



Phytochemical Content and Antioxidant Activity of Flesh Fruits *Rosa canina* Extracts Collected from Ait Ayach Midelt

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ABSTRACT

Rosa canina L. is a rich fruit on bioactive substances which provides them with high antioxidant potential, like phenolic acids, flavonoids, condensed tannins. The aim of this study was to estimate and characterize the phenolic content, flavonoids content, condensed tannins content obtained from rose hips fruits in five extracts (water, 50% v/v ethanol, 70% v/v ethanol, 80% v/v methanol, apple vinegar) to examine their antioxidant activity by two reliable methods: Total antioxidant activity and DPPH. Surprisingly, in our results, the highest polyphenol, flavonoids content and condensed tannins was observed in vinegar extract 55,51±1,16 mg GAE/g dw, 22,15±0,45 mg QE/g dw, 12,37±0,16 mg AAE/g dw respectively. Furthermore, the vinegar extract was the most effective to chelating the radical DPPH (IC50% = 0,336±0,005µg), and have the highest total antioxidant activity (7,88±0,19mg AAE/g dw). The current research showed that the vinegar was more effective than the methanol, ethanol, water for polyphenols, flavonoids, tannins extraction.

Key words: Antioxidant activity, Condensed tannins, Flavonoids, Polyphenols, *Rosa canina*.

INTRODUCTION

Rosa canina distributed natively in Europe, Asia, the Middle East and North America and fit to numerous soil and climates (Nilson, 1997).

Known in Morocco in Midelt area under the vulgar Berber name "Tabgha", this plant has several therapeutic virtues, its leaves are used in traditional medicine by the population of this region to treat erectile dysfunction, stomachic (by decoction) and against headaches, fall arrest (powder) (Benlamdini *et al.* 2014). Common preparations of rosehips include wine, juice, tea, jam, jelly and sometimes blended with salmon dry eggs (Moerman, 2002). The fruits of this plant are a precious source for the food industry and pharmaceuticals. In the course of last decade, rosehips earned interest more because they contain compounds which are known to have several anti-oxidant properties, anti-mutagenic and anti-carcinogenic (Tumbas *et al.* 2012). The rosehips have been used for the prevention and treatment of colds, flu and diabetes in many European countries (Chrubasik *et al.* 2008), in Bulgaria rosehips are generally used as an infusion (Taneva *et al.* 2016). The juice and the aqueous extract of the fruits of the eglantine have an exceptional antioxidant activity (Demir *et al.* 2001). The powder of the fruits of the rose hip can support the longevity of the skin and of cells by mechanisms implicating polyunsaturated fatty acids, support of collagen, anti-oxidant properties and anti-inflammatory (Phetcharat *et al.* 2015).

The fruits of the eglantine are well known for their high polyphenol content (Campos-Vega *et al.* 2013), these compounds constitute an important class of known natural antioxidants that exhibit various important physiological and biological properties *in vivo* and *in vitro* (Campos-Vega *et al.* 2010; Shahanas *et al.* 2019; Sonia *et al.* 2016). The antioxidant activity of *Rosa canina* fruit extract has been

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documented in several studies (Roman *et al.* 2013; Ersoy *et al.* 2015; Taneva *et al.* 2016; Murathan *et al.* 2016).

Of more the composition of the fruits of *Rosa Canina* is affected by soil and geographical factors, where from comes the objective of the present study, which aims at characterizing the wild fruit of *Rosa canina* which develops naturally in the region of Midelt by determining the contents of polyphenols, flavonoids, condensed tannins, antioxidant activity, using different organic solvents and apple vinegar like bio solvent.

MATERIALS AND METHODS

Extracts preparation

The biological material of this study consists of *Rosa canina* fruit bearing interest on the peel, these fruits are harvested in November 2016 in Ait Ayach (Zhira). Rural commune

(32°41' North and 4°44' West), is part administratively of the circle of Midelt (area of Deraa Tafilalet) and hydraulically of the basin overturning of Moulouya. This area is bounded to the north by the City of Zaida, to the east by the town of Midelt, to the south by Tounfite and the West by the Annex to Boumia and Lakbab, geographical code of 08.363.07.05. This region is located in 250 km the Atlantic Ocean and insulated by 150 km of chains of the mountain (Rhanem, 2010). Only healthy and undamaged fruits wall were subjected to determinations.

Before extraction, the fruits of *Rosa canina* were washed in the water of tap then dried in air and cleared out from seeds. Crushed the shells dried to have a powder. The technique of extraction was accomplished by five solvents as follows: distilled water, ethanol 50% (v/v) ethanol 70% (V/v), methanol 80% (V/v), vinegar extract, with maceration. The solid/liquid report was 1/10. The obtained extracts were filtered through filter paper and then were analyzed.

Wight of hips, number of seed

Twenty hips were weighed on a digital scale with a sensitivity of 0,0001g (OHAUS CORPORATION). The seeds of hips were counted (n=20).

Determination of total polyphenols content, total flavonoids content, condensed tannins

The determination of the total polyphenols in different extracts was determined spectrophotometrically following the Folin Ciocalteu method (Singleton *et al.* 1999, Miguel *et al.* 2014, Bakour *et al.* 2017). The concentration of total phenolic compounds was determined as mg of Gallic acid equivalent, using a calibration curve. The total flavonoids content was determined according to the method described by Kong *et al.* (2012). the result was expressed as mg of quercetin equivalent/g dw. The condensed tannins were determined according to the method described by Joslyn (1970), the result was expressed as mg of tannic acid equivalent/g dw.

Total antioxidant capacity TAC, radical scavenging activity

The Total antioxidant capacity of the rose hip extracts was determined by the phosphomolybdenum method as previously described by Zengin *et al.* (2012). The total antioxidant capacity of the different extracts was evaluated as equivalents ascorbic acid (mg AAE/g dw).

The scavenging activity of the different extracts for the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was measured as described by Hogg *et al.* (1961) and Miguel *et al.* (2014), the absorbance was recorded at 517nm. The tests were carried out in triplicate. The scavenging activity was estimated based on the percentage of DPPH radical scavenged using the following equation (Miguel *et al.*, 2014, bakour *et al.*, 2017):

$$IC_{50} \% = \frac{[(\text{control absorbance} - \text{sample absorbance}) / (\text{control absorbance})] * 100}{}$$

RESULTS AND DISCUSSION

Wight of hips and number of seed

The results for the weight fruit and number of seeds content from wild growing *Rosa canina* L. were presented in Table 1.

The analysis of the results obtained shows that the average fruit weight is the 0.84 ± 0.15 g and contains seeds with an average number of 19.4 ± 3.8 . Murathan *et al.* (2016), found that the weight of fresh fruit was about 34.5 ± 0.9 g with an average number of seeds was 23 ± 0.7 , Buchwald *et al.* (2007), mentioned that the weight of each fruit varies within a range of 0.95 to 3.27g and influenced by many factors such as growing conditions and location.

Total polyphenols content

The results for the total phenols content in different extracts from a powder of rose hip were presented in Table 2. The average of TPC in extracts varied within a quite wide range from 24.66 ± 2.95 to 55.51 ± 1.16 mg EAG/g dw. The highest TPC was established in vinegar extract 55.51 ± 1.16 mg EAG/g dw, while the lowest value was mentioned in 80% (v/v) methanol extract 24.66 ± 2.95 mg EAG/g dw. The statistical comparison showed that the TPC of different extracts was significantly different ($p < 0,005$), except the difference between 70%(v/v) ethanol extract and 80%(v/v) methanol extract. The apple vinegar extract appeared to be the best solvent for extracting phenolic compounds from dry flesh of fruits of *Rosa canina*.

These data were in agreement with several studies such as (Ersoy *et al.* 2015) registered an average varies between 20.12 - 32.2 mg GAE/g in the water-methanol extract. Ben Jemaa *et al.* (2017) documented an average of TPC was 21.918 ± 1.784 mg/g fruit in methanol extract. Roman *et al.* (2013), registered values between: 5.75 – 3.26 mg GAE/g in methanol/HCl extract, Yoo *et al.* (2008) mentioned 8.18mg GAE/g fresh fruit, Fattahi *et al.* (2012) and Campos *et al.* (2010) reported an average ranged from 1.76 to 2.25mg GAE/g and 0.20mg GAE/g respectively, data which are lower than in our study. Taneva *et al.* (2016) reported an average higher than ours in results. The obtained results revealed the richness of hips in phenolic compounds which make these fruits as an interesting source of the bioactive compounds.

Total flavonoids

The average of total flavonoids content registered in different extracts varied from 3.66 ± 0.02 to 22.15 ± 0.45 mg QE/g dw. High concentration of TFC was found in the vinegar extract 22.15 ± 0.45 mg QE/g. while the lowest value was found in the 80%(v/v) methanol extract.

Significant differences can be observed in the TFC for all extracts that were analyzed ($p < 0,005$).

The outcome show variability in the TFC in different extracts of shells powder of rose hips fruit are agreement

Table 1: Fruits weights and number of seeds.

Weight (g)	Number of seed	Peel color
0.84 ± 0.03	19.4 ± 0.85	Red

Table 2: Phenolic content, flavonoid content and condensed tannins of different extracts.

	Total phenolic content mg GAE/g dw	Total Flavonoids content mg QE/g dw	Condensed Tannins mg TAE/g dw
ED	40.16±0.25	19.68±0.22	9.56±0.24
EtOH50%	27.23±4.08	4.22±0.13	4.06±0.05
EtOH70%	26.06±0.91	13.55±0.06	8.07±0.21
MeOH80%	24.66±2.95	3.66±0.02	3.60±0.12
EV	55.51±1.16	22.15±0.45	12.37±0.16

Table 3: Antioxidant activity of different extracts.

	TAC mg EAA/g	IC ₅₀ % DPPH µg
ED	5.89±0.10	0.373±0.027
EtOH50%	5.22±0.05	0.441±0.015
EtOH70%	5.70±0.17	0.740±0.011
MeOH80%	5.13±0.05	0.814±0.025
VE	7.88±0.19	0.336±0.005

with Montazeri *et al.* (2011) reported an average varies between 1.2 ± 3.2 - 23.6 ± 4.2 mg QE/g in different extracts. In the study conducted by Jemaa *et al.* (2017), the average of TFC was 2.647 ± 0.040 mg Rutin Equivalent/g dw. The lower value of TFC was documented by Roman *et al.* (2013) and Fettahi *et al.* (2012), with values 1.63 ± 0.005 mg QE/g of frozen pulp and 2.02 ± 0.03 mg QE/100g respectively.

Condensed tannins

The results showed in Table 2 depicted that the condensed tannins values ranged from 3.60 ± 0.12 mg TAE/g to 12.37 ± 0.16 mg TAE/g dw. The maximum value was detected in vinegar extract (12.37 ± 0.16 mg TAE/g dw) and the minimum value was observed in methanol extract with value 3.60 ± 0.12 mg TAE/g dw.

Antioxidant activity

There are many methods to examine the antioxidant activity of plant extracts. In the present study, the antioxidant activities were evaluated by the phosphomolybdenum method and the capacity of scavenging of radicals' DPPH. The results for the total antioxidant capacity of the different extracts rose hip shells were presented in Table 3. From the obtained values, the vinegar extract showed the highest antioxidant potential evaluated by phosphomolybdenum method was the highest values of 7.88 ± 0.19 mg EAA/g dw, while the lowest value was found in 80% methanol extract. The radical scavenging activity of the different extracts of *Rosa canina* was determined from its radical quenching ability. The results are described in Table 3, the values of IC₅₀% varied between 0.336 ± 0.005 µg for vinegar extract and 0.814 ± 0.025 µg for methanolic extract. The results presented in table 3 showed a significant difference between different extracts ($P < 0.005$). Previously, different studies mentioned that the antioxidant activity of plant extracts is positively correlated with bioactive compounds (Wenzig *et al.* 2008; Sinha *et al.* 2013; Beniwal and Jood 2014).

In the current study, the remaining extracts had antioxidant activity that positively correlated the phenolic content, flavonoid content, condensed tannins ($r = 0.946$; $P < 0.05$, $r = 0.817$; $P > 0.05$, $r = 0.893$; $P < 0.05$). Results are in agreement with Roman *et al.* (2013).

Multivariate analysis

Different extracts distribution and homogeneity founded on their biochemical parameters were studied using principal component analysis, to explore the links between variables and similarities between extracts, as a powerful tool for the chemometric analysis (Forina *et al.* 1987).

The outcomes were mentioned in Fig 1 the first component explained (83.953%) and represented in its positive part: flavinoid, TAC, condensed tannins, while the IC₅₀% DPPH was the dominating parameter in the negative part.

The second principal component explained (10.703%) of the given results and represented mainly the IC₅₀% DPPH in the positive part, while the phenolic content in the negative part. Based on the studied parameters, good discrimination was made between all extracts, which were discriminated by the first component. There is one group (AE, VE) in the positive part of component 1 and EtOH50%, EtOH70%, MeOH80% in the negative part of the same component. The vinegar extract and aqueous extract are characterized by their homogeneity in term of phenolic compounds, flavonoids compounds, condensed tannins, which implicated the

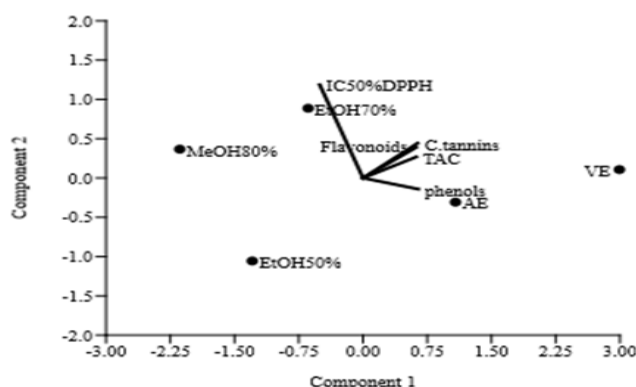


Fig 1: Principal component analysis (PCA). biplots of the analyzed extracts using the phytochemical parameters as an input: Phenols, Flavonoids, Tannins, IC₅₀ DPPH, TAC.

Table 4: Pearson correlation coefficients between bioactive compounds and antioxidant activities of different extracts of rose hip shells.

	Phenols	Flavonoids	C.Tannins	TAC mgAAE/g	IC50% DPPH µg
Phenols	1	0.847	0.880	0.946	-0.677
Flavonoids	0.847	1	0.985	0.817	-0.605
C.Tannins	0.880	0.985	1	0.893	-0.595
TAC	0.946	0.817	0.893	1	-0.595
IC50% DPPH	-0.766	-0.605	-0.595	-0.595	1

positive correlation with the total antioxidant activity and negatively with the IC50% DPPH activity. The EtOH70% and MeOH80% PV are characterized by their homogeneity in term of IC50%DPPH which implicated the negative correlation with phenolic content, flavonoids content, condensed tannins (phenols, $r = -0.677$, $P < 0.05$; flavonoid, $r = -0.605$, $P < 0.05$; Condensed tannins, $r = -0.595$, $P < 0.05$). Detailed correlations between the analyzed parameters were illustrated in Table 4.

CONCLUSION

The outcome obtained throughout this study showed large variability in the content of the bioactive substances of the fruits of *Rosa canina*. The differences in the content of phenolic compounds, flavonoids content, condensed tannins content were significant ($p < 0.05$). The different extracts obtained from wild growing rose hip fruits were examined their ability to scavenging radicals by their biological active substances and demonstrated well pronounced antioxidant activity. The significant correlation between the total phenolic content, flavonoids content and the antioxidant activity in different extracts was shown. The obtained results from all investigated extracts, the most proper solvent for extraction of substances with antioxidant potential was the vinegar as a bio product with health benefits. The incorporation of bio solvent for extraction of bioactive compounds will be perspective in design and formulation of food and cosmetic, nutraceutical products with improved added value

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