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Research Article

Effect of extraction solvents on total polyphenolic content, total flavonoid content, and antioxidant activity of Tunisian cultivated mulberry (*Morus alba* L.) fruit extracts

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Abstract

This study aims to evaluate the total polyphenolic, flavonoid contents, and the antioxidant activity of the aqueous, ethanolic, and methanolic extracts of Tunisian cultivated mulberry fruits. Analyses showed that the highest extract yield was obtained in the methanolic extracts 44.15%. The highest values of total phenolic content (TPC) were obtained in aqueous extract (6.75 ± 0.38 mg gallic acid equivalent per gram dry extract, mg GAE/g DE) followed by the methanolic extracts (6.10 ± 0.23 mg GAE/g DE), and the ethanolic extract (4.98 ± 0.55 mg GAE/g DE). Similarly, the total flavonoid content (TFC) was also detected in a significant amount with values ranging from 2.79 ± 0.21 mg quercetin equivalent per gram dry extract (mg QE/g DE) in the ethanolic extract to 3.50 ± 0.18 mg QE/g DE in the methanolic extract. The antioxidant activity of different extracts was then assessed, and the IC_{50} reached the values of 0.83 ± 0.05, 1.13 ± 0.13, and 1.78 ± 0.14 mg/mL for the ethanolic, methanolic, and aqueous extracts, respectively. The results highlight the richness of white mulberry fruits in bioactive molecules and the effect of extraction solvents on the variation of antioxidant activity. Indeed, the antioxidant properties of white mulberry fruits may be promising for potential applications in the pharmaceutical and cosmetic industries.

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Morus Alba L., total polyphenol content, total flavonoid content, antioxidant activity, effect of solvent.

1. Introduction

Aromatic and medicinal plants are recognized as an important resource of complementary and alternative medicine due to their richness in bioactive molecules with antioxidant potential and other beneficial properties [1, 2]. In fact, these plants remain an essential source of bioactive compounds that have pharmacological properties, widely used in traditional and modern medicine, as well as for uses

in phytotherapy and other industrial sectors. Moreover, due to their antioxidant, antimicrobial, and anti-cancer properties, they are used in certain foods and pharmaceutical products to help preventing diseases [3]. Among the most significant medicinal plants, the white mulberry (*Morus alba L.*) has been effectively used as a traditional medicine in Asia for the treatment of various infectious and internal diseases. It is a rich source of bioactive compounds that can promote healthy human life [4, 5].

Mulberry (*Morus alba* L.) of the Moraceae family is native to China. This plant is also widely cultivated in India, Japan, Korea, and other countries that have warm temperatures such as Mediterranean, sub-tropical and tropical environments, including African and European countries [6, 7]. It stands out from others by its low lipid content and its richness in proteins, carbohydrates, fibers and vitamins [8]. Various parts of this plant, including leaves, fruits, and root barks are used in medicine. Additionally, its fruits are valued for their health-promoting properties, nutritional value, pleasant taste and biological activities [9, 10], as well as their content of bioactive compounds that can neutralize free radicals, helping to protect the body against oxidative stress and related diseases [11]. Recent studies have reported several bioactive properties of *M. alba* L., such as antibacterial, antioxidant, anti-inflammatory, anti-obesogenic, or hypoglycemic and antihypertensive activities. The healthy properties of mulberry have been related to the content of phytochemicals in its leaves and fruits, such as flavonoïds or phenolic acids [12-14].

In China and Korea, mulberry fruits were used as a traditional folk medicine to prevent diabetes and other chronic diseases [15]. Several studies have shown that mulberry fruit extracts contain many bioactive components which are responsible for their antioxidant activities [7, 16]. Wang et al. [17] reported that mulberry fruits can protect against kidney and liver damage, strengthen the joints, improve eyesight, and treat sore throat, aging, fever, anemia, and hypertension. A study conducted by Altaf et al. [10], showed that quercetin and isoquercetin were found to be the major flavonoids present in all parts of the mulberry tree (fruit, root, shoot, and leaf). The fruits exhibited the highest antioxidant activities. The variation in antioxidant activity among the parts of the mulberry tree can be attributed to the variation in polyphenol contents, which were significantly higher in the fruits and roots. However, the differences in phytochemical composition depending on extraction solvents have yet to be studied in *M. alba* L. fruits. In this context, this study aims to evaluate the effect of solvents on the total polyphenolic content, the total flavonoid content and the antioxidant activity of white mulberry (*Morus alba* L.) fruit extracts, in order to promote this plant as a potential source of bioactive molecules with beneficial effects for human health.

2. Materials and methods

2.2. Plant material

Mature fruits of mulberry were randomly collected in May 2024, in the region of Gafsa (southwest of Tunisia, lower arid, Latitude: 9°16'1.2''N, Longitude: 34°28'1.2''E). Collected fruits were washed with water to remove any residual particulate matter and extrinsic contaminants and then dried at room temperature for two days, afterwards dried in a forced-air drier at 35 °C for 48 h, and then ground to a fine powder using a Moulinex coffee grinder and stored in a glass bottle at 4°C until use.

2.3. Preparation of mulberry fruit extracts

The dried sample (1g) was macerated in 10 mL of distilled water, ethanolic (70%) and methanolic (70%) solvents for 24 hours. Then the different mulberry extracts were filtered and dried in a forced-air dryer at 37°C. The residue was redissolved in the same solvents of maceration and made up to 5 mL [18]. The yield (%) extraction was calculated as grams of dry extract per 100 grams of dry plant weight (g DE/100 g DPW). The final extract was stored in vials at 4°C until the corresponding analyses were conducted.

2.4. Determination of total polyphenol content

The determination of total polyphenolic content (TPC) was determined by the Folin-Ciocalteu reagent method [19]. Briefly, 20 µL of the different mulberry extracts were added to 1155 µL of distilled water and 100 µL of Folin-Ciocalteu reagent (10%). Then, a 225 µL of sodium carbonate (10%) were added. After 30 min of incubation at 25 °C, the absorbance of the resulting blue-colored solution was measured at 765

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nm. A standard curve was prepared by using different concentrations ranging from 0.1 to 1 mg/mL of gallic acid. The TPC was expressed as mg gallic acid equivalents per gram of dry extract (mg GAE/g DE). All experiments were performed in triplicate.

2.5. Estimation of total flavonoid *content*

The estimation of the total flavonoid content (TFC) was measured spectrophotometrically [20]. A total of 100 µL of the different mulberry fruit extracts was mixed with 900 µL of distilled water in a test tube. After 5 min, 500 µL $AlCl_3$ (2%) was added to the mixture and incubated for 15 min. The absorbance was measured against the blank at 510 nm. Quercetin was used to prepare a standard calibration curve with different concentrations ranging from 0.1 to 1 mg/mL of quercetin. The results were expressed as mg of quercetin equivalent per g of dry extract (mg QE/g DE). All measurements were performed in triplicate.

2.6. Assessment of DPPH radical scavenging activity

The scavenging activity of different mulberry extracts was measured according to the method described by Brand-Williams et al. [21]. 500 µL of mulberry extracts, at different concentrations (12.5, 25, 37.5, 50, and 62.5 µL from the stock mother solution on mg DE/mL solvent) were added to 1000 µL of DPPH• solution (0.1 mM) and kept in the dark at room temperature for 30 min. The absorbance was measured at 517 nm against a control (500µL of solvent and 1000 µL of DPPH solution). All the assays were conducted in triplicate. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration, using the following equation:

$$\%I = [(Abs\ control - Abs\ sample) / Abs\ control] \times 100$$

The results were expressed as the inhibitory concentration of the extract needed to decrease DPPH• absorbance by 50% (IC_{50}). Concentrations are expressed in milligrams of dry extract per milliliter of solvent (IC_{50}, mg/mL).

2.7. Statistical analysis

All results are expressed as the mean ± SD of three replicates (n = 3). A one-way ANOVA, followed by Duncan's multiple range tests, was carried out to assess for significant differences between various experiments (a significant model was accepted for a p-value < 0.05) using Excel and STATISTICA software version 5.1.

3. Results and discussion

3.1. Extraction yield

Mulberry extract yields varied depending on the solvent used (Fig. 1). The highest yield was obtained with hydromethanolic extract (MFME) with a value of 44.15%, closely followed by hydroethanolic extract (MFME) at 42.6% while the lowest was obtained with water at 42.09%. Our results are higher and differ from those reported by Kobus-Cisowska et al. [14] who found the highest yield in the ethanolic extract (15.82%), while the lowest yield was in the aqueous extract (10.11%). The solvent selection plays a crucial role in the extraction yield, as its polarity influences its efficiency in extracting the phytochemical compounds [7]. The variation in polarity directly affects the type and amount of bioactive compounds extracted, thereby influencing the biological activities of these compounds [22].

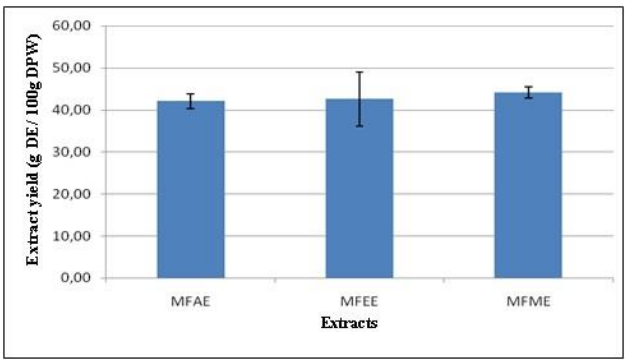

Figure 1. Mulberry fruits extract yields (g DE/100 g DPW). (MFAE: Mulberry fruits aqueous extract, MFEE: Mulberry fruits ethanolic extract, MFME: Mulberry fruits methanolic extract, g DE/100 g DPW: grams of dry extract per grams of dry plant weight).

3.2. Total phenolic and total flavonoid *contents (TPC and TFC)*

The TPC and TFC of the different mulberry fruit extracts are presented in Table 1. The results showed that the highest values of TPC were obtained in the aqueous extract, reaching a value of 6.75 mg GAE/g DE, followed by the hydromethanolic extract reaching 6.11 mg GAE/g DE. While the lower value was obtained in the hydroethanolic extract with 4.98 mg GAE/g DE. Similarly, Ali et al. [23] reported that the aqueous extract exhibited the highest value of total phenolic content of white mulberry fruits, with the value of 23.5 mg GAE/g DE.

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Table 1. Total phenolic content, total flavonoid content, and antioxidant activity of mulberry fruits aqueous, ethanolic, and methanolic extracts.

Samples	Total phenolic content (TPC, mg GAE/g DW)	Total flavonoid content (TFC, mg QE/g DW)	DPPH (IC_{50}, mg/mL)
MFAE	6.75± 0.38[a]	3.33±0.18[a]	1.79± 0.29[a]
MFEE	4.98±0.56[b]	2.79±0.21[b]	0.83±0.056[b]
MFME	6.10±0.23[a]	3.50±0.18[a]	1.13±0.118[c]

Values are expressed as means ± SD of triplicate determinations. Means with different letters within the same column are significantly different at 5% using the Duncun test ($P< 0.05$). MFAE: Mulberry fruits aqueous extract, MFEE: Mulberry fruits hydroethanolic extract and MFME: Mulberry fruits hydromethanolic extract.

Likewise, Suriyaprom et al. [11] reported that the total phenolic content in the aqueous mulberry extracts was significantly higher than the ethanolic extracts (23.36 and 23.77 mg GAE/g for aqueous extract against 13.95 and 13.97 mg GAE/g for ethanolic extract).

On the other hand, the highest value of total flavonoid content was obtained in the methanolic extract with 3.50 mg QE/g DE followed by the aqueous extract with 3.33 mg QE/g DE. However, the ethanolic extract presents the lowest value, with 2.79 mg QE/g DE. Kobus-Cisowska et al. [16] confirmed that total flavonoid content in the hydromethanolic extract was also high, and reaching 1.99 mg QE/g DE. While Ali, et al. [23] reported that the highest total flavonoid content is in the ethanolic extract, these values are significantly lower than those obtained in our study. Phenolic compounds and flavonoïds from medicinal plants present a significant potential for enhancing human heath through their different properties [24, 25]. Moreover, the amount of total phenolic and flavonoid contents depends on various factors, such as the types of solvents, extraction method and certain conditions [2, 7, 26-28].

3.3. Antioxidant activity analysis

The IC_{50} values for all extracts are presented in Table 1. DPPH scavenging activity in percent (%) of different mulberry fruit extracts is presented in Figs. 2 (a, b, and c). The ethanolic extract exhibited the strongest antioxidant activity with an IC_{50} value of 0.83 mg/ mL, followed by the methanolic extract with 1.13 mg/mL, and the aqueous extract with the lowest activity (1.79 mg/mL). These results indicate that ethanol is the most effective solvent for extracting antioxidant compounds from mulberry fruits. Our results align with those reported by Chen et al. [7], who found that the strongest antioxidant activity was in the ethanolic extract with an IC_{50} value of 0.18 mg/mL followed by the methanolic extract with an IC_{50} value of 0.29

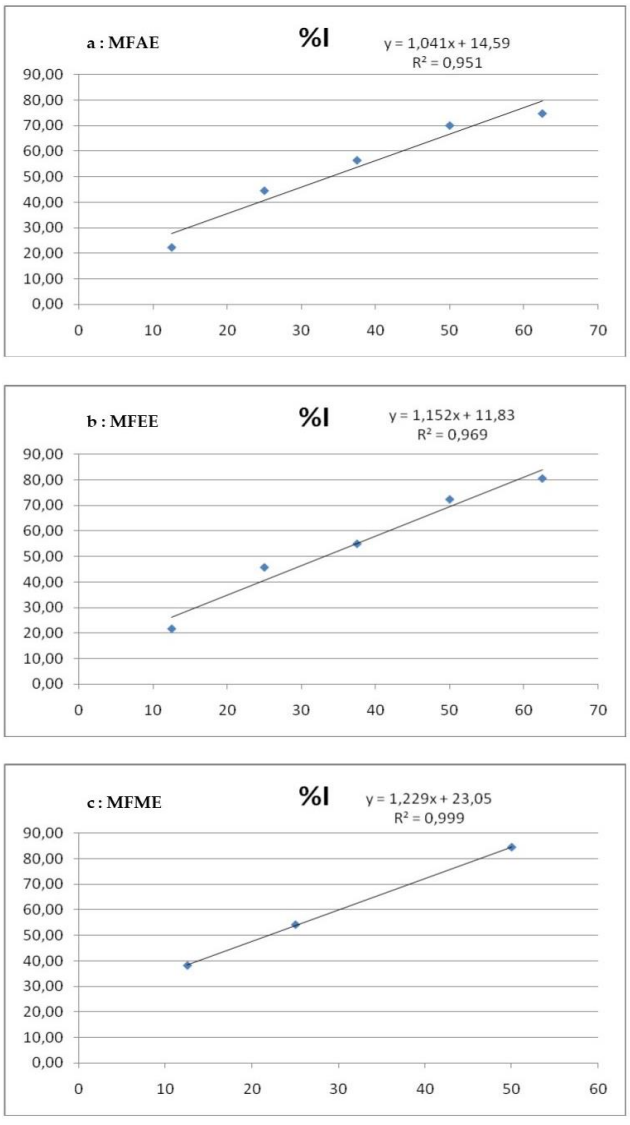

Figure 2. DPPH scavenging activity in percent (%) of different mulberry fruits extracts.
(a) MFAE: Mulberry fruits aqueous extract, (b) MFEE: Mulberry fruits hydroethanolic extract, and (c) MFME: Mulberry fruits hydromethanolic extract.

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mg/mL. These results suggest that these plants with strong antioxidant properties usually have high levels of total polyphenols [8, 27, 29]. The phenolic and flavonoids content is often correlated with the antioxidant activity of plant extracts, as these bioactive molecules play a crucial role in the neutralization of free radicals; however, antioxidant effectiveness also depends on extraction conditions and the type of solvents [16, 27, 30].

The use of Pearson's correlation coefficients revealed significant correlations between several phenolic compounds and the antioxidant tests, proving the significance of these compounds and their contribution to the antioxidant power of the plant extracts [31, 32]. The interaction or synergistic effect among the polyphenolic compounds contained in plant extract may also contribute to their antioxidant capacity. It was the greater presence of these components that was responsible for the increased antioxidant capacity of the corresponding extracts.

4. Conclusions

This study revealed a high phenolic content in the aqueous and methanolic extracts of mulberry fruits. Furthermore, the different extracts show strong antioxidant activity. These compounds contribute significantly to its antioxidant properties. This study suggests that mulberry fruits could be a promising functional food and an important antioxidant source for applications in the food and pharmaceutical industries. Further research is needed to explore their biological properties and health benefits.

Authors' contributions

Conceptualization, A.A., K.H.; Methodology, A.A., K.H., I.T.; Formal analyses, A.A., I.T., K.H., F. Z., A.F., R. A.; Investigation, A.A., K.H., I. T.; Resources, K.H.; Writing-original draft preparation, A. A., K.H.; Writing, review, editing and supervision, K.H, S.S.

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Availability of data and materials

All data will be made available on request according to the journal policy.

Conflicts of interest

The authors declare that they have no financial and commercial conflicts of interest.

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