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CHARACTERISTICS, NUTRITIVE VALUE AND ANTIOXIDANT CONTENT OF THE LIBYAN ENDEMIC (*Arbutus pavarii* Pamp.) STRAWBERRY TREE FRUITS

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ABSTRACT
Arbutus pavarii Pamp. (Ericaceae) is one of the endemic species in Libya. Samples of the non-ripened and ripened fruits of Arbutus pavarii Pamp. were collected from its natural habitat (El-Gabel El-Akhdar, Libya).Fruit characters like fruit size, weight, fruit mass and seeds per fruit were estimated. The vitamin content of A, C and E was estimated using different methods. The total phenolic and flavonoids content of the fruits was determined spectrophotometrically. The antioxidant activity was assessed using a modified quantitative (DPPH) assay at two maturation stages of fruits (green and red). The results were discussed according to the nutritional value of the fruits.

KEY WORDS: *Arbutus pavarii; antioxidant activity; DPPH; phenolics and flavonoids.*

INTRODUCTION

Arbutus pavarii Pamp. (Ericaceae) is one of the endemic species in Libya and it distributes naturally as wild plant in El-Gabel El-Akhdar area, which characterized with Mediterranean climatic conditions **(Elshatshat 2009; Elshatshat *et al* 2009; and Elabidi and Elshatshat 2017).** It is evergreen shrub or small tree, 1.5 to 3 m tall with reddish brown peeling bark. The flowering season appear from late October to February and the flowers are a good source of nectar for bees. Because of its nutritional and medicinal value, *A. pavarii* Pamp. honey is widely used for folk medicinal purposes **(El abidi and Elshatshat 2017),** in addition, other honey types which collected from other plant species **(Elshatshat and Elsilini 2017).**

The fruit takes around 8 months to ripen, and they are spherical and warty, and turn from yellow to orange to scarlet as the autumn progresses (figure 1). The strawberry fruits are edible directly as fruits or can be made into jam but the taste is somewhat insipid **(Elshatshat 2009).**

Increasing phenolic compounds in some native plants is one of the strategies of these species to avoid drought stress in arid and semi-arid zones like Libya. According to many studies, the phenolic compounds of the leaves and fruits of *Arbutus* genus, especially *Arbutus unedo* L. were reported, identified and investigated (**Ayaz*, et al.* 2000**; **Fortalezas*, et al.* 2010**; **Guimarães*, et al.* 2013**; **Hamad*, et al.* 2011**; and **Pawlowska*, et al.* 2006)**. In addition, the antioxidant properties and activities (**Isbilir*, et al.* 2012; Mendes*, et al.* 2011)**.

Because of its endemism and lack of information about the fruits of *Arbutus pavarii* Pamp., this work was conducted to shade some light on the fruit characters and the characterization of the antioxidant composition at different fruit ripening stages.

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Figure 1; Fruits of *A. pavarii* Pamp. in different stages.

MATERIALS AND METHODS

Plant material: Fruit samples of *Arbutus pavarii* Pamp. were collected from its natural habitat (El-Gabel El-Akhdar, Libya) in June and November for the non-ripened and ripened fruits, respectively.

Fruit parameters estimation: For ripened fruit parameters, sampling was conducted according to Molina et al. (2011) with some modifications, when fruits were already ripe and the plants reached their peak fruit densities. The following parameters were estimated; fruit size and weight, number of fruits per main branch, number of fruits per tree, fruits weight per branch and per tree(Kg). Mean fruit mass was determined by weighing 100 randomly ripe fruits. Fruit mass was expressed in fresh weight units.

CHEMICAL ANALYSIS

Antioxidant activity: The antioxidant activity was assessed using a modified quantitative 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay **(Amić, *et al.* 2003, Phang, *et al.* 2011).** The DPPH was prepared at a concentration of 0.004 % in methanol (HPLC grade). The alcohol extracts of the fruit of *Arbutus pavarii* Pamp. was dissolved in 50% methanol at a concentration of 0.1, 0.2, 0.4, 0.6 and 0.8 mg/mL for both plants.

Sample of the tested solution, 200 µl, was added to 6 mL DPPH solution. Blank was carried out using 200 µl methanol and 6 mL DPPH solution. Solutions were incubated in dark for 30 minutes at room temperature. The absorbance of each sample was measured against methanol at 517 nm. Gallic acid was used as a standard control at a concentration of 0.005, 0.01, 0.02, 0.04, 0.06 mg/ml. All experiments were carried out in triplicate. The scavenging activity of the samples was calculated as a percentage of free radical inhibition (I %) according to the following formula and each measurement was performed in triplicate:

$$I\% = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

Where; A_{blank} is the absorbance of the control reaction, A_{sample} is the absorbance of the extract

Vitamin A: The content of β-carotene (pro-vitamin A) was estimated according to **(Nagata and Yamashita 1992)**. The dried powdered plant sample (1 g) was vigorously shaken with a mixture of acetone-hexane (4:6, 10 mL), and filtered. To determine the *β*-carotene content, the absorbance of the filtrate was measured at four different wavelengths 453, 505, 645 and 663 nm and its amount deduced from the following:

$$\beta\text{-carotene (mg/100 mL)} = 0.216\ A_{663} - 1.22\ A_{645} - 0.304\ A_{505} + 0.452 A_{453}$$

Finally, the corresponding vitamin A content was calculated as following:

$$\text{Vitamin A (IU/100 g)} = (\beta\text{-carotene concentration})/0.6$$

Vitamin C: Extraction of vitamin C from the powdered sample (1 g) was carried out with 0.05 M solution of oxalic acid for 24 hours and away from light. The extract was then filtered, and an aliquot amount of the filtrate (2.5 mL) transferred to an amber-coloured volumetric flask and treated with oxalic acid (0.05 M solution, 2.5 mL), metaphosphoric acid / acetic acid solution (0.5 mL), sulphuric acid (5% solution, 1 mL) and ammonium molybdate (5% solution, 2 mL). The absorption of the blue color produced was measured at 760 nm, and the vitamin C content in the investigated sample calculated from a pre-established standard curve**(Hussain, *et al.* 2010).**

Vitamin E: The spectrophotometric estimation of vitamin E was performed by adopting the method of **(Nasar, *et al.* 2009)**, based on Emmorie-Engel reaction. Tocopherols reduce ferric ions to ferrous ions, which upon the addition of α, α'-dipyridyl

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produces a red coloured complex. Tocopherols and carotenes were extracted from the powdered herbal material with petroleum ether and the absorbance of the extract measured at 450 nm. A correction was made for carotenoids by addition of $FeCl_3$ and the absorbance of the solution recorded after 90 seconds, at 520 nm. The concentration of vitamin E (mg/100g dry wt) was calculated as follows:

$$\textbf{Vitamin E (mg / 100 g)} = \frac{\textbf{AT}-\textbf{AC}}{\textbf{AS}}$$

Where, AT, AC and AS represent the absorbance of test, carotene and standard samples, respectively. Taking in consideration that the IU of Vitamin E is the biological equivalent of either 2/3mg dl-α-tocopherol or 1mg of dl-α-tocopherol acetate; therefore, the concentration of Vitamin E in IU/100 g estimated as dl-α-tocopherol can be deduced from the following equation:

$$\textbf{Vitamin E (mg/100g)} = \frac{\textbf{Vitamin E (IU/100g)}}{\textbf{0.67}}$$

Total Phenols and Flavonoids: The total phenolics and flavonoids content of the fruits was determined spectrophotometrically at 765 nm following the Folin-Ciocalteu method. A standard calibration curve was plotted using gallic acid (Merck, Germany) in the concentration range 1–500 mg/L.

Changes in antioxidant activities and total phenolic content: The antioxidant activity at two maturation stages (green and red) have been examined. The hydrogen donating and/or radical-scavenging capacity of the samples was evaluated by their ability to scavenge the free radical DPPH. For each extract, five dilutions in methanol were prepared (1.93–9.67 mg/L). 2 mL of each dilution were added to 0.15 mL of a 10−3 M DPPH methanolic solution and maintained in the dark at room temperature for 1 h. The absorbance was measured at 517 nm versus a blank prepared without extract.

RESULTS AND DISCUSSION

The results of fruit characters and parameters are presented in table (1). The mean value of fruit size was (2.5 ± 0.45) cm and the weight was (6.7 ± 0.66)g while the mean value of fruit mass was (4.5 ± 0.23)g. On the other hand, the quantitative characters of the fruit production were differed in their values as they shown in table(1).

Table (1): The fruit parameters of ripe fruit of *A. pavarii* Pamp. .values are means with ± SD.

Fruit parameter	Mean value	± SD
Fruit size(cm)	2.5	± 0.45
Fruit weight(g)	6.7	±0.66
Fruit mass (g)	4.5	± 0.23
No. of fruits per branch	91	± 11
No. of fruits per tree	1872	± 257
Fruits per branch (kg)	0.610	± 0.05
Fruits per tree (kg)	8.34	± 1.53
Number of seeds per fruit	11.6	± 1.21

From the results, it is clear that the fruit characters and parameters in this study are appeared at the same levels(with some non-significant differences) and were agreed to large extant with those which reported by Molina *etal,* (2011) and Markovski (2017) for the strawberry trees *A. unedo* and *A. andrachne*, respectively.

Even though, *A. pavarii* is endemic to Libya, but it has the same fruit characters of other species which belong to the same family and the same eco-region (Mediterranean). This might reflect and exhibited geographical and climatic environmental factors, especially, annual rainfall variation in the Mediterranean region and their effect on the fruit characters.

The results of both ripe and unripe fruits were differed in their vitamins contents. But, revealed high amounts of most of them in both fruit stages as they listed in Table 2.

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Table (2):Vitamin contents of the fruit of *Arbutus pavarii* Pamp. RDA = Recommended dietary allowances (Tobias 1995, Sweetman 2002)

Vitamin	the ripe fruit	the unripe fruit	RDA
Vitamin A	1057.83 IU/ 100g	900 IU/ 100g	700-1300 IU/d
Vitamin C	120 mg/100g	250 mg/100g	65-115 mg/d
Vitamin E	33 mg/ 100g	300 mg/100g	4-19 mg/d

Vitamins are essential dietary components; which are necessary for optimal body growth and health. From the previous data, it could be concluded that, the aerial parts of the fruit of *Arbutus pavarii* Pamp. contains appreciably high amounts of the two fat-soluble antioxidant vitamins, A and E in the ripening stage exceeding the amount in the unripening stage. The fruit also showed the presence of tocopherols (vit E) with lower concentration(235 mg/kg) than of the current study **(Barros *, et al.* 2010)**.

Previous studies in *A. unedo* fruits showed very variable amounts of vitamin C, and only a few studies reported values of ascorbic acid in fresh fruits **(Alarcao-E-Silva*, et al.* 2001)**. The analysis showed vitamin C levels considerably high ranging from 120- 250 in the different stages. Studies performed by **(Alarcao-E-Silva *, et al.* 2001)** demonstrated the importance of harvest date and location on the amounts of total vitamin C.

The results revealed that the total phenolic content was highest at the red stage (457.59 mg/g) followed by the green stage (163.6mg/g)**.** On other hand, the contents of the flavonoid are similar with slight difference at the two stages (Table 3).

Table (3): Total phenolics and flavonoids in the fruit of *Arbutus pavarii* Pamp. The results are expressed as milligrams of gallic acid and rutin equivalents per 100 g of fruit.

Stage of *A.pavarii* Pampan fruit	Total flavonoids mg/g	Total phenolic mg/g
Unripe fruit (green stage)	35.1	163.6
Ripe fruit (red stage)	34.2	457.59

Changes in antioxidant activities and total phenolic content of *Arbutus pavarii* Pamp. fruit at different maturation stages (green and red) have been examined. Studies related to this point are minimal, if not non-existent. The IC50 value is the effective concentration which is required to decrease the initial DPPH concentration by 50% and the lower EC50 value reflects the better protective action. Ripe fruit extract had the lowest EC50 value. According to statistical evaluation, in fruit ripening period, the average EC50 value of DPPH scavenging activities is the following order: ripe fruit higher than the unripe. Our results for red fruits are agreement with **(Oliveira *, et al.* 2011)**, who explained the lowest EC50 value of DPPH in ripened fruits.

The results showed a good correlation between the antioxidant activity and vitamin A, E and C and phenolic contents (Table 4).

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The antioxidant activity of ripe and unripe fruits of *Arbutus pavarii* Pamp. against gallic acid in terms of IC_{50} ± SD

Sample	DPPH % Inhibition*
Alcoholic extract of ripe fruit of ***A. pavarii*** **Pamp.**	0.045 ± 0.01
Alcoholic extract of unripe fruit of ***A. pavarii*** **Pamp.**	0.066 ± 0.02
Gallic acid	0.03± 0.002

Increasing phenolic compounds and antioxidants in *A. pavarii* Pamp. is one of strategies which this plant take to avoid drought stress in El-Gabal Al-akhdar area that characterized with semi-arid climatic conditions. This lead to increase the nutritive value of the fruits of this endemic plant. The chemical composition of fruits, in which phenolic acids, flavonoids and vitamins are present, may be responsible for the reported biological properties, especially as antioxidant. All of these have suggested as a main reason to increase the utilization of fruits and/or derivatives in human nutrition in the future as source of bioactive phytochemicals. In addition, as one of natural resources of industry of these compounds.

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