Phytochemical Analysis and Evaluation of Antioxidant, Antimicrobial, and Antidiabetic Activities of Micromeria Myrtifolia

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ABSTRACT

Natural compounds have been extensively investigated for new drug discoveries. *Micromeria* genus is known to be rich in essential oils and bioactive constituents possessing significant biological activities. The current work aims to explore the phytochemical composition of *M. myrtifolia* liquid extracts and to evaluate their antioxidant, antimicrobial, and antidiabetic activity *in vitro*. The LC-MS/MS detected various phenolic compounds, especially in the aqueous extract. The aqueous extract of *M. myrtifolia* positively inhibited α-amylase activity with an IC_{50} value of 174.44 ± 0.68 mg/mL, and showed the highest antioxidant activity as it scavenged the DPPH radicals from 23.25% to 63.72% with increasing concentration from ~ 0.04 to 0.15 mg/mL. On the other hand, ethyl acetate extract illustrated the lowest antioxidant activity as the % radical scavenging activity of 77% was achieved at the concentration of 7.5 mg/mL. Tested extracts were more effective against Gram-positive bacteria in a dose-dependent manner with logarithmic CFU reduction. *Micromeria myrtifolia* methanol extract exhibited potential antibacterial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis* with an MICs value of 3.15 µg/µL and 25 µg/µL, respectively. In comparison, ethyl acetate and hexane extracts had antibacterial activity against *Streptomyces epidermidis* at MICs of 3.125 µg/µL and 12.5 µg/µL, respectively. Gram-negative bacteria were more challenging to eradicate; however, ethyl acetate, methanol and dichloromethane extracts were successful in reducing the number of *Escherichia coli* CFUs in a dose-dependent manner at MIC of 25 µg/µL for both ethyl acetate and methanol extracts and at MIC of 50 µg/µL for dichloromethane extract. While *Pseudomonas aeruginosa* only responded to hexane extract at MIC of 50 µg/µL. The antioxidant, antibacterial, and antidiabetic effects of *M. myrtifolia* extracts bear significant clinical and therapeutic implications. Furthermore, the potent antioxidant properties make *M. myrtifolia* extract promising candidates for developing nutraceuticals or pharmaceuticals, particularly for conditions associated with oxidative stress.

Keywords: *Micromeria*, *Micromeria myrtifolia*, antidiabetic, antioxidant, antimicrobial, liquid chromatography-mass spectroscopy, secondary metabolites.

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1. INTRODUCTION

Plants have been used since ancient times for their unique holistic role in providing food, clothing, drugs, shelter, etc. Thus, natural compounds are extensively investigated as a source for new drug discoveries(1). Indeed, herbal remedies have been used as syrups, infusions, ointments and medicines for more than 5,000 years, as a mine of antibiotics, antidiabetic, antineoplastic, analgesics, cardioprotective and other healing effects(1-3). The plant kingdom has provided an inexhaustible source of medicinal treasures. Accordingly, developed and developing countries have reverted to using natural herbal products due to synthetic drugs' limitation in activity and complications(4). About 70–90% of the population in developing countries continue to use ancient medicines based on plant extracts(1).

Plants secondary metabolites are powerful and promising elements, which humans depend upon. Several plant secondary metabolites, including phenolic compounds, alkaloids, phenolics, lignans, terpenes, and glycosides possess significant antidiabetic and antioxidant activities (5, 6). In recent years, herbal medicines gained a particular interest as a subject of both commercial and scientific interest(1, 7). Interestingly, plant phytochemical constituents are among the wealthiest supplies for new drug discoveries.

Lamiaceae, or the mint family, had a role in the medicinal, culinary, and gardening aspects of human history (8). It is a source of species containing phenolic compounds, which are known for their antioxidant effects, in addition to the production of commercially important essential oils worldwide, particularly in the Mediterranean area(9, 10).

Micromeria myrtifolia (*M. myrtifolia*) is one of the intensely aromatic plants of Lamiaceae family. It is well known in the traditional medicine in Lebanon and Asia, where it is often among the main botanical components for the therapeutic value of their essential oils and extracts (9). *M. myrtifolia* is the native flora of the Mediterranean region(11). It has been used as an appetite enhancer, gas remedy, antispasmodic, intestine pain and inflammation, fever, respiratory disorders, female sterility, and stimulant (11, 12). Recently, the methanol extract of the aerial parts of *M.myrtifolia* was reported to have antidepressant activity(13). In addition, *Micromeria* genus is known to be rich in essential oil content and bioactive constituents possessing substantial antioxidant, antimicrobial, antifungal, and antiviral activities(11). Also, the genus is a rich source of polyphenolic compounds. Flavonoids are among the most exciting plant bioactive constituents found in the *Micromeria* genus, with attractive activities like antioxidant, antimicrobial, and anticancer activities(14).

Recently, bacterial resistance has become a global issue. Therefore, the search for new antibacterial agents has become an urgent priority. Nature seems to be a deep well for searching for novel antimicrobial agents. Secondary metabolites derived from plants are still the pillar for developing and designing new antimicrobial agent and enhancing treatment choices(5, 15). This is due to the several attractive charms and properties of plant metabolites: they are available, have structural diversity, and are cheap(16).

Several secondary metabolites demonstrated high-level activity against pathogens and infrequently have serious side effects (16). Currently, researchers investigated the essential oil components of *M. myrtifolia* (11). Other studies investigated *M. myrtifolia* polyphenolic components and evaluated their activities(12, 13). In the current work, we aim to evaluate the phytochemical composition of the liquid extracts of *M. myrtifolia* using LC–QTF–MS/MS to explore their antioxidant, antimicrobial, and antidiabetic activity *in vitro*.

2. MATERIALS AND METHODS

2.1. Instruments and reagents

The reagents and equipment that were utilized in the current study were electric blender model No. 1083 (GFL, Germany), lyophilizer (SP Scientific, USA),

rotary evaporator (Buchi, USA Germany), UV spectrophotometer (Agilent Technologies, Woburn, MA, USA). All chemicals and reagents were purchased from Sigma, USA.

2.2. Collection and identification of plant material

The dried aerial parts of *M. myrtifolia* were purchased from a local market in Amman in March, 2022. It was identified and authenticated by Dr. Hatem Taifour, Royal Botanical Garden, Jordan. The dried plant material was ground into powder using an electric blender. The powdered material was stored in an airtight amber bottle at room temperature until further testing.

2.3. Preparation of plant extracts

Each sample (250 gm) was weighed and extracted sequentially with five solvents in the following arrangement: hexane, dichloromethane, ethylacetate, methanol, and finally, water. After each solvent extraction, the residue was placed under a stream of dry air to remove solvent traces prior to the subsequent extraction.

Generally, each solvent was added to the milled sample, and the mixture was placed in a sonicator for two hours. The mixture was then left for twenty-four hours, filtered, and dried under reduced pressure, while the aqueous extract was freeze-dried. All extracted samples were stored in a refrigerator.

2.3. Phytochemical Analysis

Extracts were analyzed using Liquid Chromatography–Mass Spectrometry (LC–MS/MS). Each extract (10 µg) was diluted with (100 µl) of the extraction solvent. Samples were centrifuged at 4000 rpm for 2 min, then filtered and injected by the autosampler. The test was carried out using the SWATH scan type. The apparatus was activated using the Ion Source Turbolon electrospray source (ion spray voltage; 5000, nebulizer gas, 50 psi; dry gas flow, 8 L/min; dry temperature, 500 °C; mass accuracy, less than one ppm; mass resolution, 50,000 FRS; the TOF repetition rate, 20 kHz). Chromatographic separation was performed using an Inertsustan C-18 UHPLC column (25 mm × 4.6 mm × 5 µm) at a flow rate of 1.0 mL/min and a column temperature of 40 °C.

2.4. Investigation of biological activities

2.4.1. Alpha-amylase Inhibitory Activity

The alpha□□amylase inhibitory activity of the aqueous and organic extracts was measured in a microtiter plate according to (17) with slight modification. A reaction mixture containing 20 µL of the tested extract ((aqueous extracts (500 mg dissolved in 1000 µL water) and the organic extract (100 mg dissolved in aqueous 6% dimethyl sulfoxide)) and 50 µL of □□amylase (10 IU/mL) (Sigma-Aldrich, USA) in 20 mM sodium phosphate buffer (pH 6.8) was incubated at 37 °C for 10 min. Following, 50 µL of 0.1% starch solution was added to each well and incubated for 10 min. Following, the reaction was terminated by adding 50 µL coloring solution (12.0 g of sodium potassium tartrate tetrahydrate in 8 mL of 2M NaOH and 96 mM of 3,5dinitrosalicylic acid solution). The microtiter plate was then incubated in a boiling water bath for 5 min and allowed to cool to room temperature. The absorbance (Abs) of the mixture was measured at 540 nm. Mean values were obtained from triplicate experiments. Negative control samples were prepared without any plant extracts.

The percent inhibition of amylase activity was calculated using the following formula:

$$Inhibition\ \% = \left(1 - \frac{A - Ao}{(X - Xo)}\right) * 100\%$$

where is,

A: the absorbance of the sample.

A_o: the absorbance of the sample blank.

X: the absorbance of the control.

X_o: the absorbance of the control blank.

2.4.2. Antioxidant activity

The antioxidant potential of *M. myrtifolia* plant extracts was determined using the DPPH (2,2-Diphenyl-1-picrylhydrazyl) method as described by N Seder and colleagues with minor modifications(18).

Initially, a DPPH solution of 0.025 mg/mL was prepared in methanol. A stock solution of 10 mg/mL of each plant extract was prepared for serial dilution in the corresponding solvent of water, ethanol, and ethyl acetate. Consequently, 100 µL of the plant extract solution at different concentrations was added to each test tube containing 1.5 mL DPPH solution. Ascorbic acid was used as a positive reference control. The reaction mixtures were vigorously shaken and incubated at 25°C for 30 minutes in a dark place. Then, the absorbance was measured at 517 nm using a microplate reader (Biotek, USA)

The percentage of inhibition of the DPPH was expressed as radical scavenging activity according to the expression:

$$\%\, INHIBITON = [(A0 - A1)/A0] * 100$$

Whereas,

A_0: absorbance of the control (DPPH solution) at 30 minutes.

A_1: absorbance of the sample at 30 minutes.

The half-maximal inhibitory concentration (IC_{50} mg/mL) was used to express the extract concentration required to reduce 50% of DPPH radicals. The test was performed in triplicate.

2.4.3. Antimicrobial activity

Four types of extracts were tested against four bacterial strains: two Gram-negative bacteria (*Escherichia coli* "ATCC 14169" and *Pseudomonas aeruginosa* "ATCC 27853") and two Gram-positive bacteria (*Staphylococcus aureus* "ATCC 25923" and *Staphylococcus epidermis* "ATCC 12228"). The antibacterial results were measured according to the turbidity by the microplate reader (Biotek, USA) at 600nm. The results were expressed as CFU/mL (Colony forming unit/ mL) of each bacterium through the eight dilutions of each extract (50-0.39 µg/µL). And detect if there is an inhibition of growth and the number of log reductions of CFU/mL also detected. The started CFU/mL of each bacterium was 10^8/mL. The MIC (minimum inhibitory concentration) was considered the minimum extract concentration able to inhibit visible microbial growth(19).

2.5 Statistical Analysis

The experimental analyses were carried out in triplicate and results were expressed as the means ± standard error of means (SEM).

3. RESULTS

3.1. Sequential Extraction and Phytochemical Analysis

The plant material was extracted sequentially by adding different solvent systems in increasing polarity. Each extract was characterized using HPLC-QTF-MS/MS. Figure 1 and Table 1 show secondary metabolites of the various extracts.

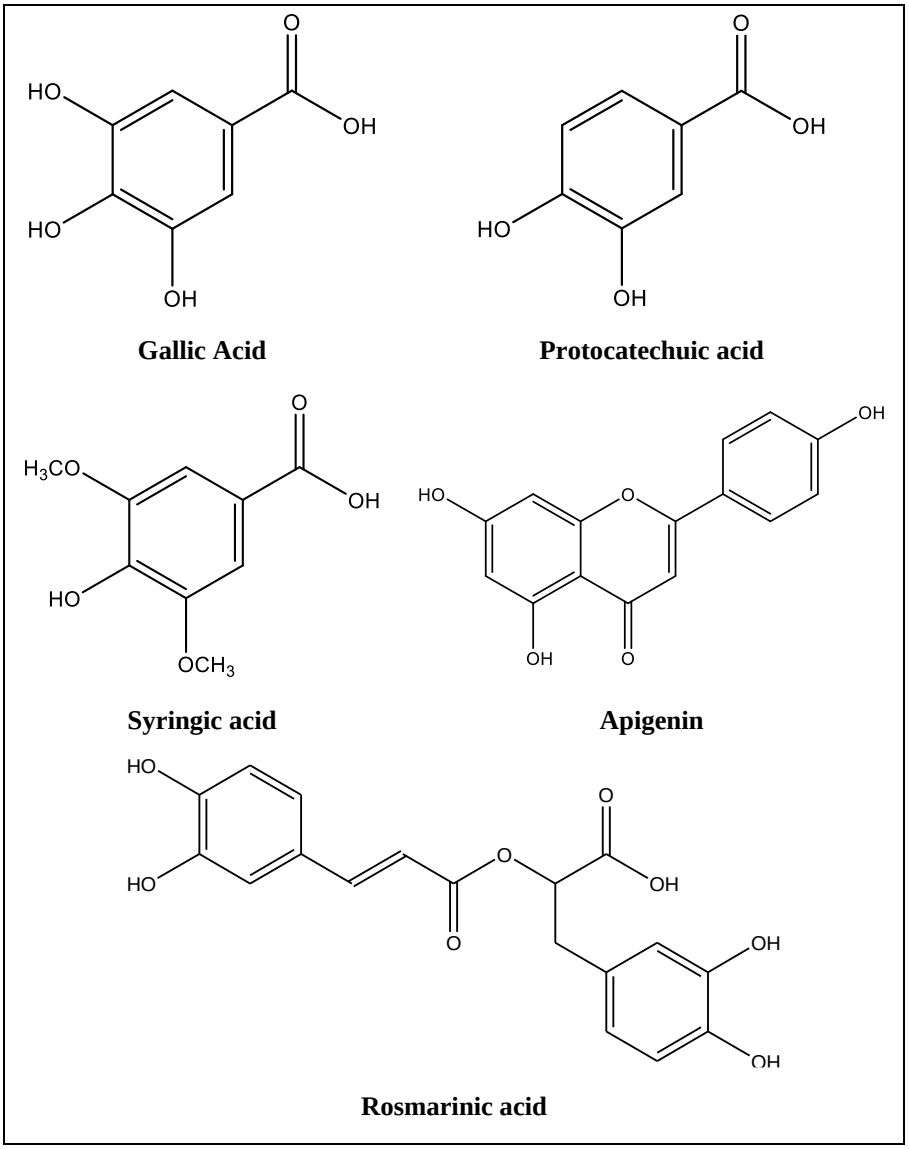

Figure 1. Structure of secondary metabolites found in different extracts

Table 1. LC-MS Analysis of *M. myrtifolia* extracts

Chemical compound	Molecular Formula	*m/z*	Water Extract	Methanol Extract	Ethyl acetate Extract	Dichloromethane Extract	Hexane Extract
Gallic acid	$C_7H_6O_5$	170.120	2.28	0.20	ND	0.49	ND
Protocatechuic acid	$C_7H_6O_4$	154.120	18.7	0.86	0.30	0.06	1.25
Chlorogenic acid	$C_{16}H_{18}O_9$	354.311	3.6	2.26	0.14	0.00	ND
Caffeic acid	$C_9H_8O_4$	180.160	21.24	2.50	1.55	2.21	ND
Vanillic acid	$C_8H_8O_4$	168.150	1.83	0.87	0.16	ND	ND
Syringic acid	$C_9H_{10}O_5$	198.170	1.99	0.34	0.48	1.06	ND
Verbascoside	$C_{29}H_{36}O_{15}$	624.600	0.45	1.22	ND	ND	ND
Taxifolin	$C_{15}H_{12}O_7$	304.250	3.77	0.33	0.02	ND	ND
Coumaric acid	$C_9H_8O_3$	164.160	13.95	2.21	ND	ND	ND
Ferulic acid	$C_{10}H_{10}O_4$	194.180	1.16	0.47	0.25	0.14	26.46
Luteolin-7--glucoside	$C_{21}H_{20}O_{11}$	448.400	0.95	15.32	0.63	ND	ND
Hesperidin	$C_{28}H_{34}O_{15}$	610.180	ND	2.76	0.16	91.57	ND
Rosmarinic acid	$C_{18}H_{16}O_8$	360.300	1.06	9.07	9.18	ND	57.91
Quercetin	$C_{15}H_{10}O_7$	302.236	ND	7.11	0.38	0.21	ND
Luteolin	$C_{15}H_{10}O_6$	286.240	28.96	25.92	68.74	2.10	8.72
Apigenin	$C_{15}H_{10}O_5$	270.052	ND	27.70	17.69	2.10	4.42
2,5-dihydroxybenzoic acid	$C_7H_6O_4$	155.034	ND	0.86	0.30	0.06	1.25

ND: not detected

3.2. Determination of α- amylase Inhibitory Activity of *M. myrtifolia*

The *in vitro* α-amylase inhibitory activity of *M. myrtifolia* extracts showed that the aqueous extract positively inhibited α-amylase activity with IC_{50} value, 174.44 ± 0.68 mg/mL. The other extracts showed inhibitory activity at very high concentrations, Figure 1. The standard positive control acarbose showed an IC_{50} of 129.62 ± 1.21 mg/mL.

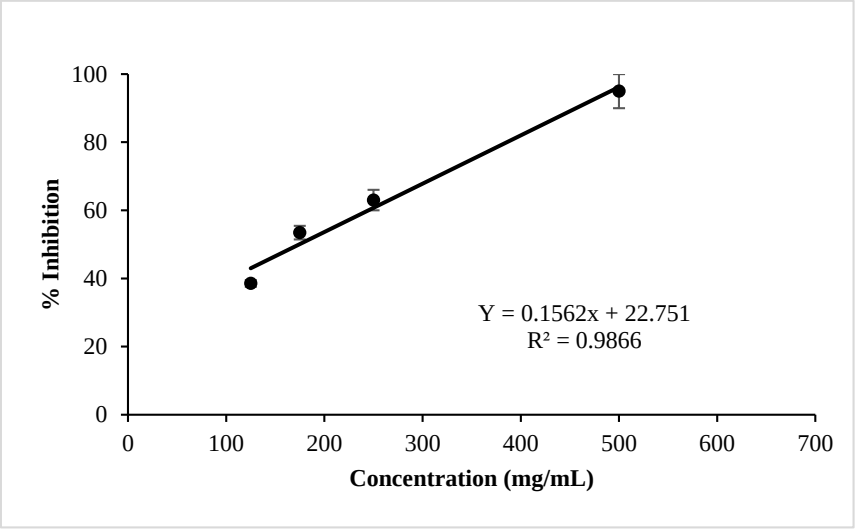

Figure 2. α- amylase inhibitory activity of the aqueous extract of *M. myrtifolia*

The other extracts were screened for their antidiabetic activity. Both hexane and dichloromethane showed 25 % inhibition, while ethyl acetate and methanol showed no activity at this concentration. No further investigation for these two extracts was accomplished due to solubility issues.

3.3. Determination of Antioxidant Activity of *M. myrtifolia* extracts

The antioxidant activity of *M. myrtifolia* extracts was investigated using a DPPH assay(20). A variation in the antioxidant activity of the plant extracts was affected by the extraction solvents applied. The aqueous extract showed the highest antioxidant activity as it scavenged the DPPH radicals from 23.25% to 63.72% with increasing concentration from ~ 0.04 to 0.15 mg/mL. Followed by methanolic extract with radical scavenging activity of ~75.5% at 0.25 mg/mL concentration. Meanwhile, ethyl acetate extract had the lowest antioxidant activity as the % radical scavenging activity of 77% was achieved at the concentration of 7.5 mg/mL.

Additionally, there was a significant variation in the IC_{50} values obtained from linear regression analyses for each plant extract ($p < 0.05$). Table 2 shows that aqueous extract had the lowest IC_{50} value of 0.11 ± 0.015 mg/mL, followed by methanol extract with IC_{50} of 0.16 ± 0.021 mg/mL. Whereas ethyl acetate extract has the highest IC_{50} value of 4.5 ± 0.36 mg/mL.

Table 2. Antioxidant activity of extracts compared to ascorbic acid

Extract	IC_{50} (mg/mL)
Aqueous extract	0.11 ± 0.015
Methanol extract	0.16 ± 0.021
Ethyl acetate extract	4.5 ± 0.36
Ascorbic acid	0.056 ± 0.06

3.4. Determination of Antimicrobial Activity of *M. myrtifolia* extracts

From the six extracts, only the hexane extract inhibited the growth of *Pseudomonas aeruginosa* at 50 µg/µL. The remaining extracts showed no log reduction in the growth of *P. aeruginosa* (Figure 3).

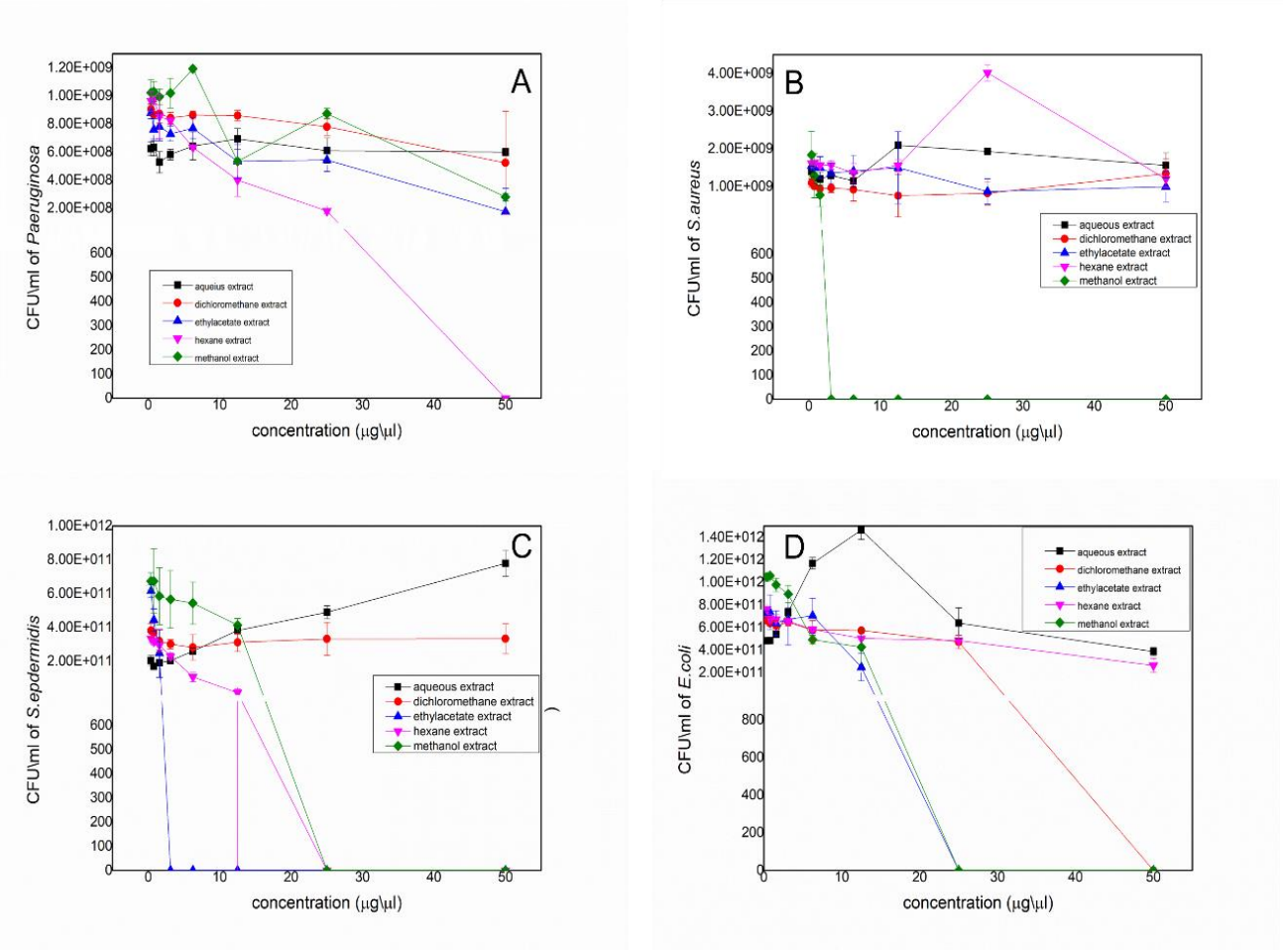

Figure 3. ***In vitro*** **antimicrobial activity of** ***M. myrtifolia*** **extracts against** ***P. aeruginosa*** **(A),** ***S. aureus*** **(B),** ***S. epidermidis*** **(C),** ***E. coli*** **(D).**

The methanol extract showed a promising result against *Staphylococcus aureus* as it could inhibit the bacteria at low concentrations. The MIC value of this extract against *S. aureus* is 3.125 µg\ µL. On the other hand, hexane extract showed an increase in the growth by one log at all the concentrations (from 0.39 µg\ µL to 50 µg\ µL). With dichloromethane extract, there was no change in the CFU\ mL (10^8) until 0.781 µg\ µL, but with the ethyl-acetate extract, the increasing CFU\ mL was seen with the third dilution (12.5 µg\ µL) as shown in (Figure 3B).

The antibacterial activity of the ethyl-acetate extract against *Staphylococcus epidermidis* was promising (Figure 3C), as the MIC value is 3.125 µg/µL. In addition, three log reductions were calculated with the last three dilutions of this extract (1.562, 0.781, and 0.39 µg/µL). The methanol and hexane extracts showed antibacterial

activity, but the MIC value was relatively high (25 µg/µL). Dichloromethane extract did not show an inhibition effect against *S. epidermidis*.

Three extracts inhibited *Escherichia coli*, with different MIC values (Figure 3D). The MIC value of methanol and ethyl-acetate extracts is 25 µg/µL, and it was for the dichloromethane extract 50 µg/µL. Unexpectedly, the hexane extract results in 3 log elevations on CFU\ mL (CFU\ mL= 1011) from the highest dilution (50 µg/µL) to the lowest dilution (0.39 µg/µL). The same elevation is seen when the concentration of methanol and ethyl-acetate extracts equal 12.5 µg/µL.

4. DISCUSSION

The present study was conducted to examine the phytochemical composition and potential biological activities of *M. myrtifolia*. Antioxidant, antimicrobial and antidiabetic activities of different extracts were investigated. The extraction was done with five solvents of different polarities, and the phytochemical composition was analyzed with LC-MS/MS in a previously developed and validated method. The aqueous extract showed significant antioxidant activity with the highest number of polyphenolics content, followed by the methanol extract. However, only the aqueous extract had significant inhibitory effects on α-amylase when compared to the other extracts.

Regarding the antibacterial efficacy of the different *M. myrtifolia* extracts, the extracts were more effective against Gram-positive bacteria in a dose-dependent manner with logarithmic CFU reduction. The methanol extract was found to have antibacterial activity against *S. aureus* and *S. epidermidis* observed at MICs of 3.15 µg/µL and 25 µg/µL, respectively. In comparison, ethyl acetate and hexane extracts had antibacterial activity against *S. epidermidis* at MICs of 3.125 µg/µL and 12.5 µg/µL, respectively. Gram-negative bacteria were more challenging to eradicate; however, ethyl acetate, methanol, and dichloromethane extracts were successful in reducing the number of *E. coli* CFUs in a dose-dependent manner at MIC of 25 µg/µL for both ethyl acetate and methanol extracts and at MIC of 50 µg/µL for dichloromethane extract. While *P. aeruginosa* only responded to hexane extract at an MIC of 50 µg/µL.

The LC-MS/MS detected a variety of phenolic compounds, with the highest number found in the aqueous extract (n=17) and the lowest number in the hexane extract (n=1). Notably, the extraction solvents and their polarities influenced the constituents and the activity of the plant extract. Rosmarinic, syringic, gallic, and Protocatechuic acids and apigenin were identified as major components in the extracts.

The results suggest that *M. myrtifolia* extracts could be used as a natural antioxidants and anti-α-amylase agent that align with their phenolic and flavonoid compounds. In addition, the extracts have potential antibacterial activity due to the secondary metabolites but do not necessarily align with their phenolic and flavonoid content.

The detection of different polyphenolic assemblies in various *M. myrtifolia* extracts (as shown in the table) underscores the significant impact of extraction solvents on the composition of these compounds and, consequently, the resulting range of bioactivities as found in other investigational studies concerned with polyphenols (21, 22).

Sarikurkcu et al., (2020) explored the pivotal role of solvent selection in the study of antioxidant phytochemical compounds and the influence of different polarities of the solvent on *M. myrtifolia* extracts (12). Their findings align with earlier research (9), which concluded that the higher the polarity of the solvent used in the extraction, the higher the phenolic and flavonoid contents of *M. myrtifolia* extracts(9).

In both earlier studies of *M. myrtifolia* extracts, three different solvents were used: water, methanol, and ethyl acetate in one study (12) and methanol, chloroform, and hexane in the other study (9). In the present study, a more comprehensive approach was adopted, employing five different solvents, including water, methanol, ethyl

acetate, dichloromethane, and hexane. The polyphenolic profiles within their respective extracts shared similarities with prior reports, albeit with some differences(12). It is worth noting that the water extract in this study showed the most diverse and highest number of polyphenolic compounds (n = 17), while in the earlier study, the water extract featured the second-highest number of polyphenols (n = 19). In that earlier study, the methanol extract had the highest number of polyphenols (n = 21). In addition, the composition of polyphenolic compounds within similar extracts from both studies were not identical. For instance, the water extract in this study had quercetin, taxifolin, and ferulic acid, while these compounds were not detected in the water extract from the earlier work. Additionally, the five extracts examined in this study did not include 3,4-dihydroxyphenylacetic acid, 3-hydroxybenzoic acid, 4-hydroxybenzoic acid, apigenin 7-glucoside, or hyperoside, all of which were present in the previous study (12).

The difference in polyphenolic content can be ascribed to differences in environmental conditions under which the plants were cultivated (23, 24) and post-cultivation processes employed, including factors related to the duration of extraction and temperature applied (12).

Polyphenols found in plants are common secondary metabolites with multiple hydroxyl groups. They are found in various parts of plants and can neutralize reactive oxygen species (ROS), which aids in preventing the growth and spread of tumors. There are concerns about the long-term use of synthetic antioxidants and their safety. Therefore, the search for safe and effective antioxidants is of paramount importance. Polyphenols are promising natural antioxidants and are explored for their high efficacy and safety (25, 26). Water and methanol extracts in this study have exhibited DPPH radical scavenging activity, which aligns with earlier studies in which the highest scavenging activity was found in the more polar extract solvent (12, 27). Chlorogenic and rosmarinic acids have been previously illustrated as the major active antioxidants in species belonging to the Lamiaceae family (28, 29). Furthermore, in a quantitative-based analysis of *M. myrtifolia* extracts, rosmarinic acid was detected in considerable amounts in both water and methanol extracts, while chlorogenic acid and caffeic acid were the second highest in water and methanol extracts, respectively (12).

The antioxidant properties of polyphenols and their free radical scavenging capacity led to a reduction of oxidative stress and the treatment of diabetes mellitus. In this study, the aqueous extract that showed antioxidant activity had α-amylase inhibitory activity. The inhibitory activity of the water extract (IC_{50}174.44 ± 0.68 mg/mL) is relatively moderate compared to that of acarbose (IC_{50} of 129.62 ± 1.21 mg/mL). The α-amylase inhibitory activity can lead to the control of postprandial hyperglycemia, the management of diabetes mellitus, and the reduction of complications associated with the disease (30). The moderate α-amylase inhibitory activity of *M. myrtifolia* water extract has the potential for a therapeutic advantage over synthetic drugs since the latter's strong α-amylase inhibitory activity is associated with digestive adverse effects (31, 32). *M. myrtifolia* could be a promising source of an effective, safe, and cheap natural therapy for diabetes. This is supported by several studies that tested the antidiabetic activity of individual polyphenolic constituents *in vivo*, such as caffeic acid in mice (33), chlorogenic acid as a single agent (34), or in combination with tetrahydro-curcumin (35), hesperidin (36), gallic acid, p-coumaric acid (37), and protocatechuic acid in rats(38). In addition, this activity is also supported by findings of α-amylase inhibitory activity of *M. myrtifolia* volatile oils (11). The observed antioxidant and antidiabetic effects of the *M. myrtifolia* water extract, in this study, prompt further investigation of the potential synergistic interactions of the polyphenols detected in it.

In earlier research on *Micromeria* species, elevated levels of polyphenols and flavonoids were linked to antioxidant activity but not to strong antibacterial activity. *M. graeca* ethanol extract was observed to have no antibacterial activity on four different bacteria strains

(namely *E. coli* ATCC 25922, *P. aeruginosa* ATCC 9027, *S. aureus* ATCC 6538, and *S. aureus* C 100459); however, it was active against resistant strains of *S.aureus* and *P. aeruginosa* when combined with medicinal antibiotics (14). The ethanol extract of *M. frivaldszkyana* demonstrated limited effectiveness against nine tested microorganisms. It was only active against one Gram-positive bacteria (*Listeria monocytogenes* ATCC 19111), while it showed no activity against the remaining eight microorganisms, including three Gram-positive bacteria, two Gram-negative bacteria, three fungi, and one yeast (39). The ethanol extract of *M. nervosa* showed no antibacterial activity, while the diethyl ether extract of *M. nervosa* exhibited high antibacterial activity against Gram-positive methicillin-resistant *S.aureus*, *S.aureus* ATCC6538, and *L. monocytogenes* ATCC19115, in addition to antileishmanial activity (40). The antibacterial activity of the *Micromeria* extracts was associated with an optimal polarity level, which does not necessarily correspond to the highest or lowest polarity. This was also observed in results for other plants studied (41). Comparatively, the oils have a more potent antimicrobial activity than other extracts, in contrast to antioxidant activity, which is higher for polar extracts than for oils.

Although the investigational studies of *Micromeria* species showed diverse results with different types of extracts and other microorganisms, the volatile oils of different *Micromeria* species have exhibited more consistent results of strong antibacterial activities. The essential oils of various species of *Micromeria* — rather than polar compounds—were primarily responsible for their antibacterial activity (42-45).

In this study, water extract had no antibacterial activity, which aligns with the above-mentioned studies. The methanol extract was the only extract with *S. aureus* antibacterial activity, hexane was the only active extract against *P. aeruginosa*, and the ethyl acetate extract was the most active extract against *S. epidermidis*. In addition, the latter had comparable inhibitory activity against *E. coli* as the methanol extract. The limited efficacy of the polar water extract could be attributed, in part, to its hydrophilic nature and loss of certain active lipophilic compounds. However, it is imperative to acknowledge that other aspects must also be considered.

CONCLUSION

Comparing our findings with previous studies on *M. myrtifolia*, our research corroborates the antioxidant capacity of the plant. However, the significant α-amylase inhibitory activity in the aqueous extract sets our study apart. To the best of our current knowledge, this work is the first to report the α-amylase inhibitory activity of *M. myrtifolia* aqueous extract and underscores its potential antidiabetic use. *M. myrtifolia* extracts antioxidant, antibacterial, and antidiabetic effects bear significant clinical and therapeutic implications. Furthermore, the potent antioxidant properties make *M. myrtifolia* extract promising candidates for the development of nutraceuticals or pharmaceuticals, particularly for conditions associated with oxidative stress.

This study opens the door to several intriguing avenues for future research. Primarily, the isolation and characterization of the specific bioactive compounds responsible for the observed effects are called for. In vivo studies are essential to confirm the bioactivity in living organisms and assure safety profiles. Mechanistic investigations into the α-amylase inhibition and the interactions between phenolic compounds are promising areas for further exploration. Furthermore, understanding the impact of varying cultivation conditions on the plant's bioactivity can inform cultivation practices for optimized phytochemical composition.

Limitations:

It is important to acknowledge the study's limitations, which include potential variations in environmental conditions affecting the phytochemical composition of *M. myrtifolia*. Differences in factors such as soil quality, climate, and geographic location could introduce

variability in plant chemistry. Additionally, the in vitro nature of our experiments may not fully replicate the complex interactions that occur in a living system. Therefore, while our findings are promising, they should be interpreted within the context of these limitations. These considerations emphasize the need for further research, including studies and investigations into the plant's bioactivity under varying growth conditions.

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التحليل الكيميائي النباتي وتقييم النشاطات المضادة للأكسدة، والمضادة للميكروبات، والمضادة لمرض السكري لنبات الميكروميرا (الزرفا)

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ملخص

تم التحقيق على نطاق واسع في المركبات الطبيعية من أجل اكتشاف أدوية جديدة. ويُعرف جنس الميكروميريا عدد الوحدات المشكلة للمستعمرات بغناه بالزيوت الطيارة والمركبات النشطة التي تمتلك أنشطة بيولوجية مهمة. يهدف هذا العمل إلى استكشاف التركيب الكيميائي النباتي لمستخلصات الميكروميريا وتقييم نشاطها المضاد للأكسدة، والمضاد للميكروبات، والمضاد لمرض السكري داخل المختبر. كشفت تقنيةالكروماتوغرافيا السائلة–مطياف الكتلة عن وجود مركبات فينولية متنوعة، وخصوصاً في المستخلص المائي. وقد أظهر المستخلص المائي قدرة مثبطة لإنزيم الألفا–أميليز بقيمة التركيز المثبط الأدنى التي بلغت 174.44 ± 0.68 ملغم/مل، كما أظهر أعلى نشاط مضاد للأكسدة حيث قام باصطياد جذور DPPHالحرة من 23.25٪ إلى 63.72٪ عند زيادة التركيز من ~0.04 إلى 0.15 ملغم/مل. من ناحية أخرى، أظهر مستخلص أسيتات الإيثيل أضعف نشاط مضاد للأكسدة، حيث تم تحقيق نسبة اصطياد للجذور الحرة بلغت 77٪ عند تركيز 7.5 ملغم/مل. وأظهرت المستخلصات المختبرة فعالية أكبر ضد البكتيريا موجبة الغرام بطريقة تعتمد على الجرعة، مع انخفاض لوغاريتمي في عدد الوحدات المشكلة للمستعمرات. وقد أظهر مستخلص الميثانول من الميكروميريا نشاطاً مضاداً للبكتيريا ضد Staphylococcus aureus و Staphylococcus epidermidis بقيم التركيز المثبط الأدنى التي بلغت 3.15 ميكروغرام/ميكروليتر و25 ميكروغرام/ميكروليتر على التوالي. وبالمقارنة، أظهرت مستخلصات أسيتات الإيثيل والهكسان نشاطاً مضاداً للبكتيريا ضد Streptomyces epidermidisعند قيم التركيز المثبط الأدنى التي بلغت 3.125 ميكروغرام/ميكروليتر و12.5 ميكروغرام/ميكروليتر على التوالي. كانت البكتيريا سالبة الغرام أكثر صعوبة في القضاء عليها؛ ومع ذلك، نجحت مستخلصات أسيتات الإيثيل، والميثانول، وثنائي كلورومیثان في تقليل عدد عدد الوحدات المشكلة للمستعمرات لبكتيريا Escherichia coliبطريقة تعتمد على الجرعة عند التركيز المثبط الأدنى بلغ 25 ميكروغرام/ميكروليتر لكل من مستخلصي الإيثيل والميثانول، و50 ميكروغرام/ميكروليتر لمستخلص ثنائي كلورومیثان. بينما استجابت Pseudomonas aeruginosaفقط لمستخلص الهكسان عند التركيز المثبط الأدنى بلغ 50 ميكروغرام/ميكروليتر. تشير التأثيرات المضادة للأكسدة، والمضادة للبكتيريا، والمضادة لمرض السكري لمستخلصات الميكروميريا إلى أهميتها السريرية والعلاجية المحتملة. بالإضافة إلى ذلك، فإن الخصائص المضادة للأكسدة القوية تجعل من مستخلص الميكروميريا مرشحاً واعداً لتطوير مكملات غذائية طبية (نيوتراسوتيكلز) أو مستحضرات دوائية، لا سيما في الحالات المرتبطة بالإجهاد التأكسدي.

الكلمات الدالة: ميكروميريا، مضاد لمرض السكري، مضاد للأكسدة، مضاد للميكروبات، الكروماتوغرافيا السائلة–مطياف الكتلة، المستقلبات الثانوية.

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