in mm. The trials were carried out in triplicate and the results were averaged (Perez 1990; Wayne 2011).

Detection of minimal inhibitor concentration and minimal bactericidal concentration

Detection of MIC for bacteria was performed following the standards of the Clinical and Laboratory Standards Institute (CLSI). Cation adjusted Mueller Hinton broth (CAMHB) was used as medium. Bacteria suspension was prepared from the colonies in the 24-hour bacterial culture according to Mc Farland 0.5 turbidity and the final inoculum concentration was diluted to 5 × 105 cfu/ml. The sterile U-based microdilution plates were placed 100 µL of the CAMHB. Soluble extracts were placed 100 µL in the first wells and serial dilutions were made respectively. Then 5 µL of bacterial suspension was added to the wells containing the extract and the plates were incubated at 37°C for 24 hours. At the end of the incubation, the lowest extracting concentrations with no growth were determined as minimal inhibitory concentration (MIC). *S. aureus* ATCC 29,213 was used as a quality control microorganism. CAMHB, DMSO and PSS were used as negative controls. Meropenem was used as a positive control (Wayne 2006).

Analysis of phenolic compounds

The approach we have previously described was used to assess the content of the bioactive extracts using HPLC-DAD (Agilent 1260 Infinity). The chemicals were separated using a C18 reverse-phase Nova-Pak analytical column (3.9 mm x 150 mm inner diameter, 5 µm) (Taşkın et al. 2021).

Statistical analysis

The findings were presented in the form of the mean values, accompanied by their corresponding standard deviations (SD), which were derived from three separate and concurrent measurements. A one-way analysis of variance (ANOVA) was conducted in accordance with established procedures to assess the presence of statistical significance among the means. Subsequently, the identification of significant differences between the means was accomplished through the utilization of a Tukey Multiple Comparison test, with statistical significance defined as p-values less than 0.05.

Results and Discussion

Antioxidant activity

The assessment of antioxidant efficacy within the various extracts, alongside ascorbic acid employed as a reference standard, was conducted via a comparative analysis of their respective IC_{50} values. The resultant findings are presented in Table 1. Notably, among the extracts derived from our experimental botanical specimen, the methanol extract exhibited the most pronounced antioxidant activity, while conversely, the petroleum ether extract displayed the least potency in this regard. Specifically, the methanol extract demonstrated the highest degree of antioxidant activity, as evidenced by its remarkably low IC_{50} value of 0.036 ± 0.002 mg/mL. In stark contrast, the petroleum ether extract exhibited a markedly inferior antioxidant performance, registering an IC_{50} value of 0.23 ± 0.06 mg/mL, thus signifying its diminished efficacy in scavenging free radicals and mitigating oxidative stress. It is noteworthy that, in comparison to the reference standard, ascorbic acid, all the extracts under scrutiny yielded IC_{50} values indicative of comparatively lower antioxidant potential (as delineated in Table 1).

Upon careful evaluation of the data derived from this investigative study, it becomes apparent that all extracts sourced from *M.myrtifolia* manifested notable copper(II) ion reduction capabilities, leading to the conversion of Cu(II) ions into Cu(I) ions. Notably, among these extracts, the methanol extract (6.535 mmtroloxE/mg extract) exhibited the most robust Cu (II) ion reduction activity, while conversely, the chloroform extract displayed the least potency in this regard. Specifically, the methanol extract demonstrated the highest Cu (II) ion reduction activity, underscoring its pronounced efficacy in catalyzing this redox reaction. In stark contrast, the chloroform extract exhibited comparatively diminished activity in reducing Cu (II) ions. It is important to highlight that when compared to the positive control group employing ascorbic acid, petroleum ether and chloroform extracts sourced from *M. myrtifolia* exhibited a lower magnitude of Cu (II) ion reduction activity. This outcome suggests that while these botanical extracts possess appreciable copper-reducing potential, their effectiveness in this regard falls short of the performance exhibited by ascorbic acid, as elucidated in Table 1, which presents the obtained results comprehensively.

The assessment of the Fe(III) ion reducing capabilities within the extracts derived from the aerial components of *M. myrtifolia* revealed that only the methanol extract exhibited discernible reducing

Table 1. Antioxidant activity, total phenolic and flavonoid contents of extract from *M. myrtifolia*.

Extracts/Standards	DPPH (IC_{50}:mg/mL)	CUPRAC (mMtroloxE/mg extract)	FRAP (mM$FeSO_4$E/mg extract)	TPC (mg GAE/g extract)	TFC (mg QuE/g extract
Petroleum Ether	0.230 ± 0.06*	1.678 ± 0.05*	2.913 ± 0.048*	57.90 ± 1.04	1.909 ± 0.035
Chloroform	0.205 ± 0.115*	1.310 ± 0.04*	3.065 ± 0.069*	117 ± 2.15	2.415 ± 0.082
Methanol	0.036 ± 0.002*	6.535 ± 0.12*	6.476 ± 0.326*	400 ± 3.35	2.519 ± 0.509
Ascorbic acid	0.004 ± 0.007	5.920 ± 0.51			
BHA			16.91 ± 0.02		

DPPH: 2,2-diphenyl-1 picrylhydrazyl; CUPRAC:cupric ion reducing/antioxidant power; BHA: butylated hydroxyl anisole; TPC, total phenolic contents; GAE, gallic acid equivalent; TFC, total flavonoid contents; QuE: quercetin equivalent; Values are mean of triplicate determination ($n = 3$) ± standard deviation; *$p < 0.05$ compared with the positive control.

activity, quantified at 6.476 ± 0.326 mm $FeSO_4$/mg extract. It is worth noting that this specific extract displayed the capacity to facilitate the reduction of Fe(III) ions, indicating its potential in mitigating oxidative processes. However, it is noteworthy that the reducing power exhibited by the methanol extract was observed to be less robust in comparison to the standard BHA, which exhibited a notably higher reducing power, quantified at 16.91 ± 0.02 mm $FeSO_4$. This result underlines the comparable limitations of the methanol extract from *M. myrtifolia* regarding its Fe (III)-ion-reducing potential compared to the reference BHA. The summarized results are presented in detail in Table 1 for illustration.

Determination of total phenolic and flavonoid contents

The quantification of total phenolic and flavonoid compounds within the extracts sourced from the *M. myrtifolia* plant was accomplished through the utilization of the FCR (Folin-Ciocalteu reagent) and aluminum chloride colorimetric methods, respectively. The obtained results were subsequently expressed as milligrams of gallic acid and quercetin equivalent per gram of extract and are succinctly summarized in Table 1. Upon a careful examination of these outcomes, it is evident that the methanol extract derived from the *M. myrtifolia* plant contained the highest concentration of phenolic (400 mg GAE/g extract) and flavonoid (2.519 mg QuE/g extract) components among all the tested extracts. Conversely, the petroleum ether and chloroform extracts were found to possess relatively lower quantities of phenolic and flavonoid compounds when contrasted with the methanol extract. Notably, the petroleum ether extract exhibited the most diminished content of phenolic and flavonoid compounds among the evaluated extracts. This observation underscores the considerable variation in phenolic composition across the different extract types, with the methanol extract emerging as the most phenolic-rich, while the petroleum ether extract displayed the least phenolic content, as discerned from the comprehensive data presented in Table 1.

Determination of enzyme inhibition activities

The evaluation of acetylcholinesterase inhibitory activities within the extracts extracted from *M. myrtifolia* was conducted following the Ellman method, and the resultant findings were subject to comprehensive analysis. Remarkably, all the acquired extracts exhibited anti-acetylcholinesterase activity, signifying their potential to modulate this enzymatic process. Specifically, it is worth noting that the methanol extract (78.935%) emerged as the extract with the most robust inhibitory activity, while the petroleum ether and chloroform extracts demonstrated an inhibitory potential that was notably close in magnitude. These significant observations are succinctly detailed in Table 2. It is of utmost significance that the inhibitory

Table 2. Anticholinesterase, anti-urease and calcium oxalate anti-crystallization activities of extracts from *M. myrtifolia*.

Extracts/standards	Acetylcholinesterase enzyme inhibition (%) (50 µg/mL)	Urease enzyme inhibition (%) (25 µg/mL)	Calcium oxalate anti-crystallization (%)	
			Nucleus test (2.5 mg/mL)	Aggregation test (2.5 mg/mL)
Petroleum ether	71.687 ± 0.846*	71.678 ± 3.01*		
Chloroform	75.826 ± 6.688*	63.261 ± 4.38*		
Methanol	78.935 ± 1.155*	71.014 ± 1.718*	18.22 ± 1.235	37.427 ± 5.908
Galantamine	83.014 ± 0.144			
Thiourea		88.691 ± 4.478		
Potassium citrate			97.94 ± 0.025	98.41 ± 3.195

Values are mean of triplicate determination (n = 3) ± standard deviation; *p <0.05 compared with the positive control.

power of these extracts is close to that of the standard compound galantamine, which is commonly employed for its acetylcholinesterase inhibitory properties. This compelling finding underscores the potential utility of *M.myrtifolia* extracts as a promising and novel therapeutic agent in the realm of Alzheimer's disease treatment. These extracts, with their evident anti-acetylcholinesterase activity, hold promise for future research and therapeutic development in the context of Alzheimer's disease management.

The urease enzyme inhibition activities within the extracts sourced from the *M. myrtifolia* were systematically assessed via the indophenol method, and the resulting data have been meticulously presented in Table 2. Notably, all the examined extracts exhibited a significant degree of urease enzyme inhibitory activity, underscoring their potential in modulating this enzymatic process. When the anti-urease activities of the extracts (25 µg/mL) and thiourea (25 µg/mL) are arranged in descending order, from the most robust to the least potent, the sequence emerges as follows: thiourea (88.691%), petroleum ether (71.678%), methanol (71.014%) and chloroform (63.261%). These findings collectively highlight the noteworthy anti-urease potential inherent in the extracts obtained from *M. myrtifolia*. Particularly, the petroleum ether and methanol extracts exhibited the most pronounced urease enzyme inhibitory activity, followed by the chloroform extract, with all extracts demonstrating activity lower than the reference compound thiourea. The chloroform extract, while displaying a slightly reduced inhibitory activity, still exhibited appreciable anti-urease properties. This comprehensive dataset underscores the potential of *M. myrtifolia* extracts in the context of urease enzyme inhibition and their prospective applications in related therapeutic research.

Determination of calcium oxalate anti-crystallization

Given the established linear correlation between radical scavenging antioxidant activity and anti-crystallization properties, a comprehensive examination of the calcium oxalate anti-crystallization activity of the methanol extract was undertaken. It's worth noting that the methanol extract, having exhibited the highest radical scavenging activity during antioxidant activity assessments, was selected for this investigation. The anti-crystallization evaluation encompassed both nucleation and aggregation tests. In the nucleation test, the methanol (18.22%) extract displayed a level of activity, albeit notably lower in comparison to the standard reference. Conversely, in the aggregation test, the methanol (37.427%) extract demonstrated a moderate degree of anti-crystallization activity. The outcomes of these assessments are presented in detail in Table 2. This data signifies that while the methanol extract may exhibit slightly reduced performance in the nucleation test when compared to the standard, it still displays noteworthy anti-crystallization properties, particularly in the aggregation phase. These findings underscore the potential utility of the methanol extract from *M. myrtifolia* in mitigating calcium oxalate crystallization processes, which could have implications in the context of renal stone prevention and related research endeavors.

Antimicrobial activity

Evaluation of the antimicrobial activity of the *M. myrtifolia* extracts was determined by the agar well diffusion method and the results are shown in Table 3. Table 3 shows that all extracts showed varying amounts of antibacterial activity against *Staphylococcus aureus* ATCC 43,300 and *Staphylococcus aureus* ATCC 29,213. It is also observed that the PE extract has antibacterial activity against *Proteus mirabilis* ATCC 14,153 and MeOH extract has antibacterial activity against *Staphylococcus epidermidis* ATCC 11,228 and *Acinetobacter baumannii* ATCC 19,606. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of extracts with observed antimicrobial activity are shown in Table 4. The MIC values of the extracts ranged between 0.39–1.56 mg/mL and MBC values ranged between 1.56–25 mg/ml.

Table 3. Antimicrobial activity of extracts from *M. myrtifolia*.

	Zone diameter* (mm)												
	Pseudomonas aeruginosa ATCC 27,853	*Staphylococcus aureus ATCC 43,300*	*Staphylococcus aureus* ATCC 29,213	*Staphylococcus epidermidis* ATCC 11,228	*Klebsiella pneumoniae* ATCC 4352	*Proteus mirabilis* ATCC 14,153	*Salmonella typhimurium ATCC 14,028*	*Acinetobacter baumannii ATCC 19,606*	*Enterococcus faecalis ATCC 29,212*	*Escherichia coli* ATCC 25,922	*Candida albicans* ATCC 90,028	*Candida tropicaliskuen 1021*	*Candida glabrata ATCC 90,030*
PE extract	0	6.18 ± 0.15	5.30 ± 0.13	0	0	11.02 ± 0.14	0	0	0	0	0	0	0
MeOH extract	0	7.91 ± 0.29	6.04 ± 0.25	9.65 ± 0.20	0	0	0	4.37 ± 0.11	0	0	0	0	0
Cl extract	0	5.43 ± 0.17	4.61 ± 0.22	0	0	0	0	0	0	0	0	0	0
DMSO	0	0	0	0	0	0	0	0	0	0	0	0	0
M	23.22 ± 0.27	28.52 ± 0.33	27.73 ± 0.14	39.32 ± 0.14	26.96 ± 0.34	26.27 ± 0.17	27.36 ± 0.22	15.22 ± 0.19	11.82 ± 0.34	27.80 ± 0.11	-	-	-
AmB	-	-	-	-	-	-	-	-	-	-	20.26 ± 0.21	12.22 ± 0.23	18.76 ± 0.19

*5 mm well diameter is removed, M: Meropenem, AmB: Amfoterisin B. -: not tested, PE: Petroleum ether, MeOH: Methanol Cl: Chloroform.

Table 4. MIC and MBC values of extracts from *M. myrtifolia*.

	MIC/MBC (mg/mL)				
	S. aureus ATCC 43,300	*S. aureus* ATCC 29,213	*S. epidermidis* ATCC 11,228	*P. mirabilis* ATCC 14,153	*A. baumannii ATCC 19,606*
PE extract	1.56/12.5	3.13/25	-	1.56/12.5	-
MeOH extract	1.56/12.5	3.13/25	0.39/3.13	-	0.39/1.56
Cl extract	1.56/6.25	0.78/6.25	-	-	-

not tested, PE: Petroleum ether, MeOH: Methanol Cl: Chloroform

Analysis of phenolic compounds

The phenolic contents of the bioactive methanol extract from *M. myrtifolia*, with a concentration of 1 mg/mL, were analyzed using an HPLC-DAD system. Quercetin, chlorogenic acid, rosmarinic acid derivative and rosmarinic acid compounds were analyzed qualitatively. The amounts of quercetin (12.99 µg analyte/mg extract), chlorogenic acid (3.55 µg analyte/mg extract), and rosmarinic acid (31.35 µg analyte/mg extract) were determined by HPLC. The data obtained showed that the rosmarinic acid was the major presence compound in the plant. The table and figure below display the phenolic contents and their respective amounts in the plant extract (Table 5, Figure 1).

The imbalance between free radical production and antioxidant defense is the fundamental cause of oxidative stress, and this condition can have a significant impact on the development of numerous chronic diseases. Studies have demonstrated that bioactive compounds confer protective

Table 5. Quantitative determination of compounds in methanol extracts of *M. myrtifolia*.

	µg analyte/mg extract
Rosmarinic acid	31.35 ± 2.40
Quercetin	12.99 ± 0.74
Chlorogenic acid	3.55 ± 0.05

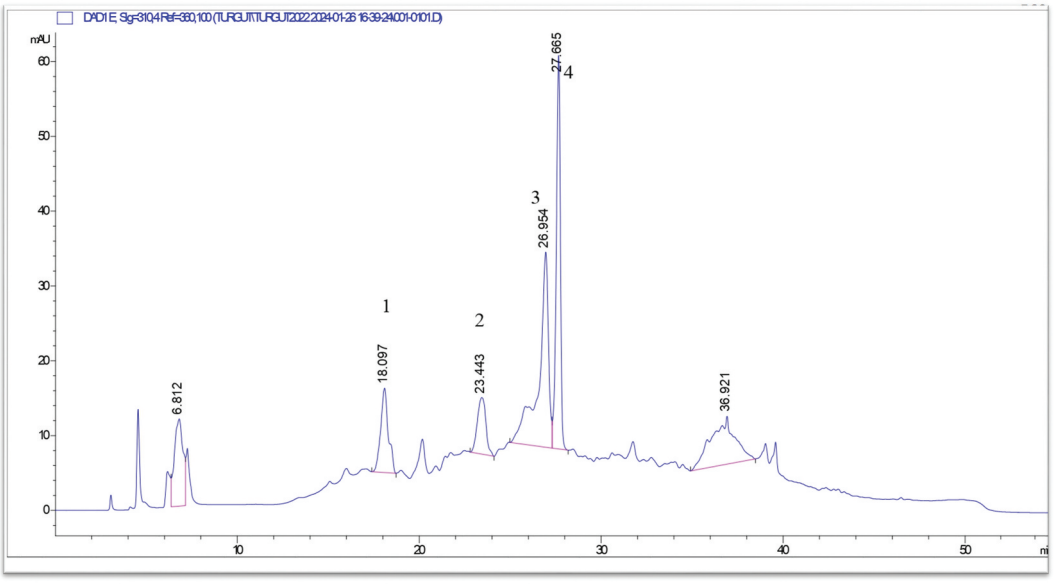

Figure 1. High-performance liquid chromatography chromatogram of phenolic compounds detected from *M. myrtifolia* extracts, 1: quercetin; 2: chlorogenic acid, 3: rosmarinic acid derivative, 4: rosmarinic acid.

effects against oxidative stress (Cam et al. 2019). In our study, the methanol extract has been identified as the extract with the highest antioxidant capacity among the extracts. The methanol extract exhibited higher activity in reducing Cu (II) ions compared to the standard. Regarding the total phenolic and flavonoid content, the methanol extract had the highest value, followed by the chloroform and petroleum ether extracts. The amount of total phenolic and flavonoid compounds is directly proportional to the antioxidant activity capacity. The data obtained in our study confirm this information as mentioned in the literature. In the literature review, different solvents were used to obtain extracts in DPPH, CUPRAC, and FRAP tests, and the highest activity was observed in the water extract, followed by the methanol and ethyl acetate extracts, with a decrease in activity. In addition, in this study, the highest total phenolic compound content was found in the water extract, while the lowest phenolic compound content was detected in the ethyl acetate extract (Sarikurkcu et al. 2020). The results obtained in this study are consistent with the finding that polar solvents have higher antioxidant capacity compared to nonpolar solvents. Furthermore, this study once again confirms the hypothesis of a relationship between the amount of total phenolic and flavonoid compounds and antioxidant capacity. In the essential oil activity test, the essential oil showed lower Cu(II) ion-reducing activity compared to the standard (Sarikurkcu et al. 2019).

There are biological activity studies of *Micromeria* species in the literature: one of these studies is on the *Micromeria imbricata* species. In that study, the aerial parts of the *M. imbricata* were used to extract a 95% ethanol solution, and this extract's in vitro cytotoxic, antioxidant, and anti-obesity properties were investigated. According to the data obtained, this extract showed the highest phenolic (125.23 ± 0.87 mg gallic acid equivalent/100 g extract) and flavonoid (112.24 ± 2.45 mg quercetin equivalent/100 g extract) contents. Additionally, the tested hydroalcoholic extract exhibited a significant DPPH scavenging activity with SC_{50} 28.4 ± 1.2 µg/mL and a remarkable lipase inhibitory activity with IC_{50} 54.2 ± 1.2 µg/mL (Al-Yousef et al. 2021). In our study, in parallel with the above study, it was determined that the methanol extract from the *M. myrtifolia* exhibited significant DPPH (IC_{50}:36 µg/mL) radical scavenging activity.

In another study, the antioxidant, antibacterial and antityrosinase properties of the ethanol extract of the *Micromeria graeca* (L.) Benth. ex Reichb. were examined. Chlorogenic, caffeic and rosmarinic acids and diosmin were also analyzed in the plant. It was determined that this species showed significant ABTS (IC_{50} = 30.5 ± 0.9 µg/mL) and DPPH (IC_{50} = 65.8 ± 2.4 µg/mL) radical scavenging activities (Brahmi et al. 2017). It was determined that the species we examined showed higher DPPH radical scavenging activity than this plant and contained chlorogenic acid in a similar way. In another study, a 70% ethanol extract of *M. croatica, M. juliana*, and *M. thymifolia* showed the following order of results: *M. croatica* > *M. juliana* > *M. thymifolia*. The extract shown significant activity in scavenging DPPH and hydroxyl free radicals, reducing power, iron chelating ability, and overall antioxidant capacity. Additionally, analyses have shown that these species include chemicals related to rosmarinic acid, chlorogenic acid, and caffeic acid (Vladimir-Knežević et al. 2011). In another study, *M.fruticosa* was analysed to contain chlorogenic acid, rosmarinic acid, quercetin, isorhamnetin, naringenin, routine and luteolin (Hamwi et al. 2021). These findings are parallel to our study facility.

The extracts we have acquired have exhibited highly effective activity when it comes to inhibiting enzymes. What distinguishes our research from others is the lack of previous studies on the inhibitory abilities of the extracts we used, particularly in relation to the enzymes acetylcholinesterase and urease. Methanol extract in the nucleation test it was low but in the aggregation test it demonstrated a moderate degree of anti-crystallization activity. Given the promising results obtained in activity assessments, we have also undertaken an examination of the phenolic content within the methanol extract. The chemical content of the methanol and water extracts of the plant has previously been analyzed (Sarikurkcu et al. 2020). It was found that the plant contains rosmarinic acid as its main constituent. In our study, the compound rosmarinic acid was found in high amounts, which is consistent with the literature. Also, in one study, the phytochemical composition of *Micromeria imbricata* was examined using UPLC-

ESIMS/MS. This extract was analyzed to contain syringic acid, rosmarinic acid, umbelliferone, kaempferol-3-O-glucuronide, isorhamnetin-O-rutinoside, caffeic acid, apigenin, caffeoylquinic acid (Al-Yousef et al. 2021). In our study, rosmarinic acid was analyzed as in the *M. imbricata* species, but unlike this species, quercetin and chlorogenic acid were also analyzed. It was thought that this phytochemical difference was due to different plant species and growing in different places. Consequently, the phenolic content is thought to be the source from which the antioxidant and enzyme inhibitory properties are derived. In addition, another uniqueness of our study is that there is no study on the antimicrobial activity of plant extracts obtained from *M. myrtifolia*. In our study, we investigated the antimicrobial activity of *M. myrtifolia* against some human pathogens such as *S. aureus*, *S. epidermidis*, *P. mirabilis* and *A. baumannii*. Among these pathogens, the activity against *S. aures* and *A. baumannii* may be instructive for further studies on antimicrobial compounds.

Conclusion

From the results of this rigorous study, the methanol extract of *M. myrtifolia* showed the highest antioxidant activity. Consistent with the antioxidant results, the methanol extract also showed the highest total phenolic and flavonoid content among the various extracts considered. In the enzyme inhibition assay, methanol extract showed the most pronounced anti-urease and anti-acetylcholinesterase activities. Petroleum ether and methanol extracts also showed antimicrobial activity against some pathogens. In conclusion, the methanol extract from the *M. myrtifolia* exhibits significant antioxidant, anti-urease, anti-acetylcholinesterase and antibacterial activities and provides empirical data indicating its potential utility as a natural reservoir of antioxidant, anti-urease, antimicrobial and anti-acetylcholinesterase agents.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Author contributions

Ideation of the concept, study design, data analysis and interpretation, manuscript writing, manuscript drafting, and review-TT, OM, RE, EM, TD, AG, KO, ÇSE, YNB, EHK. Literature search, study design, execution, data analysis and interpretation, and manuscript drafting- TT, OM, RE, EM, TD, AG, KO, ÇSE, YNB, EHK.

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