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Research Article

Assessment of antioxidant and enzyme inhibition properties of *Myrtus communis* L. leaves

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Abstract: This study investigated the antioxidant and enzyme inhibitory properties of *Myrtus communis* leaves. Three different solvents including methanol, 50%-methanol, and water were used to extract of *M. communis*. In addition, total bioactive compounds were evaluated by using total phenolic and total flavonoid content assays. In antioxidant assays, water extract displayed the highest antioxidant potential. The MeOH extract demonstrated the highest inhibitory effect against AChE (4.38 mg GALAE/g), BChE (1.58 mg GALAE/g), α-amylase (0.56 mmol ACE/g), and tyrosinase (132.20 mg KAE/g). The *M. communis* leaves extract could be used as a promising raw material source in food and medicine industries.

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KEYWORDS
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1. INTRODUCTION

Plants are rich in phytochemicals including phenolic compounds and many pharmacological activities have been reported including antioxidant, anticancer, anti-inflammatory, and anti-hyperglycemia (Chu & Chen 2006; Kumar & Pandey, 2013; Engwa, 2018). In recent decades, plant-based foods have attracted a great deal of research attention due to their health-promoting effects (Hur *et al*., 2014; Tangyu *et al*., 2019). Additionally, the use of herbal products has increased demand as a primary source of health due to their minor side effects, efficacy, and safety (Kamboj, 2000).

Myrtus communis L., also called myrtle, is a valuable medicinal plant of the Myrtaceae family. Myrtle is used in a variety of applications in several industries including food, cosmetic, and pharmaceutical (Wannes & Marzouk, 2016). The leaves of the plant are used for hypertension, hyperglycaemia, cold, rheumatic pain, and haemorrhoids (Mine *et al*., 2019). *M. comminus* leaves have been used in traditional Turkish medicine to treat conditions such as cold, obesity, and diabetes (Tuzlacı & Bulut, 2007; Tuzlacı & Sadikoglu, 2007; Sargın, 2021). Several studies have been shown that *M. communis* leaves possessed a number of therapeutic

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activities including anti-bacterial, antioxidant, anti-diabetes, and anti-cancer, (Tretiakova *et al*., 2008; Amensour *et al*., 2010; Hennia *et al*., 2018; Raoof *et al*., 2019). These activities can be explained by the presence of diverse phytochemicals including phenolic acids (gallic, ellagic, caffeic acids) and flavonol derivatives (myricetin, quercetin, kaempferol derivatives) (D'Urso *et al*., 2017).

In this study, we aimed to evaluate the potential antioxidant and enzyme inhibitory properties of *M. communis*. Total bioactive compounds were also tested.

2. MATERIAL and METHODS

2.1. Plant Materials

M. communis were gathered in September 2018 at Silifke, Mersin, Türkiye. The plant sample was identified by Dr. Evren Yıldıztugay from Selçuk University. The samples were dried in the shade and the dried materials were grounded in a laboratory mill prior to extraction.

The dried leaves (5 g) were extracted by maceration with 100 mL different solvents (50% methanol, methanol, and water) for 24 h at room temperature. The extracts were evaporated with a rotary evaporator. All samples were stored at 4 °C for subsequent analysis (Uysal *et al*. 2021).

2.2. Total phenolic and Total Flavonoid Contents

The total phenolic content was tested by Folin- Ciocalteu method. The total flavonoid content was tested by $AlCl_3$ method. (Uysal *et al*., 2017). The details of methods are indicated in Supplementary materials.

2.2. Antioxidant Assays

Antioxidant properties of the extracts were performed by ABTS, DPPH, CUPRAC, FRAP, metal chelating, and phosphomolybdenum (Uysal *et al*., 2017). The details of methods are indicated in Supplementary materials.

2.3. Enzyme Inhibitory Assays

The inhibition of tyrosinase, α amylase, α-glucosidase and cholinesterase was screened. (Uysal *et al*., 2017). The details of methods are indicated in Supplementary materials.

2.4. Statistical Analysis

The details of methods are indicated in Supplementary materials.

3. RESULTS and DISCUSSION

3.1. Total Phenolic and Total Flavonoid Contents

The extracts of *M. communis* were evaluated for total bioactive contents. The total phenolic content was higher in 50%-MeOH (145.22±4.52 mg GAE/g) and water (144.45±1.38 mg GAE/g) extracts than MeOH extract (130.24±1.52 mg GAE/g) (Figure 1). However, the highest total flavonoid content was detected in the MeOH extract (57.06±0.94 mg RE/g) (Figure 2). Abdullahi *et al*. (2020) evaluated the antioxidant, antibacterial and total phenolic content of *M. communis* leaves and total phenolics ranged between 42.12 and 189 mg GAE/g in different concentrations. Our results show a higher total phenolic content than that reported by Ozcan *et al*. (2009), who found 9.9761 mg GAE/g in the extract of *M. communis* leaves. Tumen *et al*. (2012) reported the bioactive compounds of different extracts obtained from of *M. communis* leaves and berry. The total phenolic content values of four extracts of *M. communis* leaves ranged from 38.45 to 190.85 mg GAE/g. The obtained total phenolic content value in methanol extract of *M. communis* leaves is higher than our result. According to another study, the effect of drying methods on total phenolic content of *M. communis* fruits was reported by Alkaltman

et al. (2020). The highest total phenolics value (135.07 mg GAE/100g) was determined in microwave oven-dried samples. The drying effect on the phenolic content of ethanol extract obtained from *M. communis* leaves was described by Snoussi *et al*. (2021). Our results show higher total phenolic content values than those reported for ethanol extract (25.7-55.2 mg GAE/g) in this study. Snoussi *et al*. (2021) also found total flavonoid content of *M. communis* leaves in different drying methodology methods that ranged from 11.3-28.2 mgQE/g extract. Yaghoobi *et al*. (2022) reported methanol and water extracts of myrtle leaves showed higher total flavonoid content compared other solvents (ethanol and ethyl acetate). Wannes *et al*. (2010) showed that the leaf and stem of *M. communis* var *italica* contained higher amount of flavonoid compounds in comparison to flower extract.

The variability of total bioactive compounds of *M. communis* extracts could be due to the method of extraction, climatic conditions, geographical location, and polarity of solvent used (Lee Petersen, 2003; Miliauskas *et al*., 2004).

Figure 1. Total phenolic content of *M. communis* leaves extracts. Different letters in column indicate significant differences in the studied extracts (p<0.05).

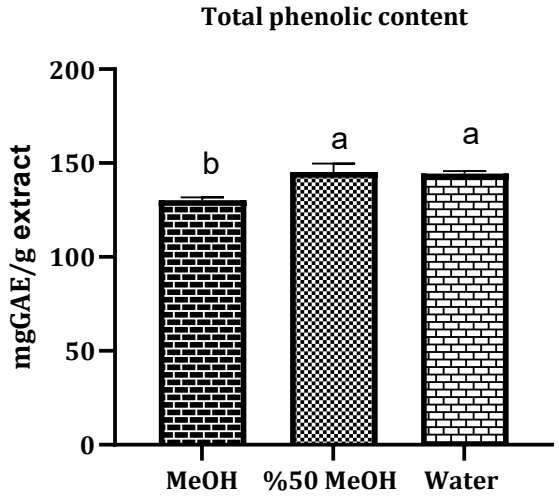
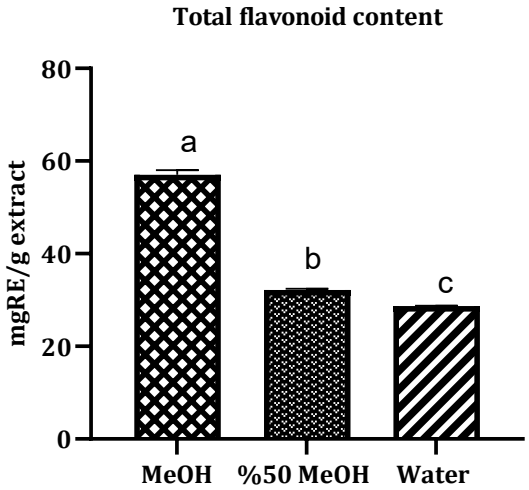


Figure 2. Total flavonoid content of *M. communis* leaves extracts. Different letters in column indicate significant differences in the studied extracts (p<0.05).



3.2. Antioxidant Capacity

Antioxidant capacity of *M. communis* leaves was assessed using complementary assays (Table 1). According to radical scavenging assays (DPPH and ABTS), the scavenger activity of the extracts decreased in the order: water> 50%-MeOH> MeOH. The reducing power activity, determined by established methods of CUPRAC and FRAP, ranged from 3.50-4.58 to 1.97-2.75 mmol TE/g, respectively. The antioxidant property of water extract was higher than that of other extracts. Antioxidant capacity of different parts from *M. communis* has been published in previous studies (Chryssavgi *et al*., 2008; Ozcan *et al*., 2009; Tuberoso *et al*., 2010; Tumen *et al*., 2012; Abdulhadi *et al*., 2020). For example, Serce *et al*. (2010) investigated DPPH activity of eight myrtles fruits. Results revealed that methanol extracts of fruits exhibited good activity, with values between IC_{50} of 2.34 and 8.24 µg/ml. Snoussi *et al*. (2021) evaluated the effect different drying methodologies on antioxidant activity of *M. communis* leaves. This study reported that the strong radical scavenging activity (DPPH IC_{50} µg/ml, ABTS IC_{50} µg/ml) was obtained from microwave dried leaves. Similarly, Alkaltham *et al*. (2021) also investigated the influence of different drying methods on antioxidant properties of *M. communis* fruits. The authors observed that dried berries extracts (DPPH: 83.01-83.55%) were more effective than the fresh berries (25.43%). In another study, the antioxidant properties of myrtle leaves cultivar were investigated by Medda *et al*. (2021). Comparison with our results is difficult because of different ways of expression of the activity.

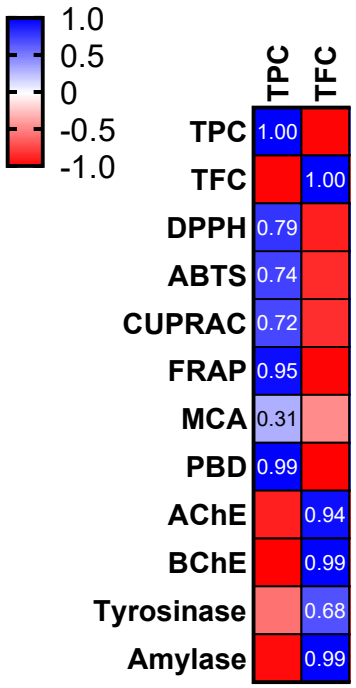
The results obtained in correlation analysis showed total phenolic content positive correlation with the antioxidant activity (Figure 3). These results are consistent with a study conducted by Medda *et al*. (2021), who found a correlation between the total phenols content and DPPH and β-Carotene.

Table 1. Antioxidant properties of *M. communis* leaves.

Assays	*Myrtus communis* -MeOH	*Myrtus communis* -50% MeOH	*Myrtus communis* -Water
DPPH (mmolTE/g)**	2.86±0.14^{c*}	3.07±0.11^{b}	3.36±0.05^{a}
ABTS (mmolTE/g)**	2.29±0.03^{c}	2.68±0.04^{b}	3.38±0.02^{a}
CUPRAC (mmolTE/g)**	3.50±0.05^{c}	3.85±0.10^{b}	4.58±0.06^{a}
FRAP (mmolTE/g)**	1.97±0.06^{c}	2.53±0.09^{b}	2.75±0.04^{a}
Phosphomolybdenum (mmolTE/g)**	4.08±0.25^{b}	4.49±0.18^{a}	4.52±0.02^{a}
Metal chelating (mgEDTAE/g)***	34.05±1.70^{c}	31.63±1.50^{b}	45.60±1.08^{a}

*Values expressed are means ±SD **TE: trolox equivalents, ***EDTAE: isodium edetate equivalents. Different letters in same row indicate significant differences in the studied extracts (p<0.05).

Figure 3. Pearson correlation values between biological activity assays and bioactive compounds (p<0.05). TPC: Total phenolic content; TFC: Total flavonoid content; ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid; DPPH: 1,1-diphenyl-2-picrylhydrazyl; CUPRAC: Cupric reducing antioxidant capacity; FRAP: Ferric reducing antioxidant power; MCA: Metal chelating ability; PBD: Phosphomolybdenum; AChE: acetylcholinesterase; BChE: butyrylcholinesterase;



3.3. Enzyme Inhibitory Properties

AChE and BChE are the major enzymes responsible for the hydrolysis of acetylcholine (Fan&Chiu, 2014). α-amylase and α-glucosidase play an essential role in the hydrolysis of carbohydrate to glucose (Wang *et al*., 2013). Tyrosinase is the key enzyme in melanin biosynthetic metabolism (Pillaiyar *et al*., 2017). Inhibition these enzymes is one of the current strategies for Alzheimer's, diabetes and skin disorders management. In recent years, natural products draw more attention because of the low side effect on the treatment of these diseases (Tuzimski *et al*., 2022, Uba *et al*., 2022). Thus, the effects of *M. communis* extracts were tested on cholinesterase, α-amylase inhibition, α-glucosidase, and tyrosinase inhibitory effect. As demonstrated in Table 2, *M. communis* MeOH extract showed the highest AChE inhibition with 4.38 mg GALAE/g. In BChE assay, only MeOH extract (1.58 mg GALAE/g) displayed the activity against BChE. The α-amylase inhibition results showed that MeOH extract had significant higher activity than %50- MeOH and water extract. None of the extracts showed inhibition effect against α-glucosidase enzyme. The order of tyrosinase enzyme inhibition effect of extracts was as follows: MeOH (132.20 mg KAE/g) > 50% MeOH (124.94 mg KAE/g) > water (71.84 mgKAE/g). Tumen *et al*. (2021) studied the inhibition effect of *M. communis* leaves and fruits on different enzymes (AChE, BChE, and tyrosinase) and reported that leaves extracts showed no activity against BChE. Ibrahim *et al*. (2021) investigated the α-amylase inhibitory effect of Egyptian *M. communis* essential oil and reported inhibitory activity. Similarly, α-amylase inhibition activity of *M. communis* essential oil (IC_{50} 29.94 µg/ml) was determined by Sen *et al*. (2020). In another study, *M. communis* leaves essential oil showed AChE inhibitory activity (IC_{50} µg/ml).

The results of the correlation analysis indicated a highly positive correlation between TPC and FRAP and PBD assays (0.95, 0.99, respectively). A weak positive correlation was noted

for the total phenolic content and metal chelating ability. For enzyme inhibition assays, total flavonoid content was strong and positively associated with AChE, BChE, tyrosinase, and amylase assays (Figure 3).

Table 2. Enzyme inhibitory activity of *M. communis* leaves

Assays	*Myrtus communis* -MeOH	*Myrtus communis* -50% MeOH	*Myrtus communis* -Water
AChE (mgGALAE/g)**	4.38±0.18[a*]	3.44±0.28[b]	2.73±0.07[c]
BChE (mgGALAE/g)**	1.58±0.15	nd	nd
Amylase (mmolACE/g)***	0.56±0.02[a]	0.21±0.02[b]	0.10±0.01[c]
Glucosidase (mmolACE/g)***	nd	nd	nd
Tyrosinase (mgKAE/g)****	132.20±0.77[a]	124.94±0.67[b]	71.84±1.27[c]

*Values expressed are means±SD **GALAE:galanthamine equivalets; ***ACE: acarbose equivalents; ****KAE:kojic acid equivalents; nd: not determined. Different letters in same row indicate significant differences in the studied extracts (p<0.05).

4. CONCLUSION

This study focuses on the total bioactive compounds, antioxidant and enzyme inhibition properties of *M. communis* leaves extracts. 50 %-MeOH (145.22 mgGAE/g) and water extracts (144.45 mgGAE/g) showed similar amounts of total phenolic content. The highest amounts of total flavonoid were found in the MeOH extract. The highest antioxidant property was determined for the water extract. (DPPH: 3.36 mmolTE/g, ABTS: 3.38 mmolTE/g, CUPRAC: 4.58 mmolTE/g, FRAP: 2.75 mmolTE/g, phosphomolybdenum: 4.52 mmolTE/g, metal chelating: 45.60 mgEDTA/g). All the extracts tested showed different level of inhibition against the AChE, α-amylase, and tyrosinase enzymes. The highest activity against AChE, α-amylase, and tyrosinase was obtained with MeOH extract, followed by 50 % MeOH, and water. *In vitro* antidiabetic activity (α-amylase and α-glucosidase inhibition assays) of *M. communis* leaves was evaluated for the first time. Overall, *M. communis* leaves extracts could be regarded a possible natural source in different industries including food and pharmaceutical.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship contribution statement

Sengul Uysal, Gokhan Zengin: Research concept and desing; Collection and/or assembly of data; **Sengul Uysal, Kouadio Ibrahime Sinan** and **Gokhan Zengin:** Data analysis and interpretation; Writing the article; Critical revision of the article, Final approval of the article.

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