**Research Paper**

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# Evaluation of antioxidant activity and the phenolic composition of Syrian Arbutus andrachne L.

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Arbutus andrachne L. (Grecian Strawberry tree) is widespread in the Mediterranean and the Black Sea Regions. This study aimed at determining total phenolic content and antioxidant activity of Arbutus andrachne parts (flowers, leaves, bark and fruit) collected from different parts of the Latakia Province (Syria). Our work is the first to conduct this experiment on the selected plants in Syria.

The fresh samples were extracted using ethanol 50% as an extraction solution, then the total contents of the phenolic compounds were determined spectrophotometrically using the Folin-Ciocalteu reagent. The results showed higher phenolic contents in flowers (38.32 mg/g) than any other parts. Antioxidant activity was determined by FRAP (ferric reducing antioxidant power) method, and the results were in accordance with those obtained with the Folin-Ciocalteu method, which the general trend of the antioxidant activity FRAP in the botanical parts was similar to the total phenolic contents, which flowers have an antioxidant activity value (19.35 μMFe2±/g) that exceeds the other parts.ANOVA analysis showed significant differences in the contents of phenolic compounds between the four parts of Arbutus andrachne L. ($p<0.05$). Therefore, a positive correlation was found between total phenolic contents and antioxidant activity for Arbutus andrachne L. ($R2=0.952$).

## 1. Introduction

Medicinal plants are the richest biological resources of traditional medicine systems, nutritional supplements, herbal medicines and pharmaceuticals (Handa et al., 2008). The World Health Organization has confirmed that traditional medicine still has a primary role in health care, especially primary health care, as it is estimated that 60% of the world's population and 80% of the population in developing countries depend on traditional medicine (Gairola et al., 2010).

Phenolic compounds are the most effective antioxidants in food as they have many health effects (Cox et al., 2005). The antioxidant activity of plant products is mainly attributed to phenolic compounds (Chua et al., 2008). Hence, the importance of a diet rich in fruits with high contents of natural antioxidants is due to its therapeutic value (Sokol-Letowska et al., 2007), and their ability to neutralize free radicals (Stratil et al., 2007).

Free radicals are continuously generated as a result of normal metabolic processes in the body and are

sometimes produced by immune system reactions to bacterial and viral infections (Stratil et al., 2007). Due to the growing concerns about the potentially toxic effects of synthetic antioxidants, consumers have turned to consume natural antioxidants coming from food and diet. Thus, several studies have focused on new antioxidants to be chosen in typical foods (Mohd Azman et al., 2016).

The Eastern strawberry tree, Arbutus andrachne L., is a medicinal evergreen, small tree naturally distributed in the Mediterranean region and Southwestern Asia (Davis, 1982). Arbutus andrachne L. also called Greek Strawberry-tree, and Qayqab in Arabic is one of the Syrian medicinal species (Aljabari et al., 2014). This evergreen tree belongs to the Ericaceae family. Several plant species belonging to the Ericaceae family have been identified due to their medicinal properties within the list of species that are included in the composition of herbal medicines (Guendouze-Bouchefa et al., 2015).

Traditionally, Arbutus andrachne L. leaves are used as an antiseptic, anti-diarrheal, treatment for urinary infections and astringency, and its depurative properties and against some cancer types (Sakar et al., 1991). The fruit also has diuretic and laxative effects (Fonseca et al., 2015). The fruit has been customarily eaten in Spain and other Mediterranean countries, and they are known for their richness in numerous nutrients, especially calcium, phosphorus and potassium (Aljabari et al., 2014). Phenolic compounds are commonly found in the fruits, leaves and roots of the Arbutus andrachne L. like phenols, catechins, quercetin, rutin and myricetin. Subsequently, leaves and flowers recorded antimicrobial and antioxidant activities (Ergun et al., 2014). However, scientific literature confirming the effects of Arbutus andrachne L. fruit is minimal, and only a few studies from Middle Eastern countries have been published about the Arbutus andrachne L. components and their biological effects (Sakar et al., 1991; Serce et al., 2010; Tawaha et al., 2007).

The present study was directed towards the identification of antioxidant ability of ethanolic extracts from the Arbutus andrachne L. accessions sampled from the province of Latakia in Syria.

## 2. Material and Methods

### 2.1. Plant material

Plant materials of Arbutus andrachne L. (leaves and flowers) were collected in Spring of 2016 (100 g of each part), the bark was collected in the Fall of the same year (100 g), and fully ripe fruit with a healthy external appearance was collected in the winter of the same year (200 g) from different sites (10 sites graduated above sea level from 600 m to 900 m) of Latakia Province in Syria.

A total of 30 samples were collected from the studied sites (3 samples from each site to form a compound sample). Samples were packed in plastic bags and carried to the laboratories in a cold system within a day (stored at - 20 °C until analysis).

### 2.2. Extract preparation

1 g of each part (leaves, flowers, and bark) were taken from Arbutus andrachne L. and were appropriately sliced to facilitate the extraction process. The fruits of the Arbutus andrachne were juiced according to the method by Mezadri et al. (2008), where the fruits were pressed with a homemade machine and then filtered through filter paper. The juice was refrigerated at -20°C until the analysis was performed. The juice was directly used when determining the total content of phenolic compounds after its extraction so that the concentration of phenolic compounds was within detectable limits for a standard gallic acid series. At the same time, the other plant parts were extracted using ethanol 50% (because of its extraction ability and inexpensiveness) at a temperature of 70°C for 30 min from the start of boiling, with a 600 rpm.

### 2.3. Determination of total phenolic content

The phenolic contents of the extracts were determined using the Foline-Ciocalteu reagent according to the method previously reported by Slinkard and Singleton (1977) using gallic acid as a standard. The results are expressed as follows: the number of milligrams of gallic acid equivalent to phenolic compounds present in 1g of fresh plant extract (mg GAE/g). All experiments were carried out in triplicates, and the results were expressed as mean ± standard deviation.

### 2.4. Determination of antioxidant capacity FRAP assay


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Ferric reducing antioxidant power (FRAP) assay was used to measure the concentration of total antioxidant. An intense blue colour appears when the TPTZ-Fe3+ complex reduces to the TPTZ-Fe2+form (2,4,6-tripyridyl-s-triazine) in the presence of antioxidants. The reduction occurs rapidly with all reductants with half-reaction reduction potentials above that of Fe3+/Fe2+ (Pellegrini et al., 2003).

FRAP assay were done with FRAP reagent (i.e. 1 mM2,4,6-tripyridyl-2-triazine [TPTZ]) and 20 mM ferric chloride in 0.25 M sodium acetate, pH 3.6. 100 ml of extract were added to 1ml of FRAP reagent and mixed thoroughly. After standing at ambient temperature (20 °C) for 4 min, absorbance at 593 nm was measured against a water blank. Calibration was against a standard curve (100–600 mM ferrous ion) produced with freshly prepared ammonium ferrous sulphate. All experiments were carried out in triplicates, and the results were expressed as mean ± standard deviation.

## 3. Statistical analysis

ANOVA contrast analysis was performed to compare the significant differences between the averages of phenolic and antioxidant values using Least Significant Difference (LSD) at the level 5% using the statistical analysis program SPSS (Version 16.0). Graphs were created using Microsoft Excel. The average of the three values (n=3) for each sample was calculated. Results were presented as means ± standard deviations.

## 4. Results and Discussion

### 4.1. Total phenols and antioxidant activity for Arbutus andrachne L.

Fresh plant material was chosen instead of dry plant material to maintain the sensitivity of the phenolic compounds. Dry plant material is at risk during the drying process and can result in a loss of the compounds but can provide results that are more accurate in terms of concentration levels (Bruneton, 1999). While reference studies used dried plant material, this is the first to use fresh plant material to determine the phenolic content of an Arbutus andrachne L.
The concentration of phenolic compounds is graded first from the flowers that recorded the highest content (38.32 mg/g), followed by the leaves (37.25 mg/g), then the bark (36.93 mg/g) and finally the fruit (3.62 mg/g) (Table 1). Our results were consistent with the results of Serce et al. (2010), who reported that the content of Arbutus andrachne fruits of phenolic compounds was 2.4 mg/g in fresh weight (fw). While Saral et al. (2017) reported that the phenolic content of Arbutus andrachne fruits was 7.29 mg/g of fw.

On the other hand, other studies that used dry plant materials indicated that the highest phenolic content of Arbutus unedo L. was in leaves (119.97 mg/g dw) (Saral et al., 2017), (197.16 mg/g dw) (Orak et al., 2011), (179 mg/g dw) (Guendouze-Bouchefa et al., 2015) and (207.84 mg/g dw) (Moualek et al., 2016). Besides, a study by Saral et al. (2017) stated that the concentration of phenolic compounds in Arbutus andrachne flowers was 43.27 mg/g of dw. Regarding phenols in bark, a study by Abidi et al. (2016) found that bark of Arbutus andrachne is rich in phenols (416.15 mg/g dw).

**Table 1.** The phenolic compounds and antioxidant activity for Arbutus andrachne L. using the FRAP method (n=3).

| | **Phenolic content**<br>**mg GAE/g** | **The antioxidant activity**<br>**µMFe$^{2+}$/g** |
|---|---|---|
| Flowers | 38.32±0.04 [a] | 19.35±0.06 [a] |
| Leaves | 37.25±0.04 [b] | 16.81±0.04 [b] |
| Bark | 36.93±0.01 [c] | 14.33±0.06 [c] |
| Fruit | 3.62±0.05 [d] | 1.02±0.02 [d] |
| LSD | 0.07 | 0.57 |
| Similar letters indicate that there is no significant difference, while different letters indicate a significant difference. | | |

The difference in the results of the mentioned studies with the current study can be attributed to the solubility of phenolic compounds governed by the physical statues of the used material (using dry compared to fresh materials), solvent type, the studied plant type, the studied plant part, in addition to the differences in geographical locations, and the extraction protocol (Saral et al., 2017). As noted by Panico et al. (2009), the role of environmental conditions such as heat, light intensity and soil composition in the accumulation of phenolic compounds found in strawberry fruits helps explain the difference in these results.

The antioxidant activity was calculated using the FRAP method for the ethanolic extracts prepared from the parts (flowers, leaves, bark and fruit) of the Arbutus andrachne L. From the results in Table (1) flowers have an antioxidant activity (19.35 µMFe2±/g) that exceeds the antioxidant activity of the leaves (16.81 µMFe2 ±/g), bark (14.33 µMFe2±/g) and fruit (1.02 µMFe2±/g). A significant difference was observed using ANOVA analysis in the total phenolic content of plant parts (flowers, leaves, bark and fruit) ($p<0.05$). Also, there was a significant difference in the antioxidant activity by the FRAP method for the plant parts of the Arbutus andrachne L.

Table (2) shows some reference studies that dealt with the antioxidant activity of some plant species belonging to the Ericaceae family, which differ by some values from the current study for several reasons, including the solvent used, the studied plant parts, and the difference of the plant species. Other studies indicated that flowers of Arbutus andrachne L. were superior in their antioxidant effectiveness compared to leaves (Okmen, 2015), and Arbutus unedo L. flowers outperformed their phenolic content and their antioxidant efficacy over fruits (Isbilir et al., 2012). This result is confirmed by the study of (Saral et al. 2017), which showed the superiority of A. andrachne L. flowers with their phenolic content and their antioxidant effectiveness (FRAP) over fruits.

### 4.2. The relationship between total phenolic content and FRAP antioxidant efficacy

The relationship between the contents of the plant parts of Arbutus andrachne L. of phenolic compounds and the antioxidant activity of the FRAP method is represented by a graph (Fig 1), based on the previous

**Table 2.** Reference studies on the antioxidant efficacy of some Ericaceae species plant extracts using FRAP method.

| Reference | Plant | solvent used | antioxidant activity (FRAP) |
|---|---|---|---|
| Pavlovic et al., 2009 | Arbutus unedo L. (Leaves) | Ethanol 70% | 5.11 µMFe$^{2\pm}$/g |
| | Erica arborea L. (Leaves) | | 3.55 µMFe$^{2\pm}$/g |
| | Erica carnea L. (Leaves) | | 3.49 µMFe$^{2\pm}$/g |
| Saral et al., 2017 | Arbutus andrachne L. (Fruit) | Methanol | 3.41 µMFe$^{2\pm}$/g |
| | Arbutus andrachne L. (Flowers) | | 104.81 µMFe$^{2\pm}$/g |
| Ruiz-Rodriguez et al., 2011 | Arbutus unedo L. (Fruit) | Ethanol | 9.86 µMFe$^{2\pm}$/g |
| Serce et al., 2010 | Arbutus andrachne L. (Fruit) | Aqueous | 22.4 µMFe$^{2\pm}$/g |


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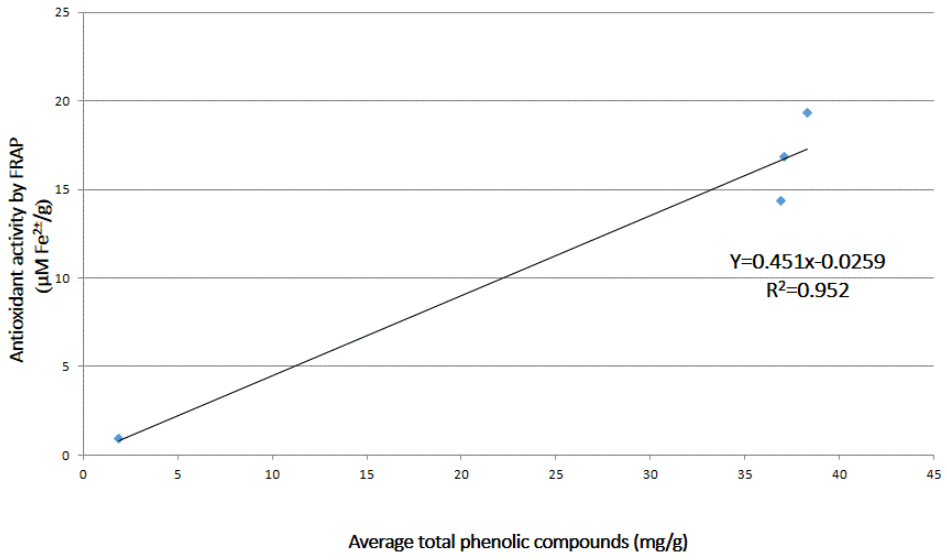


**Figure 1.** Increased antioxidant activity of plant parts for Arbutus andrachne L. FRAP method with increasing concentrations of phenolic compounds.

results presented in Table (1). A strong correlation is observed between the concentration of phenolic compounds and the antioxidant activity as shown in Figure (1), so it can be said that the more phenolic compounds in the plant part, the higher its antioxidant activity.

It is known that phenolic compounds differ from each other with their antioxidant effectiveness, and many studies have found a strong positive correlation between the total content of phenolic compounds and the antioxidant effectiveness (Bilto et al., 2015). This correlation is consistent with the current study, as the correlation between the total contents of phenolic compounds and the antioxidant effectiveness of the studied plant was strong, as the R2 coefficient reached a value equal to 0.952 by the FRAP method for Arbutus andrachne L. (Figure 1)

Finally, many studies have indicated that the physiological function of natural foods can be attributed to the antioxidant capacity of the phenolic compounds present in it (Hamzaa et al., 2012).

## 5. Conclusion

This study confirms that the flowers of A. andrachne L. contain high amounts of phenolic compounds (38.32±0.04 mg GAE/g), followed by leaves (37.25±0.04 mg GAE/g) and bark (36.93±0.01 mg GAE/g). The general trend of the antioxidant activity (FRAP) in the botanical parts was similar to the total phenolic contents, which flowers have an antioxidant activity value (19.35±0.04 µMFe2±/g) that exceeds the rest of the parts.

A positive correlation was found between total phenolic contents and antioxidant activity for Arbutus andrachne L. (R2=0.952).

## Conflict of interest

The authors hereby declare that there is no conflict of interest.

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