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 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, 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).

Know 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 pharmacies. 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 egliantive have an exceptional antioxidant activity (Demir *et al.* 2001). The powder of the fruits of the rosehip 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 egliantive 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 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
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(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 Ciocalteau 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):

$$IC_{50} \% = \frac{(control \ absorbance - sample \ absorbance)}{(control \ absorbance)} \times 100$$

**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±0.15g and contains seeds with an average number of 19±3.8. Murathan et al. (2016), found that the weight of fresh fruit was about 34.5 ±0.9g 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 ± 0.45mg 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±0.85        | Red        |
with Montazeri et al. (2011) reported an average varies between 1.2 ± 3.2 -23.6 ± 4.2mg 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 dw 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: flavoind, 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

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

| Extract | 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.**

| Extract | TAC mg EAA/g | IC50% 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   |

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