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Effect of solvents on variation of Polyphenolic content and antioxidant activity of Tunisian *Myrtus communis* L leaves and berries extracts

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Abstract
This study aims to evaluate the influence of solvent type (water, ethanol, and methanol) on the total polyphenolic content (TPC), flavonoid content (TFC), and antioxidant activity of *Myrtus communis* L. leaves and mature berries collected from Tunisia. The highest extract yield was obtained from ethanolic leaf extracts (15.83 g DE/100 g DW). Berry extracts were found to be richer in polyphenols and flavonoids. The highest TPC (34.82 mg gallic acid equivalent per gram dry extract, mg GAE/g DE) and TFC (19.07 mg quercetin equivalent per gram dry extract, mg QE/g DE) were recorded in the ethanolic and methanolic berry extracts, respectively. All the extracts demonstrated strong antioxidant activity, with the ethanolic berry extracts showing the highest activity, reflected by an IC_{50} value of 3.51 µg/mL. Overall, the results emphasize that myrtle berries, followed by leaves, are a rich and valuable source of polyphenols and antioxidants, highlighting their potential as natural additives and functional ingredients in food and beverage production, as well as in the cosmetics industry and the development of therapeutic products.

Keywords: *Myrtus communis* L., solvent, total polyphenolic content, flavonoids, antioxidant activity

1. Introduction

Wild-growing medicinal and aromatic plants are a good source of bioactive compounds including essential oils and volatile organic compounds [1], as well as phenolic acids, flavonoids, tannins, and alkaloids [2]. They are also a good source of minerals and vitamins [3]. This richness gives them significant socio-economic value, due to their pharmacological potential in medicinal products, and in the aroma, flavour, and food industries [4]. Given the diverse climate of Tunisia, the local herbal knowledge includes a wide variety of wild medicinal plants [5]. Among these species *Myrtus communis* L. (Myrtaceae) is an aromatic and medicinal shrub native to the Mediterranean region [6]. In Tunisia, it thrives in the understory of *Quercus suber* L. (cork oak) forests, particularly in association with *Erica arborea* L., *Arbutus unedo* L. *and Phillyrea angustifolia* L. [7]. Its leaves are leathery, glossy, and highly aromatic. In summer, it is covered with white, fragrant, honey-producing flowers. Its edible fruits are black berries covered with a waxy bloom [8]. Previous studies on the phytochemistry of myrtle have primarily focused on evaluating the chemical composition of *M. communis* essential oils (EOs), as well as their biological potential. In Morocco, Chraibi *et al.* (2021) [9] studied the composition of the essential oils (EOs) from its aerial parts and their antibacterial effects. Furthermore, the antioxidant, anti-inflammatory, antimicrobial, and antiproliferative activities of the Algerian species have been reported by Touaibia (2017) [10], Hennia (2019) [11], and Mohamadi (2021) [12]. In Tunisia, the variability of the EOs composition in *M. communis* wild populations have been reported by Messaoud *et al.* (2005) [13]. The leaves, stems, flower buds, and flowers are characterized by the significant antioxidant activity of their EOs [14, 15]. Regarding the phytochemical composition and biological activities of myrtle extracts, methanolic extracts, particularly from the berries, have been evaluated by Messaoud and Boussaid (2011) [16] and by Wannes and Marzouk (2013) [17], and from the leaves by Yangui *et al.* (2021) [18], while ethanolic extracts were studied by Snoussi *et al.* (2012) [19]. The phenolic content, as well as the antidiarrheal and antioxidant activities of aqueous extracts, have only been evaluated in *M. communis* seeds [20].

On the best of our knowledge, studies on the polyphenolic content of the Tunisian *M. communis* leaf extracts and their antioxidant activity are scarce. Moreover, no previous research has investigated the impact of solvent on the variation of the polyphenol content and antioxidant activities of both leaves and berries from the same myrtle population in Tunisia. The main objective of this study is to examine the effect of different solvents (water, methanol, and ethanol) on the total polyphenolic content and antioxidant activity of *M. communis* leaf and berry extracts.

This work aims to provide valuable information that could support the valorization of extracts from this medicinal and aromatic species in pharmacognosy, as well as explore its potential applications in the development of functional foods and natural products.

2. Materials and Methods

2.1 Plant material

Leaves and mature dark blue berries were randomly collected in November 2024 from a wild population of *M. communis* in the Aïn Draham forest (36°46′34″ N, 8°41′05″ E), located at an altitude of 800 m in north western Tunisia, characterized by a humid upper bioclimate. The harvested material was initially air-dried at room temperature for ten days, followed by drying in a forced-air oven at 35 °C for 48 hours. Once the samples reached a constant weight, they were finely ground into a powder and stored in glass containers at 4 °C until needed.

2.2 Plant extracts preparation

A mass of 1 g of dried *M. communis* leaf and berry powders was soaked in 10 mL of 70% methanol, 70% ethanol, and water solutions, and left to macerate for 24 hours at ambient temperature. The obtained extracts were filtered using Whatman filter paper, air-dried for five days, and then further dried in an oven at 37 °C (24 hours). The residue was re-dissolved in the same solvents and adjusted to a final volume of 10 mL [21]. The resulting extracts; aqueous extracts (AE), ethanol extracts (EE), and methanol extract (ME) of leaf (L) and berry (B) were stored in vials at 4 °C until analysis. The extract yield was calculated as grams of dry extract per 100 grams of dry weight (g DE/100 g DW).

2.3 Determination of Total Phenolic Content (TPC)

The total phenolic content (TPC) in the extracts was quantified using the Folin-Ciocalteu reagent method, following Singleton and Rossi (1965) [22]. To 20 µL of each extract, 1155 µL of distilled water and 100 µL of 10% Folin-Ciocalteu reagent were added. The mixture was stirred vigorously, and then 225 µL of 10% sodium carbonate solution was incorporated. After incubating for 30 minutes at 25 °C, the absorbance of the resulting blue solution was measured at 765 nm using a spectrophotometer. A standard curve was constructed using gallic acid concentrations ranging from 0.1 to 1 mg/mL. The TPC was expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g DE).

2.4 Determination of total flavonoids content (TFC)

The total flavonoid content (TFC) was determined using the aluminium chloride colorimetric method [23]. A 125 µL volume of the different *M. communis* leaves and berries extracts was combined with 625 µL of distilled water, followed by the addition of 38 µL of 5% $NaNO_2$ solution. After 6 minutes, 75 µL of 2% $AlCl_3$ solution was added, and the mixture was allowed to stand for an additional 5 minutes. Following this, 250 µL of 1 M NaOH and 137 µL of distilled water were introduced. Absorbance was recorded at 510 nm. A standard calibration curve was constructed using quercetin, with concentrations ranging from 0.1 to 1 mg/mL. The results were then expressed as milligrams of quercetin equivalents per gram of dry extract (mg QE/g DE).

2.5 Determination of DPPH free radical scavenging activity

The scavenging activity of the obtained extracts was measured according to Brand-Williams *et al.* (1995) [24]. A volume of 500 µL of each extract, with concentrations ranging from 5 to 20 µL, was mixed with 1000 µL of 0.1 mM DPPH• solution and incubated in the dark at room temperature for 30 minutes. The absorbance was measured at 517 nm against a control (500 µL of solvent and 1000 µL of DPPH solution. The radical scavenging activity (RSA) was determined as the percentage of DPPH discoloration, calculated using the equation below:

$$\% \text{ InhibitioN}=[(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100$$

The results were expressed as the inhibitory concentration (IC_{50}) of the extract required to reduce DPPH• absorbance by 50%. Concentrations were reported in micrograms of dry extract per milliliter of solvent (µg/mL).

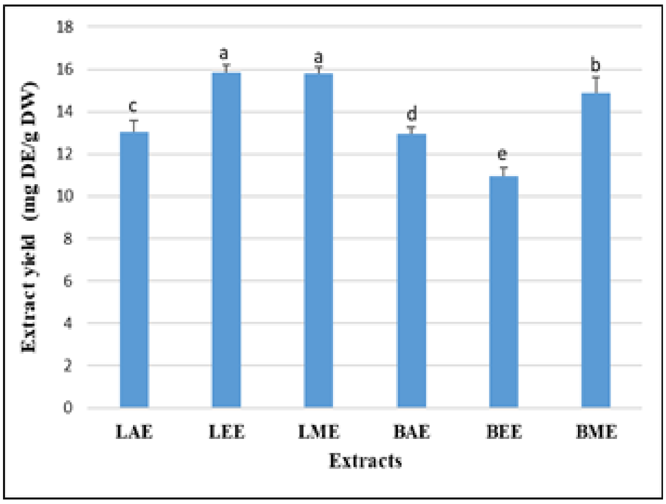
2.6 Statistical Analysis

All results are expressed as the mean values±standard deviation (SD) of three replicate. Variance analysis (ANOVA) was performed on measured data. The *Duncan's* test of multiple range was then applied to highlight the significant differences at $p<0.05$. All experiments were performed in triplicate (N=3).

3. Results and discussion

3.1 Extraction yield

M. communis extract yields varied depending on the type of plant part (leaf or berry) and the solvent used (Figure 1).



LAE: leaves aqueous extract, LEE: leaves ethanol extract, LME: leaves methanol extract, BAE: berries aqueous, g DE/100 g DW: grams of dry extract per 100 grams of dry weight. Different letters are significantly different at 5% using the *Duncun* test ($p<0.05$).

Fig 1: *Myrtus communis* leaf and berry extract yields (g DE/100 g DW)

The highest yields were obtained from leaf extracts, ranging from 13.02±0.56 to 15.83±0.37 g DE/100 g DW, surpassing those of berry extracts, which varied from10.93±0.40 to 14.89±0.72 g DE/100 g DW. The highest yield was recorded from ethanolic leaf extracts (LEE), with a value of 15.83±0.37 g DE/100 g DW), closely followed by methanolic leaf extracts (LME) at 15.79±0.31 g DE/100 g DW.

3.2 Total phenolic content and total flavonoid content

Results of the total phenolic (TPC) and flavonoid contents (TFC) are presented in the Table 1.

Table 1: Total polyphenol and total flavonoid contents in leaf and berry extracts of *Myrtus communis* wild-growing in Tunisia

Organs	Extracts	TPC (mg GAE/g DE)	TFC (mg QE/g DE)
Leaf	LAE	31.54±2.66 [b]	2.73±0.34 [e]
	LEE	27.11±0.38 [e]	4.88±1.07 [d]
	LME	31.77±1.11 [b]	12.22±1.05 [c]
Berry	BAE	29.50±2.40 [c]	4.45±0.35 [d]
	BEE	34.82±2.02 [a]	17.07±1.26 [b]
	BME	28.22±0.21 [cd]	19.07±0.88 [a]

Note: values are expressed as means ± Standard Deviations (SD) of triplicate determinations. Means with different letters within the same column are significantly different at 5% using the *Duncan* test (p<0.05). LAE: leaves aqueous extract, LEE: leaves ethanol extract, LME: leaves methanol extract, BAE: berries aqueous extract, BAE: berries ethanol extract, BME: berries methanol extract, TPC: total phenolic content, TFC: total flavonoid content, mg GAE/g DE: milligrams of gallic acid equivalents per gram of dry extract, mg QE/g DE: milligrams of quercetin equivalents per gram of dry extract

3.2.1 Total phenolic content (TPC)

The highest TPC values were recorded in the berry extracts of *M. communis*, particularly in the ethanolic extract, reaching a maximum of 34.82 mg GAE/g DE. Leaf extracts are also rich in polyphenolic compounds, although their concentrations are lower than the maximum value obtained in the berry extracts. The TPC of the hydro-methanolic leaf extract reached 31.77 mg GAE/g DE, followed by the aqueous leaf extract at 31.54 mg GAE/g DE.

When comparing our results with previous studies, notable differences emerged. In fact, the total phenol content reported by Messaoud and Boussaid (2011) [16] in 70% MeOH extracts of black-blue and white mature berries of Tunisian myrtle (63.20 and 53.00 mg GAE/g DE, respectively) exceeded our values. However, the berry, its separated seeds, and the pericarp extracts, reported by Wannes and Marzouk (2013) [17], of *M. communis* from the Haouaria region (Northeast Tunisia, altitude 141.43 m), were found to be less rich in TPC than those recorded in our study, both in fruit and leaf extracts. The highest value was recorded in the seeds (23.87 mg GAE/g DW), followed by the berry (13.73 mg GAE/g DW). The pericarp of the berry was the least rich in TPC (2.76 mg GAE/g DW). On the other hand, for the Algerian myrtle, leaf extracts were found to be richer in TPC than the stems and fruits (119.23, 112.96 and 70.26 mg GAE/g DM, respectively) [25]. Indeed, the variation in TPC within the same species is influenced by factors such as the stage of maturity, environmental conditions (including geographic location and bioclimatic zone), and genotype [26, 27, 28].

3.2.2. Total flavonoid content (TFC)

The methanolic and ethanolic extracts of the berries were found to be richer in flavonoids than aqueous leaf extract (Table 1). The TPC in the BME reached a maximum value of 19.07 mg QE/g DE, followed by the flavonoid content of the BEE (17.07 mg QE/g DE). The methanolic leaf extract (LME) is also rich in TFC, but with a lower value (12.22 mg QE/g DE). The TFC values in the ethanolic leaf extract, as well as in the aqueous extracts of both leaves and berries, are low and do not exceed 4.88 mg QE/g DE. Indeed, *M. communis* berry extract is an excellent source of flavonoids. The methanol extract of fruits reached a maximum value of 25.60 mg RE/g DW [16]. Yangui *et al.* (2021) [18] confirmed that TFC in the hydro-methanolic leaf extract of myrtle is very high and varies significantly depending on the genotype. The TFC values reached 41.01, 63.41, and 88.08 mg RE/g extract in the three studied genotypes. According to these authors, myricetin and quercetin, known for their antioxidant, anti-inflammatory, and potential health benefits, are the most abundant flavonoid in the leaf extracts of myrtle.

3.3. Antioxidant activity analysis

Table 2 presents the antioxidant activity results of aqueous, ethanolic, and methanolic extracts of *M. communis* assessed through the DPPH assay.

Table 2: Antioxidant potential of *Myrtus communis* leaf and berry extracts

	Organs					
	Leaf			Berry		
Extracts	LAE	LEE	LME	BAE	BEE	BME
DPPH (IC_{50}, µg/mL)	6.70±0.26 [d]	5.75±0.23 [c]	5.63±0.14 [c]	5.35±2.18 [c]	3.51±0.08 [a]	4.90±0.35 [b]

Note: Values are expressed as means ± Standard Deviations (SD) of triplicate determinations. Means with different letters within the same line are significantly different at 5% using the *Duncan* test (p<0.05). LAE: leaves aqueous extract, LEE: leaves ethanol extract, LME: leaves methanol extract, BAE: berries aqueous extract, BAE: berries ethanol extract, BME: berries methanol extract**,** DPPH**.** 2, 2-diphenyl-1-picrylhydrazyl, IC. Inhibitory concentration

The results demonstrated that both the leaf and berry extracts of *M. communis* exhibit strong antioxidant activity, as indicated by very low IC_{50} values, which do not exceed 6.70 µg/mL. The anti-radical activity of berry extracts was found to be more important than that of leaf extracts. For the berry extracts the total antioxidant activity followed the order ethanol greater than methanol greater than water, while for the leaf extracts the order was methanol greater than ethanol greater than water. Thus, the radical-scavenging activity is primarily influenced by the type and quality of the extract rather than its quantity. The ethanolic extracts of myrtle berries (BEE) showed the lowest IC_{50} value of 3.51 µg/mL, reflecting the highest antioxidant activity. This is directly correlated with their high content of polyphenolic compounds (TPC = 34.82 mg GAE/g DE). In fact, polyphenolic extracts derived from various plant sources exhibit significant antioxidant properties, playing a crucial role in mitigating oxidative stress-related diseases. Due to their potent bioactive compounds, these extracts are increasingly utilized as natural preservatives and functional ingredients in food products [29]. Particularly, compounds such as flavonols and anthocyanins are believed to contribute to the antioxidant properties of *M. communis* extracts [30]. Our results do not align with those reported by Yangui *et al.* (2021) [18], who found an IC_{50} value of 3.81 µg/mL in the methanolic extracts of Tunisian myrtle leaves, which is considerably lower than the value of 5.63 µg/mL obtained in our study using methanol as a solvent. Even with the same type of extract and plant organ, the radical scavenging activity can vary due to the genotype, the environmental conditions, and the extraction methods [31]. However, our results align with those of Messaoud and Boussaid (2011) [16], who found that the berries, and particularly their seeds, exhibit stronger antioxidant potential.

4. Conclusion

Extracts from the berries, followed by those from the leaves of Tunisian wild myrtle, grown in the northwest of Tunisia, appear to be an excellent source of polyphenols and flavonoids, with significant antioxidant potential. These extracts could serve as a valuable natural product beneficial to human health making them a promising alternative to synthetic antioxidants in various applications, including food, cosmetics, and pharmaceuticals.

5. Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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