Research Article

Improvement of the antioxidant potential: impact of drying and extraction techniques on polyphenols in *Arbutus unedo* L. leaf aqueous extract

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Article history:
Submitted 17 December 2023
Accepted 19 January 2024
Published 01 March 2024

Abstract
The search for alternatives to chemical pharmaceutical products remains an urgent and crucial step for humanity. In this regard, the use of plants presents an ideal approach for such biological studies. *Arbutus unedo* L., which belongs to the family Ericaceae, is one of the species that holds significant importance in traditional pharmaceutical uses. In Algeria, the use of this species in traditional treatment is generally rare. Therefore, this study aims to highlight its medicinal importance and the proper method for extracting its bioactive elements. To achieve this, the biological activity of leaves including total phenolic content (TPC), DPPH assay, and total flavonoid content (TFC), were assessed under four different drying temperatures (shade-drying, sun-drying, 40°C and 60°C) and two extraction methods after maceration (centrifugation, filtration). The results have indicated that these processes have affected the final accumulation of polyphenols and antioxidant activity in the leaf extracts. Additionally, it has been highlighted that the centrifugation method extracts a higher biochemical amount, especially after drying at 40°C. Furthermore, *Arbutus unedo* L. leaves should be considered a promising source of natural compounds to be used as ingredients in various fields.

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How to cite:
1. Introduction

Medicinal plants have an important place in therapeutic and cosmetic uses worldwide (Zemour et al., 2019; Labdeli et al., 2019; El Zerey-Belaskri et al., 2022; Sousa et al., 2022; Lenzi et al., 2022). Throughout history, various cultures and traditional systems of medicine have relied on the healing properties of plants to treat illnesses and promote well-being. Medicinal plants play a deeply rooted role in the culture of indigenous communities in Africa, where they are extensively utilized for the treatment of various diseases (Frimpong et al., 2021; Haris et al., 2023; Wanga & Nyamboki, 2023; Abebe et al., 2022). These plants contain a wide range of phytochemicals that exhibit pharmacological and therapeutic effects (Dutta et al., 2023; Nafeh et al., 2023; Kumar et al., 2023; Kim & Kang, 2023). It has been demonstrated in previous studies that medicinal plants have potential antiaging and antioxidant activities (Zemour et al., 2019; Chaikhong et al., 2023; Yazan et al., 2021; Çör Andrejc et al., 2022). Additionally, natural products are associated with fewer side effects when compared to synthetic drugs, making them a potential alternative solution (Nisar et al., 2018; Youssef et al., 2023; Bafandeh et al., 2023; Ahdaa et al., 2023).

Among these plants, the strawberry tree, scientifically known as *Arbutus unedo* L., is a plant native to the Mediterranean Region that represents a significant source of biologically active compounds (Ateş et al., 2022; Morales, 2022; Morgado et al., 2018; El Mekkaoui et al., 2023; Tenuta et al., 2019). It is recognized for its fruits and leaves, which have been traditionally used for their positive effects on health. For many years, it has been employed in folk medicine to alleviate a range of health issues, including urological and kidney problems, dermatological conditions, cardiovascular disorders, and gastrointestinal diseases (Bebek Markovinovic et al., 2022; Scarano et al., 2022; Abidi et al., 2016). *Arbutus unedo* L. (local name in Algeria: Lanj) is a plentiful medicinal plant found in various regions of Algeria. However, in these areas, the traditional therapeutic practices associated with it became less and less transmitted and tends to disappear (Senouci et al., 2019).

Phenolic compounds and flavonoids have the potential to serve as alternative bioactive agents within the pharmaceutical and medicinal domains, contributing to the enhancement of human health and the prevention and treatment of various diseases (Sun & Shahrajabian, 2023). Phenolic compounds exhibit significant potential in combating diverse human viruses, and they additionally possess immunomodulatory and anti-inflammatory properties (Tirado-Kulieva et al., 2022).

It is commonly recognized that plant resources undergo significant natural fluctuations due to the genetic makeup and environmental factors affecting their growth and quality characteristics (Pacheco-Hernández et al., 2021). Also, the drying method effect on this quality has been extensively reported in several papers (Guclu et al., 2021; Nurhaslina et al., 2022; Lee et al., 2022). Drying process plays a significant role in inhibiting enzymatic reactions, preventing the growth of microorganisms, and reducing weight to facilitate cost-effective transportation and storage (Baibuch et al., 2023; Singhal et al., 2020). The specific drying conditions, particularly the temperature and duration of the process, have an impact on the final extract composition (Santos et al., 2022; Lang et al., 2019; El Gamal et al., 2023).

Therefore, the aim of this study is to offer novel insights into the characterization of strawberry tree leaf extracts from Northwest Algeria (Tissensilt), particularly focusing on their polyphenolic compounds and associated activities. Herein, this is essential to obtain comprehensive and representative information on the composition of the leaves and to understand the impact of different drying methods on this extract. Another objective of this study is to determine the most suitable drying method for producing *Arbutus unedo* extract powder with optimal properties while minimizing production costs.
2. Materials and methods

2.1. Collection of plant and drying process

Spontaneously growing leaves of *Arbutus unedo* L. were collected in March 2023 from a semi-arid area in the Larbaa forest (35°54'20"N, 1°30'41"E, 1498m), located in Tissemsilt, in western Algeria (Figure 1). This region is characterized by low rainfall and high temperature, typical of a semi-arid climate.

Only healthy leaves were collected randomly according to the method of transect, from 10 shrubs for the intended laboratory analyses. After that, the fresh leaves were divided into four batches with three replicates (Figure 2). The two first batches were dried in an oven at 40°C and 60°C for 4 hours, while the other batches were air-dried for 8 days under shade and sun (Figure 2). Three repetitions were carried out for each sample. After this drying step, the leaves were finely powdered and stored at a temperature of 4°C until they are used for extraction (filtration, centrifugation) after maceration. The studied parameters were conducted at Laboratory of the Faculty of Sciences and Technology at Tissemsilt University (Algeria).

![Figure 1. Map of the study area](image)

![Figure 2. Experimental setup (R: number of repetition)](image)
2.2 Preparation of the aqueous extract

5g of the dried powder was mixed with 100 ml of distilled water at room temperature for 24 hours. Subsequently, for the extract recovery, two extraction techniques were employed. The first technique involved centrifugation at 3000 rpm for 15 minutes (El-Desouky, 2021), while the second technique corresponds to traditional filtration through filter paper (Figure 2).

2.3 Parameters determination

Quantitative yield of the extract

This parameter is determined by referring to the method of Alghoraibi et al. (2020). 100 μl of the extract was deposited on empty glass slide, and the weight was measured with a precision scale after evaporation under 35 °C. The extract yield was calculated relative to the weight of the powder used (5 g) as indicated below:

\[
\text{Yield of extract (\%)} = \frac{\text{Weight of the sample of the obtained extract (g)} \times 100}{\text{Weight of the sample powder used (g)}}
\]

Determination of total polyphenols content (TPC)

The method described by Moualek et al. (2016) was used to determine the content of polyphenols, using gallic acid as a standard and the Folin-Ciocalteu reagent. 1ml of Folin-Ciocalteu reagent and (diluted ten times) was added to a tube containing 200 μl of aqueous extract. Then, after shaking the mixture for 3 minutes, 800 μl of Na\textsubscript{2}CO\textsubscript{3} (75 g/l) was added to the solution, and the tubes were incubated at 25 °C in the dark for 45 minutes. The assay was performed using a spectrometer, measuring the absorbance at 760 nm using Cary 60 UV-Vis spectrophotometer (Agilent, USA). The results are expressed as milligrams of gallic acid equivalents per one hundred g of extract (mg GAE/100 g of extract).

Antioxidant activity (%)

The method used to determine the antioxidant activity was recommended by Zemour et al. (2019) with slight modifications, and it is based on the spectrophotometric measurement of the reduction of the DPPH radical (2,2-diphenyl-1-picrylhydrazyle). In this method, 200 μl of extract was mixed with 800 μl of pure methanol and 2 ml of DPPH (0.5 mg/1 ml in pure methanol). After 15 minutes of incubation, the assay was performed at an absorbance of 517 nm using Cary 60 UV-Vis spectrophotometer (Agilent, USA). The antioxidant activity of the extract was calculated using the following formula:

\[
\text{Antioxidant activity (\%)} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100
\]

Determination of total flavonoid content

The total amount of flavonoids present in the leaf extract was determined using the aluminum chloride spectrophotometric assay according to Moualek et al. (2016). To perform this assay, 1 ml of the plant extract was mixed with 1ml of a methanolic solution containing 2 % aluminum trichloride (AlCl\textsubscript{3}). The mixture was then incubated for 10 minutes, and the absorbance of the reaction mixture was measured at 430 nm, using Cary 60 UV-Vis spectrophotometer (Agilent, USA) and methanol as a blank reference. A standard curve of quercetin was used to convert the absorbance readings into milligrams of quercetin equivalent per 100 g of extract.
Statistical analysis

All analysis were performed in triplicate. The results were presented as mean ± SD (standard deviation). A two-factor analysis of variance (ANOVA II) was conducted to assess the effect of drying and extraction methods on the biochemical quality of aqueous extracts of the strawberry leaves. The used statistical software was OriginPro2022.

3. Results and discussion

3.1 Yield of extract

According to the Table 1, the drying method significantly influenced the yield of the obtained extract ($p<0.05$). However, there is no effect of the extraction method on this parameter ($p>0.05$).

Table 1. Analysis of variance of the yield, total phenolic content (TPC) antioxidant activity (AA) and the total flavonoid content (TFC) of Arbutus unedo leaves aqueous extract

<table>
<thead>
<tr>
<th>Factors</th>
<th>Yield of extract</th>
<th>TPC</th>
<th>AA</th>
<th>TFC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extraction (E)</td>
<td>0.119ns</td>
<td>0.135ns</td>
<td>0.000***</td>
<td>0.12ns</td>
</tr>
<tr>
<td>Drying (D)</td>
<td>0.026*</td>
<td>0.039*</td>
<td>0.002**</td>
<td>0.11ns</td>
</tr>
<tr>
<td>Interaction D*E</td>
<td>0.231ns</td>
<td>0.091ns</td>
<td>0.000***</td>
<td>0.16ns</td>
</tr>
</tbody>
</table>

*ns* Non significant at $p>0.05$; * significant at $p<0.05$; ** significant at $p<0.01$; *** significant at $p<0.001$

The results showed that high yields were obtained from samples maintained on drying in the shade and filtered (2.3 %). Indeed, drying at 60 °C resulted in average values, with a yield of approximately 2.21% for filtration and 2.24 % for centrifugation, compared to other drying methods. However, the samples dried at 40°C exhibited the lowest yield, with values of 2.08% for filtration and 1.62% for centrifugation. It appears that the amount of extract yield increased with increasing temperature and deceasing time. It can be suggested that quick drying through thermal drying preserved the phenolics and other constituents from degradation via microbial attacks and enzymatic processes, hence increasing the yield (Anwar et al., 2013; Vanielie et al., 2019). The results of Anwar et al. (2023) revealed that the extraction yield from cauliflower (*Brassica oleracea*) was higher after drying at 40°C. However, oven drying at 60°C provided the optimum extract yield for the leaves of *Scurrula ferruginea* (Vanielie et al., 2019). Additionally, the extraction method had a significant influence on the final extract yield (Tenuta et al., 2020).

Table 2. The yield, total phenolic content (TPC) antioxidant activity (AA) and the total flavonoid content (TFC) of Arbutus unedo leaves aqueous extract

<table>
<thead>
<tr>
<th>Methods</th>
<th>Parameters</th>
<th>TPC (mg GAE/100 g of extract)</th>
<th>AA (%)</th>
<th>TFC (mg QE/100 g of extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extraction after maceration</td>
<td>Drying</td>
<td>Yield of extract (%)</td>
<td>2.21±0.09a</td>
<td>380.60±13.86a</td>
</tr>
<tr>
<td></td>
<td>At 60°C</td>
<td>380.60±13.86a</td>
<td>85.76±0.93c</td>
<td>326.58±17.04a</td>
</tr>
<tr>
<td></td>
<td>At 40°C</td>
<td>391.77±16.83a</td>
<td>81.60±0.75c</td>
<td>339.28±1.28a</td>
</tr>
<tr>
<td></td>
<td>Sun-drying</td>
<td>376.27±11.68a</td>
<td>81.60±0.75c</td>
<td>339.28±1.28a</td>
</tr>
<tr>
<td></td>
<td>Shade-drying</td>
<td>362.44±7.58a</td>
<td>82.57±1.17a</td>
<td>309.75±2.98a</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>2.19±0.03</td>
<td>377±6.35</td>
<td>81.91±0.94</td>
</tr>
</tbody>
</table>
Continued Table 2...

<table>
<thead>
<tr>
<th>Methods</th>
<th>Parameters</th>
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<tbody>
<tr>
<td></td>
<td>Zhao et al. 2006</td>
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**3.2 Polyphenols total content (mg GAE/100 g extract)**

The obtained results (Table 1) indicated that the polyphenols total content is affected by the drying method \( p<0.05 \). According to the results (Table 2), the extreme values of TPC were obtained for the leaves dried at 60 °C using the filtration method, with average values around 355.44 (mg GAE/100 g of extract) and 577.05 (mg GAE/100 g of extract) for the samples dried at 40 °C using centrifugation method respectively. Moreover, during the drying process under shadow and sunlight, the examined strawberry tree leaf extract showed TPC values of 362.44 and 376.27 mg GAE/100 g of extract after filtration, respectively. Similarly, using the same drying methods, the TPC values were approximately 393.41 and 372.56 mg GAE/100 g of extract after centrifugation.

Polyphenolic compounds are the most prevalent secondary metabolites present in the natural world. They display a multitude of distinct properties and exert various biological effects (Bié et al. 2023). To recover the maximum quantity of these compounds for ensuring their functional properties, the use of appropriate extraction methods would be recommended. In this regard, several studies highlighted that the extraction method affect significantly the quantity and quality of the polyphenolic compounds (Shi et al. 2022; García-Ramón et al. 2023). This study revealed that the optimum TPC resulted from a drying temperature of 40 °C. Here, García-Ramón et al. (2023) highlighted that when using 40 °C, the unripe avocado peel extracts exhibited the highest values of TPC (44.24 mg GAE/g peel dw), TFC (786.08 mg QE/g peel dw) and antioxidant capacity (564.82 μmTE/g peel dw).

According to our results, the extract of strawberry tree leaves showed significant levels of total polyphenols regardless of the drying and extraction methods used. Several studies have confirmed the importance of these compounds in this plant (Hmaidosh et al., 2020; Juříć et al., 2020; Erkekoglou et al., 2017; Bertsouklis et al., 2021). Therefore, the TPC was about 192 (mg GAE/g DW) and 836.51 (μg GAE/mg extract) in Algeria (Bakchiche et al., 2013; Laouicha et al., 2020), 32 (mg GAE/g DW) in Tunisia (Habachi et al., 2022) and 37.3 (g GAE/g of DW) in Morocco (Mrabti et al., 2017). A study conducted in Croatia revealed a composition ranging between 553 and 850 (mg GAE/100 g DW). However, this phytochemical composition can vary depending on environmental conditions (Miguel et al., 2014; Zemour, 2022). The extraction method has been found to be influential. Indeed, the extract of *Arbutus unedo* L. leaves showed varying levels when using ethanol extraction or boiling water extraction (Mrabti et al., 2017). Furthermore, the drying method can affect the variation of total polyphenol accumulation (Roslan et al., 2020; Sahin et al., 2017). According to Zhang et al. (2009), thermal drying was found to facilitate the release of cell wall phenolics or bound phenolics by breaking down cellular components. As a result, this process led to an increased yield of the extract.
3.3 Antioxidant activity (DPPH Radical Scavenging Assay)

The antioxidant activity (Table 1) of the strawberry tree leaf extract varies depending on the drying and extraction methods (p<0.05). Thus, a high activity was observed for the extract obtained after drying the leaves at 40°C and recovered by centrifugation (90.81 %), while the lowest activity (77.72 %) was recorded for leaves dried at a temperature of 40 °C and subjected to filtration after maceration. The average recorded antioxidant activity is approximately 81.91 % and 85.69 % for filtration and centrifugation, respectively. Oxidation is a chemical reaction that can produce free radicals, leading to chain reactions that can damage cells (Rassem et al., 2018; Chaudhary et al., 2023; Martemucci et al., 2022). This antioxidant activity has been previously reported in *Arbutus unedo* extract leaves by several scientific studies (Doudach et al., 2023; Ait lhaj et al., 2022; Habachi et al., 2022). Indeed, the maximum scavenging activity is about of 79.23 % (Moualek et al., 2016). Kachkoul et al. (2019) have founded that the aqueous extract of this species exerted a value of 202.64 µg/mL (IC₅₀). Strawberry tree components, particularly fruits and leaves, have been subjected to numerous and varied extraction procedures to obtain phenolics-rich fractions with high antioxidant power (Erdogan and Uysal, 2020; El Cadi et al., 2020; Lehfa et al., 2023). However, our research has revealed the effect of the drying method on the expression of this antioxidant activity, a finding consistent with other scientific studies (Złotek et al., 2021; Benjamin et al., 2022; Saifullah et al., 2019). In one of these studies it has been revealed that sun drying induced an increase in the antioxidant activity of bamboo extracts, with a higher value of 4.73 µg/mL compared to the lowest value estimated at 2.92 µg/mL, which was obtained through freeze-drying (Benjamin et al., 2022). Also, the DPPH radical scavenging activity exhibits higher value rather than at low temperatures (i.e. 50, 60 and 70°C) (Saifullah et al., 2019). It is interesting noted that Stephenus et al. (2023) revealed that the DPPH inhibition activity was higher when the drying temperatures were consistently increased from 40 to 50°C in *Phaleria macrocarpa* fruits extract.

3.4 Total flavonoid content (TFC)

The drying and filtration methods have no effect on the total flavonoid content of the studied extract (p>0.05). According to the results in Table 2, the estimation of flavonoid content revealed high values of 334.35 (mg QE/100 g of extract) and 489.13 (mg QE/100 g of extract) for the aqueous extract of leaves dried at 40 °C and extracted by filtration and centrifugation, respectively.

After filtration, the lowest flavonoid value is recorded for the batch dried in the shade (309.75 mg QE/100 g of extract). Similarly, after drying the leaves at 60°C and subjecting them to centrifugation after maceration, the flavonoid content remains low (327.38 mg QE/100 g of extract) compared to the other drying methods. As expected, this study highlighted the high amount of the flavonoid in this species. Previous study confirmed these results (Tenuta et al., 2020; Moualek et al., 2016). It has been demonstrated that these values may varies according to the extraction method (Tenuta et al., 2020). Generally, drying at 60°C could contribute to a decrease in the flavonoid content of the extracts. Sharma et al. (2015) and Stephenus et al. (2023) also suggested that this condition might be due to the degradation of flavonoids, as a result of the increased temperature.
According to Figure 3, a strong positive correlation was revealed between TPC and TFC (R=0.98). However, the TPC and TFC R-values of the Pearson correlation coefficient for the DPPH were 0.49 and 0.48 respectively. These positive correlations have been pointed up by numerous studies (Pande et al., 2018; Muflihah et al., 2021).

4. Conclusion

Algeria is one of the countries characterized by the richness and diversity of its flora, which plays a crucial role as a vast phylogenetic reservoir. For this reason, the study of its medicinal and nutritional importance remains of paramount importance. Therefore, the objective of this study was to evaluate the biological activity of the aqueous extract of Arbutus unedo L. leaves growing in the province of Tissemsilt (Western Algeria). The main parameters studied focused on the total polyphenol content (TPC) and its conjugated antioxidant activity (DPPH) and total flavonoid content (TFC). The results obtained revealed the richness of this species in polyphenols. It was demonstrated that the aqueous extract of strawberry leaves exhibited strong free radical reducing activity. This study also aimed to examine the effect of the drying method and the extraction method after maceration. It was found that the above parameters were influenced by the drying and extraction methods studied. In conclusion, due to its biological activity, this plant could play an important role in traditional and modern uses. Therefore, its use in nutritional, pharmaceutical, and cosmetic fields is recommended. On the other hand, drying at a temperature of 40°C and centrifugation are essential steps that could play a critical role in conducting scientific research involving an aqueous plant extract.

Acknowledgement

We would like to thank the Dean of the faculty and all the personnel of the Laboratory of the Faculty of Sciences and Technology at Tissemsilt University (Algeria).

Author’s declaration

The authors declare no conflict of interest.

ZK, CKMA, ATA and LM carried out laboratory work and analysed data. ZK, LA, MM, MB and ZH advised about the laboratory technique and conducted manuscript proofreading before submission. All authors read and approved the final version of the manuscript.
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