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Nutritional, phytochemical and antioxidant evaluation and FT-IR analysis of freeze dried extracts of *Ecballium elaterium* fruit juice from three localities

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Abstract

This study was designed to investigate chemical composition, the total phenolic content, flavonoid content, antioxidant activity and to analyze through FT-IR spectroscopy method the freeze-dried extract of *Ecballium elaterium* fruit from three different localities. The highest level of phenolic and flavonoid contents was recorded for the fruit juice from the Cap-Bon region, with 106.4 ± 0.4 mg GAE/g and 6.5 ± 0.2 mg QE/g, respectively. Antioxidant activity varied in dose-dependent manner with IC_{50} values for DPPH scavenging of the freeze-dried fruit juice extracts from Cap-Bon, Kef and Sidi Bouzid were 38.6 ± 0.2, 50.1 ± 0.7, and 50.7 ± 0.2 µg/mL, respectively. The results from the FRAP test showed that the freeze-dried extracts of Cap-Bon exhibited potent activity, followed by those from Kef and Sidi Bouzid. Similar trend were revealed for $ABTS^{\bullet+}$ test, with the fruit juice extract from Cap-Bon (IC_{50} = 0.6 ± 0.0 mg/mL). Furthermore, a good positive correlation was observed between the total phenols and three assays, especially DPPH. The freeze-dried extracts of fruit juice from Cap-Bon showed strong ability to act as antioxidants and can be considered as promising natural source of bioactive compounds. FT-IR analysis of each freeze-dried extract confirmed its richness on polyphenols and biological active functional groups.

Keywords: *Ecballium elaterium*; chemical composition; antioxidant; FT-IR analysis.

Practical Application: *Ecballium elaterium* have been used as a natural medicine in Mediterranean and African countries.

1 Introduction

Fruits and vegetables are important sources of vitamins, dietary minerals, fibers, and antioxidative compounds. They are a rich source of biologically active compounds, known as phytochemicals, which are an essential and beneficial part of the human diet. Antioxidants are abundant phytochemicals that prevent some of the processes involved in the development of various degenerative disorders and diseases, including cancer and cardiovascular diseases (Denny & Buttriss, 2007). Recent research has, therefore, become increasingly interested in the search for natural antioxidants from natural origins for use in the treatment and control of several human health disorders and diseases (Ding et al., 2010).

Ecballium elaterium, a wild plant species belonging to the cucurbitacea family, has traditionally been used for various medicinal purposes. The plant, commonly known as squirting cucumber, is widely distributed in the Mediterranean region (Greige-Gerges et al., 2007). It is a perennial, fleshy, rough hairy plant with 30-100 cm long stems and greenish-yellow flowers (Salhab, 2013). It has large, juicy, berry-like, and ovate-oblong fruits that detach themselves explosively at maturity, scattering seeds and juice. *E. elaterium* is well known as a medicinal plant in Tunisia, frequently consumed in infusion, mixture of fruit or even in aerosol in cases of fever or flu (Bizid et al., 2014). This plant has been widely used in traditional medicine to treat various health disorders and diseases, including constipation, rheumatism, and jaundice (Toker et al., 2003; Attard et al., 2005; Bohlooli et al., 2012), hepatoprotective and proapoptotic effect (El Naggar et al., 2015). The fresh juice fruit has also been applied into the nostrils for the treatment of sinusitis, chronic jaundice and rheumatism (Jaradat et al., 2012; Sargin et al., 2013; Salhab, 2013). The fruit juice from this plant has been reported to be rich in lipids, proteins, sugars, and minerals (Greige-Gerges et al., 2007) and to contain several bioactive compounds, such as triterpenoïdes (cucurbitacins), carbohydrates, tannins, gum and peptides (Attard & Attard, 2008). The major active compound in this juice, cucurbitacin B, has been reported to exhibit attractive anti-ulcerous and anti-inflammatory properties (Agil et al., 1999).

Despite the large flow of data on the promising potential of *E. elaterium*, to the authors' knowledge, no study has so far investigated the physicochemical composition and antioxidant ability of the fruit juice from *E. elaterium*. Accordingly, the present study was undertaken to determine the physicochemical composition and total phenolic and flavonoid content of freeze-dried extracts of *E. elaterium* fruit juices from various

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Tunisian origins. The antioxidant properties of the extracts were also evaluated using 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2, 2'-azinobis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), and ferric reducing power (FRAP) assays. FT-IR spectrum profiles of various solvent extracts were analyzed to evaluate the IR finger print in different solvent extracts.

2 Materials and methods

2.1 Chemicals and reagents

DPPH (2,2-Diphenyl-1-picrylhydrazyl), quercetin, trichloroacetic acid, ascorbic acid, ferric chloride ($FeCl_3$), BHT (butylated hydroxytoluene), ferrous sulfate ($FeSO_4$), aluminium chloride ($AlCl_3$), Folin–Ciocalteu reagent, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid), sodium carbonate (Na_2CO_3), potassium ferrocyanide (K_3 Fe $(CN)_6$), potassium persulfate ($K_2S_2O_8$), ethanol, and methanol were purchased from Sigma-Aldrich (USA). All chemicals and solvents were of the analytical grade.

2.2 Plant materials and extraction

Fruits juices of *E. elaterium* were obtained from mature fruits collected in June 2013 from three regions in Tunisia: Cap-Bon, Kef and Sidi Bouzid, at different altitudes (Table 1). After the fruit specimens were authenticated by a recognized botanist, they were deposited at the local herbarium of the biology department of the faculty of Sciences, Sfax, Tunisia. The juices were squeezed from the fruits and then filtered through Whitman paper. A clear juice was obtained, which was then lyophilized using freeze-dryer at a temperature of −50 °C and a pressure of about 121 mbar through a lyophilizer lab (CRIST, Alpha 1-2 LD plus, Germany) and then stored at −20 °C for further use.

2.3 Chemical analysis

The protein concentration in the juice was determined by the Kjeldahl method in accordance with the AOAC method number 984.13 (Association of Official Analytical Chemists, 2000). Crude protein content was estimated by multiplying the total nitrogen content by the factor of 6.25. Crude fat content was determined gravimetrically after the Soxhlet extraction of dried samples with hexane. The moisture and ash contents were evaluated according to the AOAC standard methods 930.15 and 942.05 (Association of Official Analytical Chemists, 1999a, b), respectively. Carbohydrate levels were calculated by subtracting the total sum of protein, fat, ash and moisture from 100% dry weight sample (Judprasong et al., 2013). The fruit juice energy value (expressed in kJ) was estimated by multiplying the percentages of protein, fat and carbohydrate by the factors 16.7, 37.7 and 16.7, respectively (Association of Official Analytical Chemists, 1990).

2.4 Determination of mineral contents

Analyses of mineral contents in freeze-dried extracts were carried out using an Atomic Absorption Spectrometer (Perkin Elmer A Analyst 200) according to the method of Association of Official Analytical Chemists (1999c). Sample (1 g) of each extract was dissolved in 1 mL of 70% nitric acid. Then it was transformed to a volumetric flask and diluted to 100 mL with deionised water. The solution was then subjected to analysis. Sample content of each element were determined by comparing absorbance to a standard linear regression curve from standard solutions. The concentration of minerals was calculated and expressed as mg/100 g.

2.5 Total phenolic content

Total phenolic content was determined using the method of Li et al. (2007). Two hundred µL of diluted sample were added to 1 mL of diluted Folin-Ciocalteu reagent (diluted ten times with distilled water). After 4 min, 800 µL of Na_2CO_3 (75 g/L) were added. Absorbance was measured at 765 nm after 2 h of incubation at room temperature. Gallic acid was used for the standard calibration curve. The results were expressed as mg of gallic acid equivalents (GAE) per gram of extract (mg GAE/g). All tests were performed in triplicate.

2.6 Total flavonoid content

Flavonoid content was determined as described by Bahorun et al. (1996). The quercetin calibration curve was prepared by mixing 1 mL of samples and quercetin methanolic solution with 1 mL of aluminium trichloride solution (2% in methanol). After 10 min, absorbance was measured at 415 nm. The results were expressed as mg of quercetin equivalents (QE) per gram of extract (mg QE/g). All samples were prepared and analyzed in triplicate.

2.7 Antioxidant activity assay

DPPH free radical scavenging activity: The DPPH radical scavenging activity of the extracts was determined according to the method described by Barros et al. (2007), with depends on the reduction of the bleaching of a purple-coloured methanolic solution of the stable radical 2, 2-diphenyl-1-picryl-hydrazyl to a yellow coloured diphenyl-picrylhydrazine. Sample amounts of freeze-dried extract were mixed with 1 mL of DPPH solution

Table 1. Climatic conditions of *Ecballium elaterium* at different altitudes in Tunisia.

Altitude (growing location) (m)	Longitude (E)	Latitude (N)	Average rainfall (mm/year)	Minimum temperature (°C)	Maximum temperature (°C)	Type of climate
1 (Cap-Bon)	10°44'15"	36°27'21"	344.0	3.8	22.3	Semi-arid
582 (Kef)	8°42'17"	36°10'27"	361.4	−2.8	41.6	Semi-arid
354 (Sidi Bouzid)	9°29'05"	35°02'17"	288.8	13.2	25.8	Arid

Source: Infoclimat (2013); data were averaged for the locality in 2013.

(2 mg dissolved in 50 mL methanol). The samples were kept in the dark for 30 min at room temperature, and absorbance was measured against a blank at 517 nm. DPPH radical scavenging activity was calculated using the following Equation 1:

$$(\%)\ \text{Scavenging activity} = [(A_0 - A_1) / A_0)] \times (100) \quad (1)$$

Where A_0 refers to the absorbance of the blank and A_1 to the absorbance of the sample. The BHT and ascorbic acid were used as positive controls. All tests were performed in triplicate.

ABTS free radical scavenging activity: The spectrophotometric analysis of the $ABTS^{\bullet+}$ scavenging activity was estimated according to the method of Re et al. (1999). The ABTS stock solution was prepared by mixing a 7 mM of ABTS at pH 7.4 (5 mM NaH_2PO_4, 5 mM Na_2HPO_4 and 154 mM NaCl) with 2.5 mM potassium persulfate followed by storage in the dark at room temperature for 16 h before use. On the same day of the analysis, the $ABTS^{\bullet+}$ solution was diluted with methanol to an absorbance of 0.7 ± 0.0 at 734 nm. An amount of 20 µL of sample was added to 2 mL of the diluted $ABTS^{\bullet+}$ solution. After 6 min of incubation in the dark, absorbance was measured at 734 nm. Trolox was used as a positive control, and the formula as for DPPH radical scavenging activity was also adopted for the calculation of ABTS radical scavenging activity. All samples were prepared and analyzed in triplicate.

Ferric reducing antioxidant power (FRAP) assay: The reducing power of the extracts was determined according to the method described by Ammar et al. (2015) with some modifications. Briefly, 250 µL of each fruit extract at different concentration were mixed with a phosphate buffer (500 µL, 0.2 M, pH 6.6) and potassium ferricyanide (500 µL, 1%). The mixtures were then incubated at 50 °C for 20 min. An amount of 500 µL of trichloroacetic acid (10%) was added to each sample, and the mixtures were centrifuged at 1.006 × g for 10 min. After that, 750 µL of the upper layer were mixed with 750 µL distilled water and ferric chloride (50 µL, 0.1%). The mixtures were incubated for 10 min in the dark. Absorbance was measured at 700 nm against a control that consisted of all the reagents without the test sample. All tests were performed in triplicate.

2.8 Fourier Transform Infrared Spectroscopy (FT-IR) spectra analysis

Infrared spectra of freeze-dried extracts from fruit juice of *E. elaterium* were recorded on a Perkin Elmer FT-IR Spectrum 100 fitted with an ATR accessory. The powdered sample of extract were loaded on the sample chamber of FT-IR spectrophotometer and scanned at room temperature (25 ± 2 °C) with a scan range from 500 to 4000 cm^{-1} at a resolution of 2 cm^{-1}.

2.9 Statistical analysis

The data represent the means of three measurements ± standard deviation (SD). Statistical analyses of the data were performed using the SPSS version 17.0 software (SPSS Inc., Chicago, IL, USA). The results were analyzed by one-way analysis of variance (ANOVA) followed by *Tukey* test for multiple comparisons. Differences were considered significant at $p < 0.05$.

3 Results and discussion

3.1 Chemical analysis

The results obtained for the compositional content of protein, fat, carbohydrate, energy value, moisture, ash and pH of *E. elaterium* fruit juices are shown in Table 2. The fruit was approximately 4 cm in length and 2 cm in width. The protein, fat, moisture and ash contents in the juice varied with the region of origin. While the highest amount of protein (9.2 ± 0.4 g/100 g) and fat (3.5 ± 0.2 g/100 g) contents were recorded for the freeze-dried extracts of Kef and Cap-Bon respectively, the highest levels of moisture (87.9 ± 2.2 g/100 g) and ash (3.8 ± 0.2 g/100 g) contents was registered for the freeze-dried extracts of Sidi Bouzid and Cap-Bon, respectively. Fruit juice from Cap-Bon showed the highest level of carbohydrate (3.6 ± 0.0 g/100 g), followed by fruit juice from Kef (3.4 ± 0.0 g/100 g). In addition, the energy values, ranged from 219.1 ± 0.6 to 338.7 ± 0.3 kJ/100 g indicate a weaker carbohydrate content, with the highest level was obtained for the fruit juice from Kef. Also, we noticed that *E. elaterium* fruit juices extracted from the three regions exhibited an acidic pH ranging between 5.1 and 5.5.

The phytochemical analysis performed on *E. elaterium* fruit juices from three different regions (Cap-Bon, Sidi Bouzid and Kef) in Tunisia revealed large variations in terms of content (protein, fat, moisture, ash, carbohydrate) and pH and energy value, which could be attributed to different agro-climatic conditions and soil composition in the regions as well as other physiological and environmental factors.

The presence of high amounts of ash in the three fruit juices, particularly in the variety from Sidi Bouzid, indicated that the fruits had high deposits of mineral elements and justified the presence of adulterants. Moisture and fat were, however, present in adequate amounts to allow for the fresh conditions required for the cure of the disease (Othman et al., 2014) and to constitute a good source of energy, respectively. The results obtained in terms of protein, fat, moisture and ash contents were slightly higher than the ones previously reported by Greige-Gerges et al. (2007). The difference in the chemical composition between the

Table 2. Nutrient composition of *Ecballium elaterium* fruit juice.

Growing location	Protein (g)	Fat (g)	Moisture* (g)	Ash (g)	Carbohydrate** (g)	Energy value (kJ)	pH
Cap-Bon	8.6 ± 0.3 ^{b}	3.5 ± 0.2 ^{a}	80.3 ± 1.0 ^{b}	3.8 ± 0.2 ^{a}	3.6 ± 0.0 ^{a}	336.5 ± 0.5 ^{b}	5.1
Kef	9.2 ± 0.4 ^{a}	3.3 ± 0.9 ^{a}	81.3 ± 1.9 ^{b}	2.5 ± 0.2 ^{b}	3.4 ± 0.0 ^{b}	338.7 ± 0.3 ^{a}	5.4
Sidi Bouzid	4.2 ± 0.5 ^{c}	2.6 ± 0.1 ^{a}	87.9 ± 2.2 ^{a}	2.3 ± 0.1 ^{c}	2.8 ± 0.0 ^{c}	219.1 ± 0.6 ^{c}	5.5

Values are the means of triplicate experiments ±SD; * Moisture based on 100 g fresh weight, all other parameters based on 100 g dry weight; ** Calculated by difference; For each test, different letters in same column followed by a different letter (a, b, c) indicate significant difference ($p < 0.05$) of *Ecballium elaterium* at three different altitudes. Results are ranked in ascending order; a > b > c.

three extracts of fruit juices of *E. elaterium* under investigation were statistically significant ($p < 0.05$).

3.2 Mineral content of the fruit juices

The freeze-dried of fruit juices consisted of different minerals at different levels as shown in Table 3. Macroelements (Ca and Mg) and microelements (Fe and Cu) were analyzed. To the best of our knowledge, this is the first report undertaken the mineral composition of *E. elaterium* fruit juice. Amongst the macroelements, the highest concentration was observed mainly for the fruit juice from Sidi Bouzid. Mg was the most abundant mineral present in all fruit juices ranging from 12.1 ± 0.0 to 32.3 ± 0.0 mg/100 g. The highest concentrations of Mg (32.3 ± 0.0 mg/100 g) and Ca (12.2 ± 0.0 mg/100 g) were obtained for the freeze-dried extract from Sidi Bouzid. The level of Fe varied with locality with the highest concentration was determined in fruit juices from kef (1.0 ± 0.0 mg/100 g) followed by Cap-Bon (0.7 ± 0.0 mg/100 g), while the highest content of Cu element was found in fruit juice from Cap-Bon (0.3 ± 0.0 mg/100 g).

Fruits are important contributors of minerals in the diet, and which that the mineral composition and their content of fruits depended on the growing conditions and the stage of maturity (Konczak & Roulle, 2011). It was considered that calcium and magnesium have a potential relevance in health (Morales-de la Peña et al., 2011) and have essential roles in a variety of body functions including heart, muscle, nerve and immune systems maintenance (Nyanga et al., 2013). The highest content of magnesium in all fruit juices confirmed the antioxidant power of *E. elaterium*, because this element is a cofactor in almost all phosphorylation reactions and is considered as an indirect antioxidant (Lukaski, 2004).

The variation in the results of chemical and mineral composition of fruit juice extracts of *E. elaterium* from different locations may have been due to the influence of climatic changes, production factors, maturity state and species and variety of fruit (Halilova & Yildiz, 2009; Mahdavi et al., 2010).

3.3 Total phenolic and flavonoid contents

Phenolics and flavonoids are a major class of bioactive constituents and have beneficial effects on human health. The present study aimed to determine the total content of phenolic and flavonoid in *E. elaterium* fruit juice expressed in mg of gallic acid equivalents (GAE) per gram of extract (mg GAE/g) and mg of quercetin equivalents (QE) per gram of extract (mg QE/g), respectively. As summarized in Table 4, the total phenolic and flavonoid contents of *E. elaterium* fruit juice depended on the zone where it has grown, with the highest content recorded for the fruit juice of the region of Cap-Bon, which displayed 106.4 ± 0.4 mg GAE/g and 6.5 ± 0.2 mg QE/g, respectively.

Our investigation indicate the dependence of polyphenolic content on altitudes which is in good agreement with previous study showing that phenolic and flavonoid contents of *E. elaterium* fruit juices vary significantly with region, geographical conditions and maturity stage (Kondakova et al., 2009). Also, higher phenolic content allowed to lower altitude (Mditshwa et al., 2013; Mphahlele et al., 2014) and lower total flavonoids content may be related to high temperatures by inhibiting biosynthesis and enhancing degradation of flavonoids as observed in fruit harvested in higher and medium altitude with high maximum temperatures.

The results presented in this study showed the richness of the *E. elaterium* fruit juice from Cap-Bon in terms of phenolic and flavonoid contents, which are the main contributors to the antioxidant activity of fruit juices (Luo et al., 2010; Zakaria et al., 2011; Chai & Wong, 2012; Schvab et al., 2015). The presence of phenolic and flavonoid is crucial to evaluate antioxidant activity. This is particularly due to their redox properties and chemical structure, which play an important role in absorbing

Table 3. Mineral content of *Ecballium elaterium* fruit juice.

Growing location	Minerals content (mg/100 g dw)			
	Magnesium (Mg)	Calcium (Ca)	Iron (Fe)	Copper (Cu)
Cap-Bon	19.4 ± 0.0 [b]	5.1 ± 0.0 [c]	0.7 ± 0.0 [b]	0.3 ± 0.0 [a]
Kef	12.1 ± 0.0 [c]	6.2 ± 0.0 [b]	1.0 ± 0.0 [a]	0.1 ± 0.0 [a]
Sidi Bouzid	32.3 ± 0.0 [a]	12.2 ± 0.0 [a]	0.2 ± 0.0 [c]	0.2 ± 0.0 [a]

Values are the means of triplicate experiments ± SD; For each test, different letters in same column followed by a different letter (a, b, c) indicate significant difference ($p < 0.05$) of *Ecballium elaterium* at three different altitudes. Results are ranked in ascending order; a > b > c.

Table 4. Total phenolic content, total flavonoid content and DPPH and ABTS scavenging activities of freeze-dried extracts of *Ecballium elaterium* fruit juices.

Growing location	Total phenolic content (mg GAE/g)	Total flavonoid content (mg QE/g)	IC_{50} DPPH (μg/mL)	IC_{50} ABTS (mg/mL)
Cap-Bon	106.4 ± 0.4 [a]	6.5 ± 0.2 [a]	38.6 ± 0.2 [b]	0.6 ± 0.0 [c]
Kef	78.7 ± 0.6 [c]	0.6 ± 0.3 [c]	50.1 ± 0.7 [a]	0.8 ± 0.0 [a]
Sidi Bouzid	86.2 ± 0.9 [b]	3.9 ± 0.1 [b]	50.7 ± 0.2 [a]	0.6 ± 0.0 [b]
Ascorbic acid	–	–	28.6 ± 0.6	*
BHT	–	–	25.3 ± 0.9	*
Trolox	–	–	*	0.4 ± 0.0

Results are presented as means ±SD; Different letters in same column indicate followed by a different letter (a, b, c) significant difference ($p<0.05$) of *Ecballium elaterium* at three different altitudes. Results are ranked in ascending order; a>b>c; –: not determined; *: not used as standard.

and neutralizing free radicals, quenching singlet and triplet oxygen, and decomposing peroxides (Čanadanovic-Brunet et al., 2008; Li et al., 2008; Skotti et al., 2014).

3.4 Antioxidant activities of extracts

DPPH radical scavenging activity: The antioxidant activity of the *E. elaterium* fruit juice from the three localities was evaluated by the DPPH assay (based on the principle that a hydrogen donor is an antioxidant) and compared to the standard ascorbic acid and BHT. The results presented in Figure 1 revealed large variation in antioxidant activity, and that this variation was dose-dependent. The antioxidant activities of the fruit juices were expressed in terms of percentage of inhibition and IC_{50} values (concentration of sample required to scavenge 50% of free radicals). The findings indicated that the lower the IC_{50} values were, the higher the antioxidant capacity of the fruit juice extract became. At the highest concentration of 500 µg/mL, the fruit juice from the region of Cap-Bon manifested the highest DPPH$^{\bullet}$ scavenging ability (85.8%), followed by the fruit juices of Sidi Bouzid (75.4%) and Kef (67.0%), respectively. Radical scavenging activity was also expressed in IC_{50} values presented in Table 4, and noted to vary according to the region. The best radical scavenging activity was observed for the fruit juice extract from Cap-Bon (38.6 ± 0.2 µg/mL), followed by the extracts from Kef (50.1 ± 0.7 µg/mL) and Sidi Bouzid (50.7 ± 0.2 µg/mL), respectively. Those values were higher than the ones obtained for BHT (25.3 ± 0.9 µg/mL) and ascorbic acid (28.6 ± 0.6 µg/mL). The differences in the IC_{50} radical scavenging activity between the freeze-dried extracts of fruit juice of *E. elaterium* under investigation were statistically significant ($p < 0.05$). In the present study, fruit harvested at lower altitude significantly showed higher antioxidant activity than those harvested at medium and higher altitudes. Higher antioxidant activity has been attributed to higher total polyphenolic compounds present in fruit juice of Cap-Bon region. The difference of fruit juices in the DPPH scavenging activity could be explained by many factors, such as the ripening state of the fruits and environmental differences (Egea et al., 2010; Dou et al., 2013).

Furthermore, the results indicated that the *E. elaterium* fruit juice extracts from three Tunisian regions had good DPPH scavenging activity, particularly the variety from the Cap-Bon region, which is consistent with the results recorded for polyphenol and flavonoid contents. This, in fact, corroborates the results previously reported in the literature where several bioactive compounds present in this fruit juice, including triterpénoïdes (cucurbitacins), carbohydrates, tannins, gum, peptides and leucoanthocyanins, were reported to enhance antioxidant activity (Attard & Attard, 2008; Bernard & Olayinka, 2010). Many researchers have shown that an increase in DPPH inhibition of radicals may be explained by several factors, such as the higher antioxidant power of polyphenols at an intermediate state of oxidation, the increase in reducing sugar and formation of Maillard reaction products, known to have a great antioxidant activity, which is often exerted in a chain-breaking and DPPH type mechanism (Madrau et al., 2009; Herch et al., 2014; İncedayi et al., 2016).

ABTS Free Radical Scavenging Activity: The ABTS radical cation decolorization assay is an excellent tool produced by a reacting ABTS solution with potassium persulfate. It is commonly applied to estimate the total antioxidant activity and to reflect the ability of antioxidant species to donate electrons or hydrogen atoms (Leong & Shui, 2002). The results obtained for the scavenging ability of the fruit juices with regard to ABTS free radical are expressed as percentages are shown in Figure 2, and the IC_{50} values are illustrated in Table 4.

The scavenging potential of the fruit juices was noted to correlate well with increasing concentrations, with the greatest ABTS$^{\bullet+}$ scavenging activity obtained at 1 mg/mL for the fruit juice

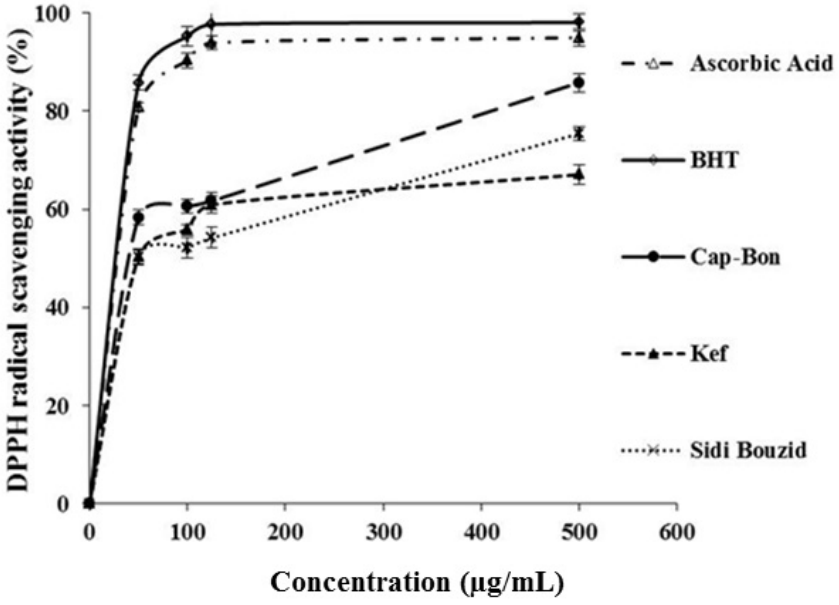

Figure 1. DPPH radical scavenging activity of freeze-dried extracts of *Ecballium elaterium* fruit juices.

extract from Cap-Bon (78.9%), followed by the fruit juice extracts from Sidi Bouzid (70.8%) and Kef (56.8%), respectively. The results also indicated that they were lower than the ones recorded for the positive control Trolox (93.4%). Furthermore, the results revealed that the fruit juice extracts showed variability in terms of scavenging ability, following the same trend observed for IC_{50} values (0.6 ± 0.0 mg/mL, 0.6 ± 0.0 mg/mL and 0.8 ± 0.0 mg/mL for Cap-Bon, Sidi Bouzid and Kef, respectively). This test indicated that the three fruit juice extracts exhibited lower levels of antioxidant activity compared to the positive control Trolox (IC_{50} = 0.4 ± 0.0 mg/mL) with significant differences ($p < 0.05$) between all fruits juices.

The scavenging ability of $ABTS^{\bullet+}$ could presumably be attributed to the presence of phytoconstituents in the fruit juices, which can act as electron donors, by reacting with free radicals, and therefore, terminate radical chain reactions (Suárez-Jiménez et al., 2015). The analysis of $ABTS^{\bullet+}$ radical scavenging activity indicated that the fruit juice extracts contained compounds that have efficient abilities to scavenge free radicals by forming resonance-stabilized phenoxyl radicals. This activity was also noted to vary according to the region of origin, with the highest levels of scavenging activity being recorded for the fruit juice extract from Cap-Bon.

Ferric reducing power assay: The results presented in Figure 3 show the reductive capability of the fruit juice as compared to ascorbic acid. This assay is based on the reduction of Fe^{3+} ferricyanide complex to the ferrous form Fe^{2+} by donating an electron to free radicals and converting them into more stable forms (Kadri et al., 2011). An increase in the absorbance at 700 nm of the reaction mixture indicated increased reducing

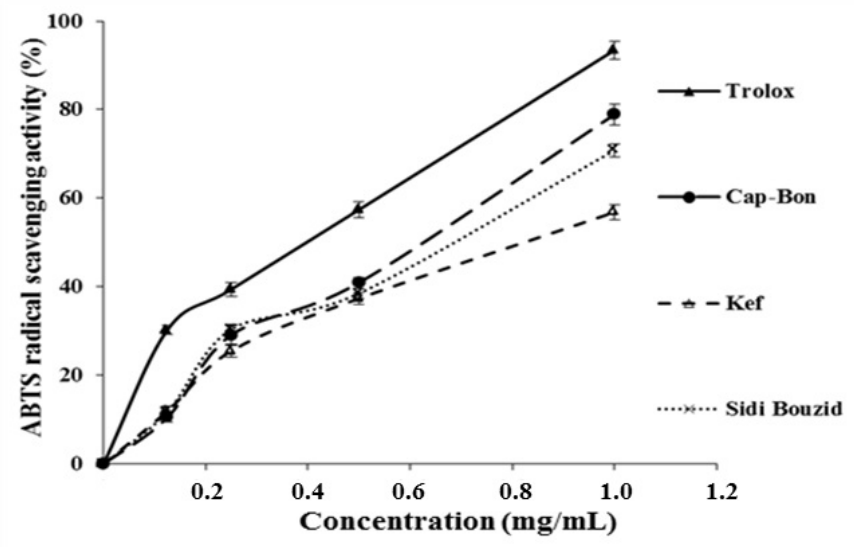

Figure 2. ABTS radical scavenging activity of freeze-dried extracts of *Ecballium elaterium* fruit juices.

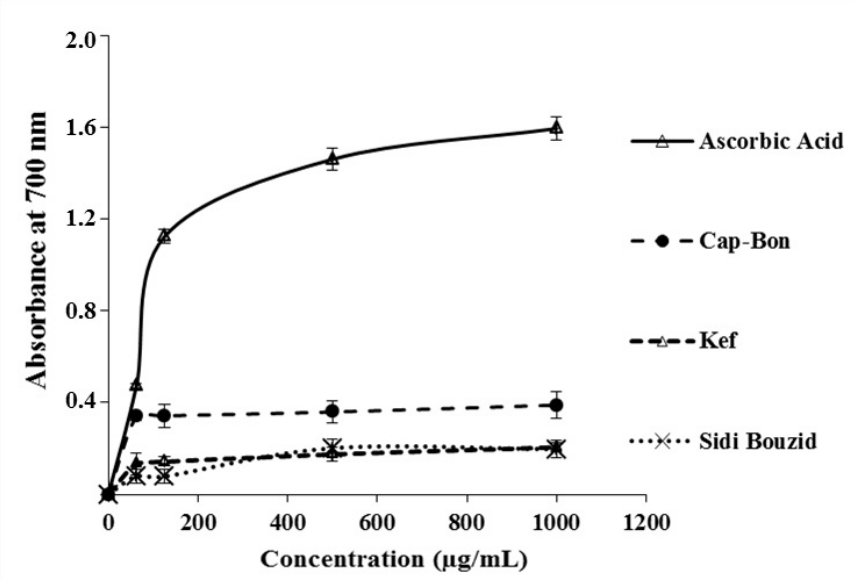

Figure 3. Reducing power effect of freeze-dried extracts of *Ecballium elaterium* fruit juices.

power. Among the three localities, the highest antioxidant potential was observed for the fruit juice from Cap-Bon (0.4) as compared to those of Sidi Bouzid and Kef (0.2), which was about four times lower than the positive control (1.6).

As far as reducing power was concerned, the results clearly indicated that the abundance of antioxidant constituents in the fruit juice extracts induced higher rates of reducing power, which varied from one region to another, with the highest value being recorded for the fruit juice extract from Cap-Bon. The results from this assay were similar to those obtained for the DPPH radical scavenging activities. The presence of hydrogen donation and the number and position of hydroxyl groups of phenolic compounds were also noted to affect the antioxidant activity (Leja et al., 2007).

The results from the three assays (DPPH, FRAP and ABTS) also showed significant correlation in terms of the antioxidant capacity of the fruit juices. High correlation coefficients (R^2) were observed between the total phenolic contents and the DPPH, FRAP and ABTS assays, reaching 0.98, 0.95, and 0.91 respectively, which confirmed that phenolics were the major contributor to antioxidant activity. On the other hand, a good correlation was found between total flavonoid content and DPPH ($R^2 = 0.80$), ABTS ($R^2 = 0.99$), and FRAP ($R^2 = 0.80$). These strong correlations further confirmed the strong antioxidant potential of phenolic compounds. The antioxidants activities obtained from DPPH, ABTS and FRAP assays were well correlated with each other, with excellent correlation coefficient, and the R^2 value for DPPH–ABTS, DPPH–FRAP and FRAP–ABTS were 0.74, 0.99 and 0.74, respectively. These results implied that the antioxidants in these extracts were capable of reducing oxidants (ferric ions) and scavenging free radicals (DPPH and ABTS).These strong correlations further confirmed the strong antioxidant potential of phenolic compounds. The differences in the antioxidant properties of the different fruit juice extracts could presumably be attributed to the differences in phenolic and flavonoid compositions, which exhibited several biological effects (Canas et al., 2008). Also the concentration of polyphenols may be influenced by the extent of fruit's maturity (Kondakova et al., 2009). The variations of TPC in the present analysis of freeze-dried extracts of *E. elaterium* fruit juices among different locations could be explained by the influence of different parameters such as temperature and different prevalent environmental factors (Wang & Zheng, 2001; Miguel et al., 2014).

3.5 FT-IR spectral analysis

FT-IR spectral analysis data for the fruit juice extracts revealed the existence of multiple functional groups in the extracts. The spectral features of the extract were shown in Figure 4a-c.

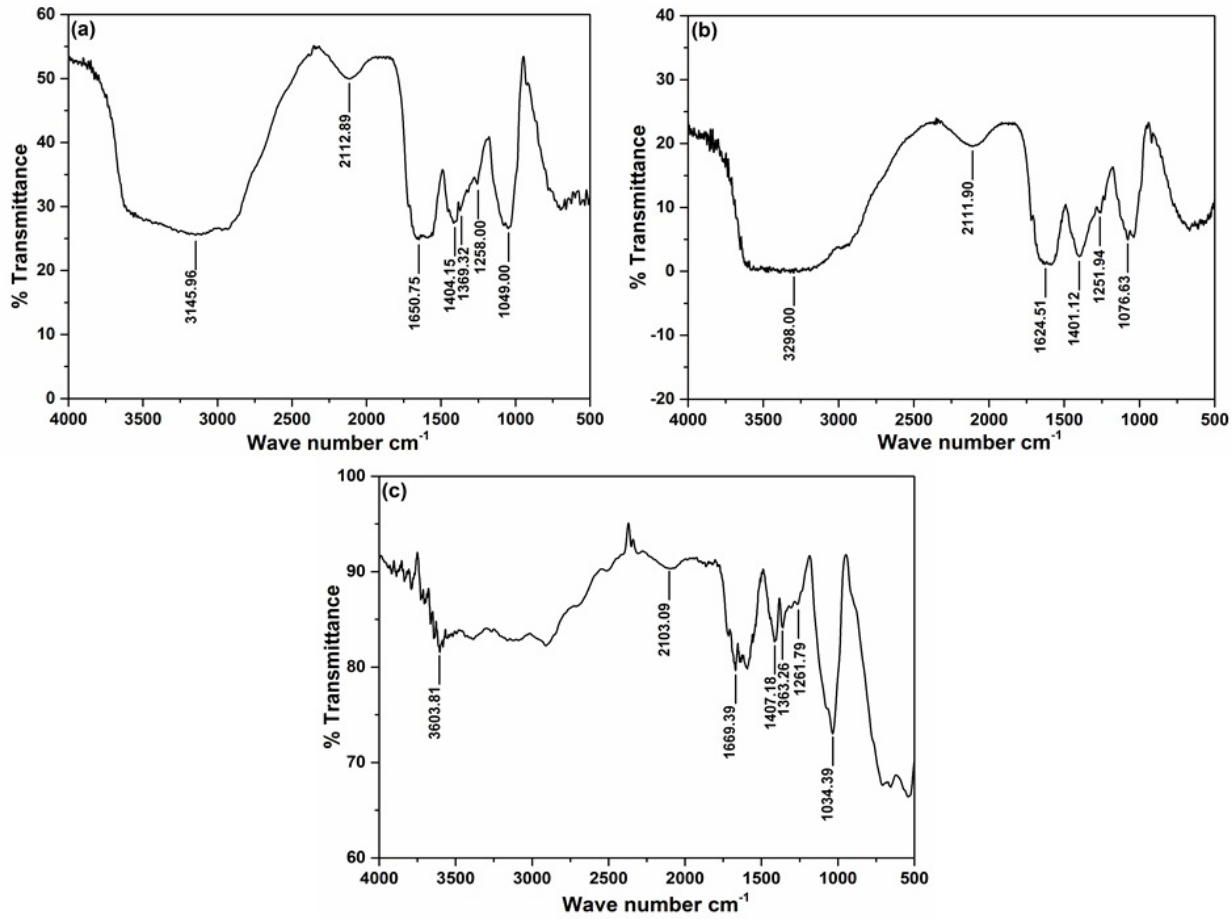

Figure 4. FT-IR spectrum of Freeze-dried extracts of *Ecballium elaterium* fruit juices from Cap-Bon (a); Kef (b) and Sidi Bouzid (c).

For all extracts, the very strong absorption bands were observed at 3145.9, 3298.0 and 3603.8 cm^{-1} of freeze dried extracts of fruit juices from Cap-Bon, Kef and Sidi Bouzid respectively, could be attributed to N–H stretching of proteins and O–H stretching of carbohydrates and water, which indicate hydroxyl groups existed in all extracts. The bands at 2112.8, 2111.9 and 2103.0 cm^{-1} of extracts from Cap-Bon, Kef and Sidi Bouzid respectively are due to the –C≡C group. The bands between the wavenumbers of 1800-750 cm^{-1} reflected the biochemical compositions, especially the moieties of carbohydrate, lipid, protein and polyphenols in plant. The absorptions around 1650.7, 1624.5 and 1669.3 cm^{-1} could be assigned to ring C–C stretch of phenyl (Lu et al., 2011), which is present at high levels in the polyphenolic components of *E. elaterium* fruit juices. Absorptions peak at 1404.1, 1401.1 and 1407.1 cm^{-1} for extracts from Cap-Bon, Kef and Sidi Bouzid respectively, corresponding to CH_3 asymmetric deformation (Agarwal et al., 2006). Only for extracts from Cap-Bon and Sidi Bouzid, the presence of peak at 1369.3 and 1363.2 cm^{-1} respectively, is due to the in-plane C–O stretching vibration combined with the ring stretch of phenyl (Schulz & Baranska, 2007). The minor bands obtained at 1258.0, 1251.9 and 1261.7 cm^{-1} from Cap-Bon, Kef and Sidi Bouzid respectively, were ascribed to O–H (–COOH) variable angle vibration (Zhao et al., 2014). The wavenumber region between 1200 and 950 cm^{-1} contains functional groups mainly from carbohydrates, while absorptions peak at 1049.0, 1076.6 and 1034.3 cm^{-1} are attributed of $–CH_2OH$ groups of carbohydrates (Lu et al., 2011). The various functional groups observed in fruit juice extracts of *E. elaterium* reflected the biochemical compositions, especially the phenolic compounds, carboxylic acids, alcohols, carbohydrates, and proteins in the plant, responsible for several medicinal properties and biological activities which is confirmed by our investigation in chemical composition. The presence of phytochemicals carrying hydrogen functional group –OH bonded found that the hydroxyl functionality is an integral part of most of phenolic phytochemicals such as polyphenols and flavonoids to provide a relative ranking of extracts in term of antioxidant activity.

Therefore, the presence of characteristic functional groups that are responsible for various medicinal properties may be influence considerably the biological properties and contribute significantly to their solubility, partition coefficient, stereochemistry and inherent acid–base properties (Knittel & Zavod, 2008). From the results obtained in the present study, it could be concluded that the *E. elaterium* fruit juice extracts in relationship with their phytoconstituents (total phenolic and flavonoid contents,…) may act as source of therapeutic agent. The diversity of functional groups observed probably indicate the presence of carbohydrates, carotenoid, glycogen, amino acids, amides, starch, calotropin, calotropogenin, phosphates, lipids, glycogen and cellulose. The richness of *E. elaterium* fruit juice extracts –OH group enhances its ability for forming hydrogen bonding capacity and confirmed therefore, the higher potential of its antioxidant activity (Diaz et al., 2012).

4 Conclusions

In conclusion, the present study is the first to report on the chemical composition and antioxidant activity of freeze-dried extracts from fruit juice of *E. elaterium* growing in Tunisia. The findings revealed variability in chemical composition, high phenolic and flavonoid contents, and potent antioxidant activity, which depended on the area of origin, with relatively advanced properties for the freeze-dried extracts from Cap-Bon. A strong positive correlation was observed between the total phenolic content and values of antioxidant activity. Considering the promising properties and attributes of *E. elaterium* fruit juice, further studies, some of which are currently underway in our laboratories, are needed to to isolate investigate the active compounds responsible for antioxidant activity and evaluate their potential use as supplements in functional foods and pharmaceutical formulations. The various functional groups observed in the different extracts probably confirmed by FT-IR analysis indicate the richness of *E. elaterium* fruit juice in polyphenols.

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