

## Full Length Research Paper

## Evaluation of biochemical compounds *Rosa canina* L. in North of Iran (Ramsar and Tonekabon Heights)

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Dog ROSE with the scientific name *Rosa canina* L. is shrub, perennial and wild in dry areas, rocky plains and in the pastures. The most important substances are acids, phenolic compounds such as tannin. Fruits are famous for high vitamin C and antioxidant properties. They have high amount of carotenoid, lycopene, carotene and xanthophylls. These materials are used for treatment of patients with lung cancer. The purpose of this research is to study phytochemicals in different population of *R. canina* L. grown in Caspian climate. *R. canina* was collected in September and October, 2011 from three sites (totally 30 samples), in North of Iran (Mazandaran and Guilan Provinces). Results indicated that Harris province has the highest ascorbic acid (10.4 mg/g fresh weight) and total phenol (26.54 mg gallic acid/ml). Ascorbic acid showed a positive correlation with total phenol and flavonoid, but negative one with seed oil. The highest concentration of oil (11.1%) and flavonoid (0.59 mg routine equivalent/ml) was measured in Tonekabon. Correlation table revealed that these two parameters are positive.

**Key words:** *Rosa canina* L., ascorbic acid, total phenol, flavonoid, seed oil.

### INTRODUCTION

Dog rose with the scientific name *Rosa canina* L. is shrub, perennial and wild in dry areas, rocky plains and in the pastures. This is a native plant of Europe and North of Alborz Mountain in Iran. Its height depends on local climate, which is between 3 and 5 m. Some European countries released drugs such as viroma and diviroma in the drug market. Ingredients of this fruit can reduce uric acid (Omidbeigy, 2001; Zargari, 1996; Klasterska and Natarajaan, 1974). This is used for the treatment of inflammation of the kidneys. Oil seed of dog rose has many applications in personal care and cosmetics industry (Chrubasik et al., 2006). The most important ingredients of dog rose are natural antioxidants, acids and phenolic compounds such as tannins (Okuda, 2005). Recent studies showed that there is a close relation between the antioxidant compounds such as flavonoid and anti-cancer properties (He et al., 2006; Kharasmi,

2008). Chemical compounds that are found in fruits are phenolic compounds, anthocyanin, flavonols, ascorbic acid, and galactolipid (Nowak, 2006; Vriesendorp, 2007). Fruits are famous for high vitamin C, high antioxidant properties (Ercisli, 2007; Chrubasik et al., 2008), and also high amounts of carotenoid, lycopene, carotene and xanthophyll that are useful for treatment of patients with lung cancer (Liand and Tan, 1994; Winther, 2008).

Climatic conditions have unusual effects on different plants. Investigation of the role of climatic factors on the active ingredients of medicinal plants is necessary (Ercisli and Guleryuz, 2006). The most important environmental factors that have a major effect on the quality and quantity of active ingredients are light, temperature, rainfall, day length, latitude, soil characteristics, height, and nutrition (Serteser et al., 2008). Generally, climate consists of climatic and edaphic factors. The role and

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influence of each of them on growth, development, yield and the active ingredients of medicinal plants should be noted. Some chemicals which are produced in plants are affected by various environmental factors. The final products of metabolic activity are important and valuable (Chessa, 2005). Results showed that antioxidant activities of 50% aqueous methanol extracts of *R. canina* are mainly free radical scavenging and hydrogen peroxide scavenging activities (Serteser et al., 2008; Christensen et al., 2008). On the other hand, *R. canina* extracts are affected by the inhibition of growth and biofilm formation in methicillin-resistant *Staphylococcus aureus* (MRSA) (Quave et al., 2008). Such activities can be attributed to the different ingredients in this species: phenol, carotenoid, vitamin C, pectin, sugar, organic acids, amino acids and essential oils (Ercisli, 2007). Several factors such as genotype, climate, region, harvesting time and altitude, may depend on different chemical composition and consequently, activities. The purpose of this research is to study phytochemical compounds in different population of *R. canina* L. grown in Caspian climate. These compounds include ascorbic acid, total phenol, flavonoid and total seeds oil that are influenced by factors like genetic, physiological characteristics of plants and environment. This study examines the effects of climatic factors such as height and distribution of *R. canina* L. in unusual regions. For this purpose, samples were collected from three regions with 30 samples in each of them. The effect of climatic factors and relation between their productions was considered.

## MATERIALS AND METHODS

### Plants and growth conditions

Samples of *R. canina* were collected in September and October, 2011 from three sites (totally 30 samples) in North Iran (Mazandaran and Guilan provinces). Thirty mature fruits from each genotype were randomly chosen and measured. Rainfall and temperature of contour maps showed that the average rainfall and temperatures were the same (Figure 1 and Table 1). Temperatures ranged from 20 to 25°C. Fruit samples were placed in ice during transportation to the laboratory of Islamic Azad University, Tonekabon Branch (IAUTB).

### Determination of ascorbic acid contents

After the production of fruit juice (dissolving 50 g of fruit in 100 ml methanol), 1.5 ml of the extract was filtered with 0.5 ml dichloroindophenol and 0.1 ml metaphosphoric acid. Absorption was read in the wavelength of 520 nm by spectrophotometer. The concentration of ascorbic acid was calculated with a standard curve (Yam and Cheung, 2007).

### Determination of total phenol contents

The total phenol contents in the hydro distillation-aqueous phase extracts and soxhlet extracts were determined using the Folin-Ciocalteu reagent and gallic acid as standard as described by

**Table 1.** Information on the collection sites of the 30 populations of *Rosa canina* L. included in the analysis.

Site	Province	Altitude (m)	N
Ramsar-Javaherdeh	MAZ	1317-1644	10
Haris -Jenatroudbar	MAZ	1481-1635	10
Tonekabon- Dohezar	MAZ	816-1259	10

MAZ: Mazandaran, North of Iran.

Dewanto et al. (2002). The sample (0.5 ml) and 2 ml of sodium carbonate (75 g/L) were added to 2.5 ml of 10% (v/v) Folin-Ciocalteu reagent. After 30 min of reaction at room temperature, the absorbance was measured at 765 nm in a Shimadzu 160-UV (Tokyo, Japan) spectrophotometer. The results are given as gallic acid equivalent/ml extract.

### Determination of flavonoid contents

Flavonoid was measured according to Popova et al. (2004) with some adjustments. In short, 1 ml of plant extract or standard was mixed with 1 ml aluminum trichloride in methanol (2%) and the volume was made up to 25 ml with methanol. The mixture was left for 40 min and the absorbance at 420 nm was measured in a Shimadzu 160-UV (Tokyo, Japan) spectrophotometer. The results are given as mg routine equivalent/ml extract.

### Determination of seed oil

After separation and cleaning seeds, 15 g of each sample was milled and hexane was used as solvent. The oil of Soxhlet extraction was extracted for 6 h. The oil's percentage was measured.

### Statistical analysis

Obtained data was analyzed by correlation and completely random design, using SPSS software version 13 with the level of statistically significant  $P < 0.05$ .

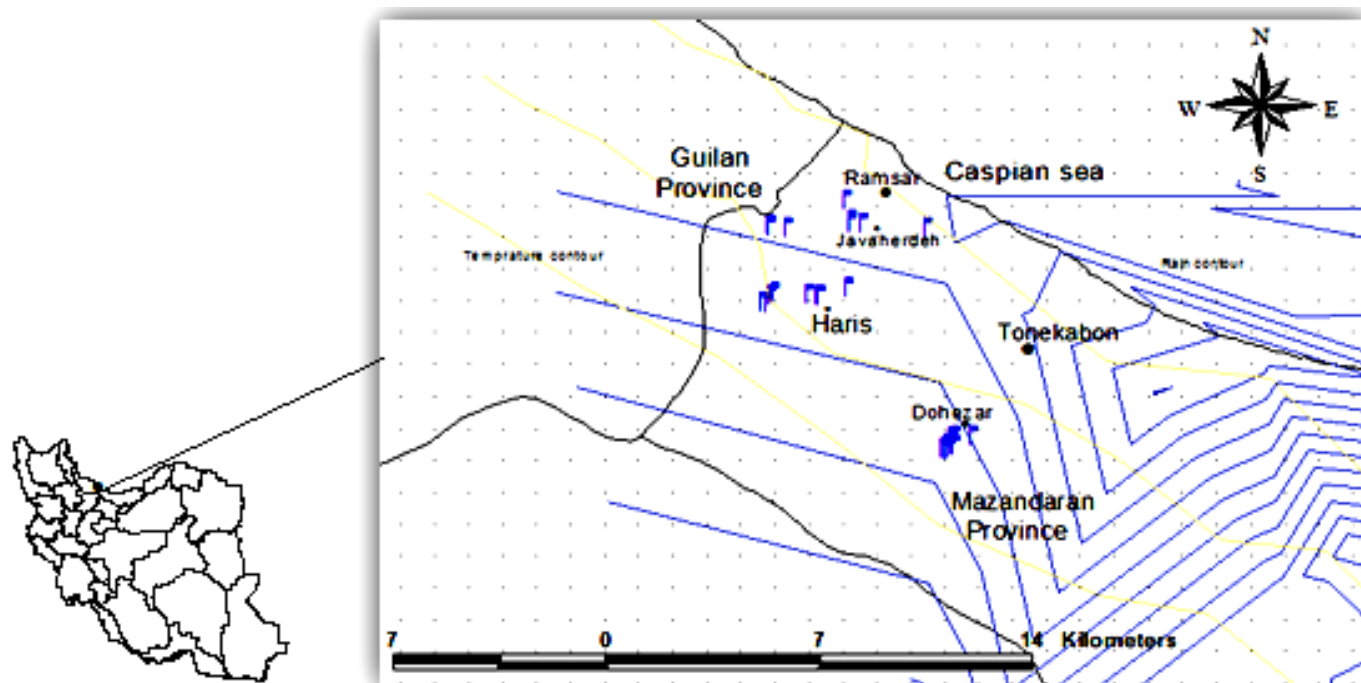
## RESULTS

Changes in ascorbic acid showed (Figure 2) that there was significant relationship between the samples and Haris (10.4 mg/g fresh weight) has the highest production in the fruit tissue. This parameter is according to the correlation table (Table 2), a negative correlation with the amount of oil and is positive against the total flavonoid and phenol. On the other hand, it has a good regression coefficient ( $R^2=0.72$ ) with total phenol, respectively that was expressed as  $Y=2.707+0.283X$ . This equation determines with change in the total phenol, can change ascorbic acid levels (Figure 3). Evaluating the change in total phenol was determined (Figure 4), such that changes in the areas were significant and the Haris produced (26.54 mg gallic acid/ml) the highest amount of total phenol. This parameter showed a negative correlation (Table 2) with the amount of oil and flavonoid. It means that with the increase in total phenol, the rate is reduced

**Table 2.** Correlation coefficient amount various traits.

Dependent variable	Total flavonoid (mg routine/ml)	Total oil (%)	Total phenol (mg gallic acid/ml)
Ascorbic acid (mg/g fresh weight)	-0.548**	-0.860**	0.994**
Total phenol (mg gallic acid/ml)	-0.561**	-0.869**	-
Total oil (%)	0.837**	-	-

\*\*Correlation is significant at the 0.01 level.



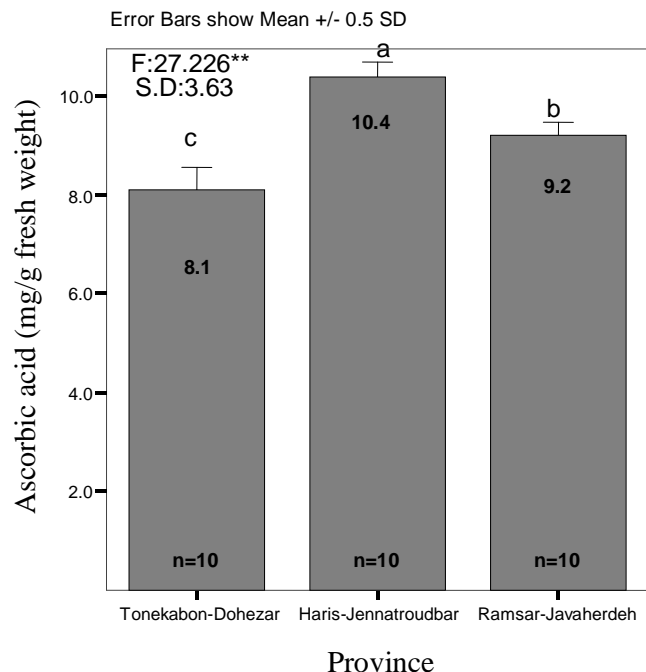
**Figure 1.** *Rosa canina* L. collected samples from north of Iran.

reduced in two variables mentioned. This relationship had been with regression coefficient ( $R^2=0.77$ ) for seed oil, which can be expressed with  $Y=41.09-1.93X$  (Figure 5), and with flavonoid ( $R^2=0.70$ ) and the equation  $Y=21.39+25.55X-42.43X^2$  (Figure 6). Measurement of flavonoid (Figure 7) indicates that the significant changes and maximum content were obtained in Tonekabon province (0.59 mg routine equivalent/ml). The correlation table (Table 2) showed that the variable amount of oil positively correlated with the regression coefficient ( $R^2=0.81$ ) which can be expressed with the equation  $Y=8.223-2.17X+10.13X^2$  (Figure 8). Varying the oil (Figure 9) specified that this variable is also significant and the highest percentage of oil (11.1%) was extracted in Tonekabon. The cluster analysis (Figure 10) indicates that ecotypes are divided into three groups. The first group includes samples that have the highest total phenolic and ascorbic acid, and the third groups are the highest seed oil and flavonoid.

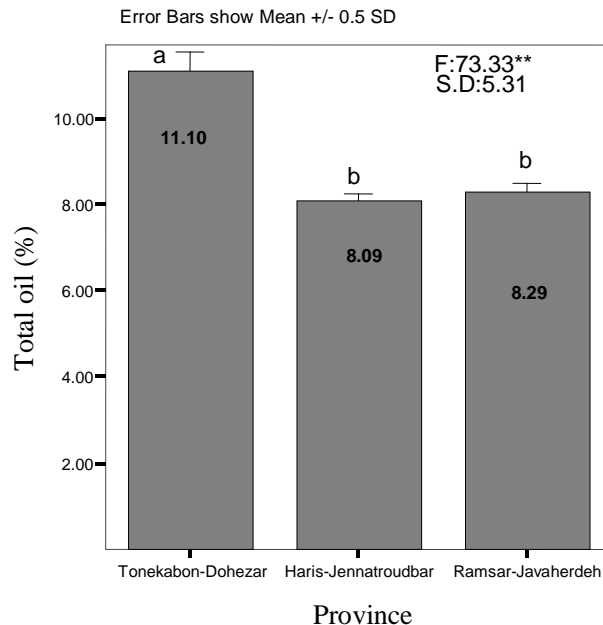
## DISCUSSION

Dog rose fruit (rose hip) is a great source of total phenol, vitamin C (Ascorbic acid), flavonoid, seed oil, sugar and mineral elements (Uggla et al., 2005). Rose hips can be used either fresh or dried. Fresh fruit utilization is for production of jam, jelly, marmalade, syrup, and soft drinks while the dried fruits and roots are excellent for making tea (Uggla et al., 2005). Changes of 106 to 2712 mg/100 g ascorbic acid in rose species were reported in the studies conducted in different agro-climatic regions of Turkey (Yoruk et al., 2008).

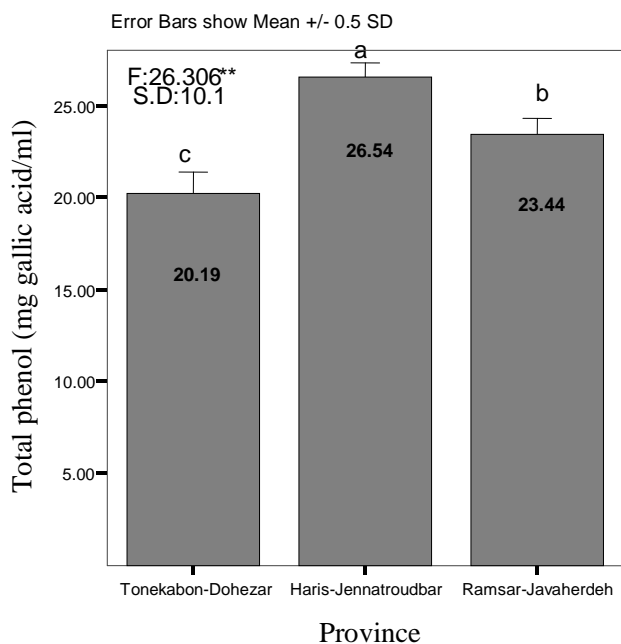
Reports showed that ascorbic acid amount in rose hip varied between 629 to 967 and 211 to 417.5 mg/100 g, respectively (Nojavan et al., 2008). The variations in ascorbic acid contents can be the result of different altitude, ecological factors, species, variety, and harvest time (Dogan and Kazankaya, 2006). The previous results which focused on seed oils of other rose species showed



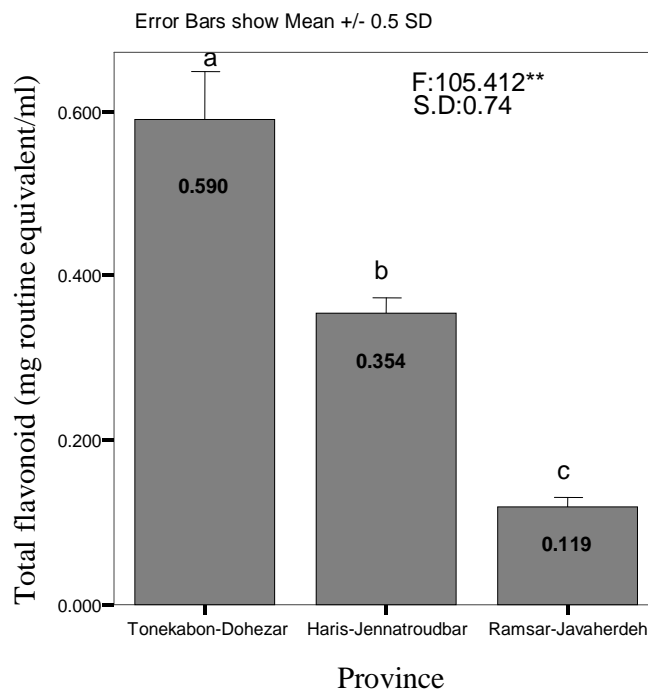
**Figure 2.** Variation of ascorbic acid (mg/g fresh weight) in provinces. Each bar represents a mean of ten individuals with standard deviations (SD) represented. Analysis of variances (ANOVAs) statistical analysis was performed by SPSS (version 13.0- SPSS Inc., Chicago, IL, USA) using Duncan's post hoc test ( $P < 0.05$ ). Bars are standard error.



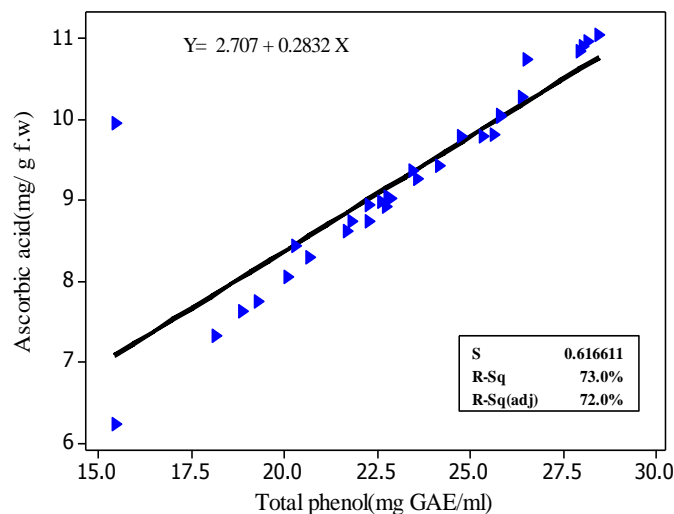
**Figure 4.** Variation of total oil (%) in provinces. Each bar represents a mean of ten individuals with standard deviations represented. Analysis of variances (ANOVAs) statistical analysis was performed by SPSS (version 13.0- SPSS Inc., Chicago, IL, USA) using Duncan's post hoc test ( $P < 0.05$ ). Bars are standard error.



**Figure 3.** Variation of in total phenol (mg gallic ac/ml) in provinces. Each bar represents a mean of ten individuals with standard deviations represented. Analysis of variances (ANOVAs) statistical analysis was performed by SPSS (version 13.0- SPSS Inc., Chicago, IL, USA) using Duncan's post hoc test ( $P < 0.05$ ). Bars are standard error.



**Figure 5.** Variation of flavonoid (mg routine equivalent/ml) in Provinces. Each bar represents a mean of ten individuals with standard deviations represented. Analysis of variances (ANOVAs) statistical analysis was performed by SPSS (version 13.0- SPSS Inc., Chicago, IL, USA) using Duncan's post hoc test ( $P < 0.05$ ). Bars are standard error.



**Figure 6.** Variations in amount of total phenol and ascorbic acid contents in collected samples. FW: Fresh weight; GAE: gallic acid equivalent.

that the most aggregation is in *R. canina*. Soner et al. (2009) reported the oil content of *R. canina* seeds to be 7.15%, while Demir and Ozcan (2001) found the crude seed oil contents of two different species belonging to *R. canina* as 1.2 and 1.6%. They suggested that rose seeds should be utilized instead of being wasted, because of their rich sugar and fatty acid contents (Yoruk et al., 2008). In previous researches, the total phenolic contents which were measured in the leaves of *Rosa* species were from 57 to 152 mg gallic acid equivalent/g dry weight (Nowak and Gawlik-Dziki, 2007). It is similar to current research. Such variability is because of several factors like altitude, developmental stage and climate. The most important factor is the level of phenol and flavonoid. These factors are also important on the type of flowers (Mohamed and Gerasopoulos, 1996; Toberman et al., 2008). This study has focused on analyzing the chemical composition of ascorbic acid, total phenol, flavonoid and seed oil from wild populations of *R. canina* collected in the North of Iran. Genetical, physiological, or environmental influences may affect them, but studies to specific influences, have not been well documented. Genetic ecotypes analyses will provide better results than a close relation between secondary metabolites, environmental factors and their interactions.

## Conclusion

The results of this study recommend that temperature, light, height and soil nutrients are important environmental factors to improve the efficiency of secondary metabolites' production in *R. canina* plants which are grown in different provinces and heights. These factors can significantly increase the ascorbic acid, total phenol,

flavonoid and seed oil concentrations. In this study, the superior ecotype was determined with height and location of growth. The investigation will be continued, and the ecotypes of different regions by molecular markers will be analyzed.

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