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Original Article

Study on Rooting and Changes of Some Secondary Metabolites of Dog Rose Grown in Lorestan Province

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Abstract

The dog rose medicinal shrub is one of the hardest rooting plants in Iran. This study was laid out in order to study on rooting and secondary metabolites of dog rose growes in different regions of Lorestan province. This study was performed in two experiments. In the first experiments, the effect of different concentrstion of IBA was evaluated on rooting ability of dog rose. This study was performed by factorial design with two factors: 1- cuttings at three levels (cuttings from regions including Abestan, Kakarza and Shul-Abad) 2- Indole-3-butyric acid (IBA) at 4 levels (0, 200, 1000 and 4000 ppm) with three replications in March 2016. In the second experiment, antioxidant ability and secondary metabolites produced by rooted cutting of dog rose cultivated in three region of lorestan province was studied. After rooting of doge rose hardwood cuttings were planted in two hot and cold regions of Lorestan province and secondary metabolites was measured after fruit harvesting. The results showed that the maximum rooting was recorded in 4000ppm IBA by 61, 59 and 60.5% for Abestan, Kakareza and Shul-Abad regions, respectively. In three regions, the minimum rooting percentage was recorded in control treatment by of 11.9, 8 and 11.3% for them, respectively. Also the higher number of roots and leaves per cutting was recorded in 4000ppm IBA. Based on the results doge rose was not entrance in fruit set stage in warm Tajere-Sadat region. At Ab-sefid and Kuh-Kela regions that fruit set was performed, highest phenolic and flavonoid was founded in fruit skin by 17.12 and 3.23% for Ab-Sefid and 16.72 and 2.29% for Kuh-Kela regions respectively. Also lowest condensed tannins (0.6 and 0.65%) and highest DPPH activity (362 and 454 µg/mL) was founded in dog rose fruit and seed at Ab-Sefid and Kuh-Kela regions, respectively.

Keywords: Antioxidant capacity, Condensed tannins, Flavonoid, Rooting

Introduction

Rosa sp mostly had about 200 species and 20,000 cultivars as wild rose type. This wild ornamental and medicinal plant is an important genus of economically floriculture industry. Dog rose (*Rosa canina* L.), is a perennial shrub and belongs to the Rosaceae family. More than one hundred species of *Rosa* have been reported so far. This shrub grows as wild plant in nature on rocks and bushes in some dry areas [1]. The largest distribution of these species is in Asia, Europe, the Middle East and the United States [2]. Iran is one of the most important centers of rose germplasm. It fruit is one to two centimeters long and contains numerous nuts that are surrounded by the ovary [2]. The fruit of dog rose is rich of vitamin C that is about 12 times more than oranges. This fruit contains other types of vitamins including A, B1, B2, B3 and K and pectin, tannins, malic acid, galic acid, citric acid, carotenoids, flavonoids and fiber [3]. Minerals of calcium, phosphorus, potassium, magnesium, iron, copper, zinc, etc. are also present in dog rose fruit significantly [4].

Roses as the main ornamental plants are one of the dominant cut flowers in the world [5]. Based on Izadi *et al* [6] roses are conventionally propagated by cutting and grafting more than other method, so that grafting and

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cutting are indeed the better propagation methods for commercial rose propagation. However, Balaj *et al* [7] reported that the for quick propagation of roses whereby cuttings of rootstock developed a technology by Netherland Scientists and grafting of the scion cuttings are performed in one action where wild rose varieties. However, the success of this technology depends on many factors such as types of cuttings, nutrient status and temperature age, portion of the branch, growing media and moisture condition [6]. In order to better rooting and establishment of cutting plants, the concentration of plant growth promoting hormones is very important. Now days, the main growth hormones like auxin are used commercially for rooting of many ornamental plants including roses [8]. The previous findings showed that to plant growth regulator most instrumental in rooting of cuttings is auxin [9]. Auxin hormone stimulates root growth and development in cutting plant. Indole-3-butyric acid (IBA) and Naphtalin Acetic acid (NAA) are two main derived auxin hormones that typically used for rooting of cuttings plants in many plant species like roses [10]. The effect of IBA and NAA depends on the concentration and the age of cutting plants [11].

Biological antioxidants are natural molecules that can prevent the uncontrolled formation of free radicals and activated oxygen or inhibit the reactions that carry out using those [12]. The Dog rose fruit has anti-inflammatory, antioxidant properties and medicinal products. The anti-inflammatory properties of the dog rose fruit have been attributed to a variety of substances, including vitamin C and galactolipids, as well as flavonoids, etc. [13].

All parts of the flowers, leaves and fruit of dog rose are found to possess antioxidant properties, anti-infectious, antimicrobial, antifungal and antipyretic activity [14]. In dog rose many components such as Tannin, polyphenols, Condensed tannins, Flavonoids, Terpenoids, Saponins etc. are present in it leaves, stem and root and fruit, which are in antioxidant system of this useful shrub [15]. These compounds are having natural habitat used for make of modern drugs [16]. However, an antioxidant is able to scavenging of free radicals in biological systems. There are many reactive oxygen species forms that are present in biological systems from a wide variety of sources and had dangerous effect on these biological systems. Some of dangerous effect of these free radicals may oxidize nucleic acids, proteins, lipids or DNA that can result to biological function block [15]. Some chemical compounds such as phenolic acids, polyphenols and flavonoids as non-enzymatic antioxidants can scavenge free radicals and block the oxidative damages in live cells [15]. The free radical scavenging are depend on activity of enzymatic and non-enzymatic antioxidants which can remove them. DPPH (1, 1-diphenyl-2-picryl-hydrazil) radical is one of the main antioxidant capacity indicator in live cells that can assesses the antioxidant properties in all live organisms. In biological studies, plant-derived antioxidant compositions such as phenolic compounds, flavonoids and condensed tannins may reduce the risk oxidative damages. Proper use of medicinal herbs requires accurate scientific information and understanding of the chemical constituents in them, as it is the presence of chemical constituents that cause the therapeutic effect of the herbs. Since dog rose is traditionally consumed in Iran and is endemic to the native species, it is important to study the secondary metabolites of these plants. Due to the appropriate geographical conditions of Iran, this has enabled the cultivation of different species of medicinal plants. Recently, special attention has been paid to medicinal plants such as dog rose shrub in the diseases treatment [17]. Therefore, the main aim of this study is rooting potential of dog rose cutting using different concentration of IBA and followed antioxidant study of different fruit parts of this main medicinal plant.

Material and Methods

This study was laid out in two separate experiments: rooting experiment and Biochemical study.

The farms were located in two location of Khorramabad (Abestan, Tajere-Sadat and Kakareza) and Aligudarz (Shul-Abad and Ab-Sefid) in Lorestan province, Iran. Aligudarz located in 33 05N and 49 29E with heath of 2022 m from sea surface. Also Khorramabad located in 33 29N and 42 21E with heath of 1200 m from sea surface.

Rooting Experiment

This experiment was performed in order to investigation of rooting in dog rose shrub by factorial design based on randomized complete block design with three replicates. The factors were hardwood cuttings in March and softwood cuttings in June, cutting region (including Abestan, Kakarza and Shul-Abad in farm) in Lorestan province and application of Indole-3-butyric Acid (IBA) at 4 levels (0, 200, 1000 and 4000 ppm) using hardwood cuttings of dog rose shrub.

Each experimental unit consists of 5 cuttings in which the cuttings will be planted in plastic pots containing animal manure, sand and soil at 3: 1: 1 ratio as rooting medium. The concentration of 2 per thousand solutions of varying concentrations are prepared separately and 50% ethyl alcohol is used as the solvent of the hormone and cuttings are treated with auxin for 10 seconds and were then treated with auxin hormone and the cuttings were transferred to the rooting medium. The planting bed was irrigated daily. After 2 to 3 months, all cuttings were removed from the planting bed and percentage of rooting

in each treatment, number of roots per cuttings; number of leaves in each cutting was measured.

Second Experiment

The second study was laid out in order to investigation of changes in secondary metabolites in dog rose using factorial design based on randomized complete block design with three replications. Factors were region (including Tajere-Sadat in farm, Kuh-Kela in farm and Ab-Sefid in nature) in Lorestan province and plant section (fruit, fruit skin and seed) . After that, the fruits were collected, air dried and prepared for chemical study.

Extraction

Amount of 5g of the sample powder was added into 2 mL Erlenmeyer flask and then add 5 mL of 2% methanol (the ratio of sample to methanol solution should be 1: 1). After 72 h for completing the extraction process, the sample mixture and methanol were filtered using a filter paper. The methanol solution was then transferred to a vacuum rotary apparatus to extract the methanol from the extract and concentrated. Finally, the pure extract was poured into a small vessel and used for further measurement of phenolic compounds, flavonoid compounds and antioxidant activity.

Total Polyphenol Content

The amount of 5g of dry plant material was homogenized in 50 mL of solvent solution [methanol 80%] for 72 h. The mixture was crossed from filter paper then used for measurement of total phenol, flavonoid and antioxidant capacity. Plant extract (0.5 mL) were mixed with 2.5 mL of 10 % Folin-ciocalteu reagent, and incubated at room temperature for 2 min. The samples were centrifuged prior to absorbance measurement. After cooling, the absorbance measured at 765 nm with spectrophotometer. An external standard curve was prepared using gallic acid (10–250 $mg.mL^{-1}$; Roth, Karlsruhe, Germany) (figure 1-A). Each determination was performed in triplicate [18].

Flavonoid measurement

In this experiment the total flavonoid content was measured by aluminum chloride chromatography. Amount of 0.5 mL of methanolic extract was added to 0.1 mL of aluminum chloride 10% in methanol and 0.1 mL of 1M potassium acetate (2.4 mL/10 mL of distilled water). Then 1mL of distilled water was added to a Falcon tube. The mixture was placed in the dark condition for a half hour at room temperature and finally absorbed at 415 nm [15]. The results were measured with reference to standard curve (Fig. 1-B).

DPPH Assay

A volume of 50 mL of various methanolic dilutions of the rose extracts and of ascorbic acid were mixed with 150 mL of a 100mM methanolic solution of DPPH. After 30 min absorbance of the samples were read at 517 nm in a microplate reader. Quercetin (EC_{50} 1.56 mg.mL-1) was used as a positive control. Each dilution was tested in triplicate [19].

Condensed Tannins Determination

To determination of condensed tannins 1% vanillin solution was prepared in 7 mM sulfuric acid in ice bath. Then 1mL of this solution was added to 1 mL of the extract and incubated at room temperature for 15 minutes. The absorbance of the samples was read at 500 nm. The calibration curve was plotted using the Katkin standard (Fig. 3-C).

Data Analysis

At the end of experiment, the data were analyzed using SAS statistical software version 9.1 and the means were compared using Duncan's multiple range test.

Results and Discussion

Rooting Study

The results of analysis of variance showed that the effect of IBA and location treatments were significant on rooting percentage, number of roots per cutting and number of leaves per cutting but their interaction was not significant on them (Table 1).

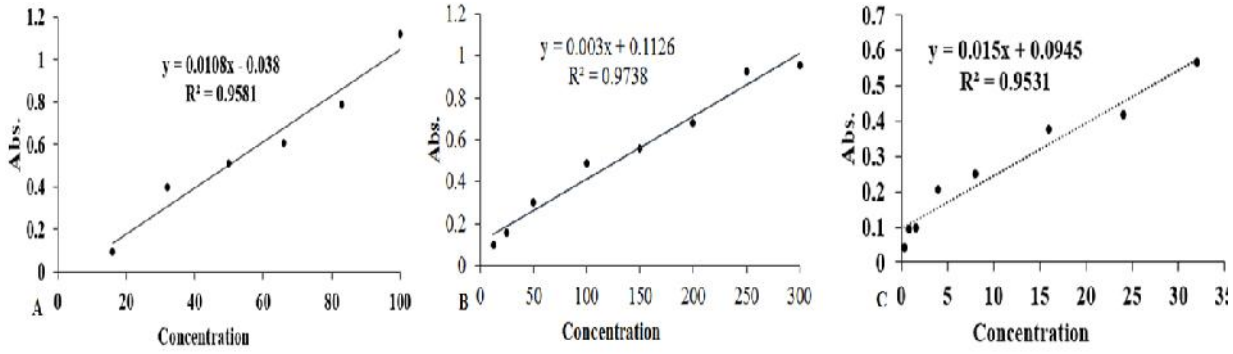

Fig. 1 Standard curves for measurement of total polyphenol content (A), Flavonoid (B) and condensed tannins (C)

Fig. 2 Rooting process in dog rose via application of IBA

Table 1 ANOVA table for rotting properties of dog rose under different IBA concentrations at three regions of Loretan province

S.O.V	Rooting percentage	Number of roots per cutting	Number of leaves per cutting
R	0.0065	0.55	0.21
IBA	0.054**	4.21*	1.55**
Location	0.032*	2.1**	0.9*
IBA*Location	0.009	0.62	0.32
Error	0.0075	1.1	0.4
CV	12.1	8.12	11.25

The results showed that rooting percentage increased with increasing of IBA concentration in three experimental regions. The maximum rooting percentage was obtained in 4000ppm IBA in amounts of 61, 59 and 60.5% for Abestan, Kakareza and Shul-Abad regions, respectively. The minimum rooting percentage was recorded in 0ppm IBA in rate of 11.9, 8 and 11.3% for Abestan, Kakareza and Shul-Abad regions, respectively (Figure 2 and Table 2).

There were no significant differences between three regions. The number of roots per cutting increased linearly with increasing of IBA concentration. The results showed that three regions of Abestan, Kakareza and Shul-Abad had same response to increasing of IBA concentration.

Among all integrated treatments, the maximum number of roots per cutting was founded in 4000ppm IBA at Shul-Abad region (14 roots per cutting). Among three regions, there are no significant differences for four concentration of IBA. However, the results showed that the minimum roots per cutting were founded in Abestan region at no application of any IBA concentration (2 roots per cutting) and had no significant differences in comparison with other regions (Table 2).

The results showed that in three regions the number of leaves per cutting increased linearly with increasing of IBA concentration. Although, there are no significant differences between three regions, in all regions the maximum number of leaves per cutting was founded in 4000ppm IBA concentration.

In Shul-Abad region the highest number of leaves per cutting (5.7 leaves per cutting) was obtained in 4000ppm IBA concentration and had no significant difference with 1000ppm IBA concentration (4.8 leaves per plant). Among all integrated treatments, the minimum leaves per cutting was recorded in Shul-Abad region and no application of any IBA concentrations (2.9 leaves per cutting).

Table 2 Mean comparison of rotting properties of dog rose under different IBA concentrations at three regions of Lorestan province

Region	IBA concentration (ppm)	Rooting percentage	Number of roots per cutting	Number of leaves per cutting
Abestan	0	11.9 d	2 c	3.2 c
	200	22 c	7 b	4.1 bc
	1000	43.3 b	8 b	4.8 b
	4000	61 a	11 a	5.1 a
Kakareza	0	8 d	3.2 c	3.2 b
	200	19.1 c	4.2 bc	3.5 b
	1000	39.7 b	6 b	4.4 a
	4000	59 a	12.7 a	4.5 a
Shul-Abad	0	11.3 d	2.5 c	2.9 c
	200	20 c	5.2 c	4.4 b
	1000	41 b	9.3 b	4.8 ab
	4000	60.5 a	14 a	5.7 a

There are no significant differences for means with same letters

In Abestan and Kakareza regions the minimum leaves per cutting was founded in no application of IBA in rate of 3.2 leaves per cutting for both regions (Table 2).

In the present study, rooting percentage, number of roots per cutting and number of leaves per cutting were increased significantly by increasing of IBA concentrations, so that in all three regions the maximum rate of rooting percentage, number of roots per cutting and number of leaves per cutting were obtained in 4000ppm IBA concentration (Table 2).

Among three regions, the maximum rooting percentage, number of roots per cutting and number of leaves per cutting were recorded in 4000 ppm IBA concentration at Abestan, Shul-Abad and Shul-Abad respectively. However, the growth of the above ground parts of dog rose was affected by all IBA concentrations and the differences between all IBA were significant for three measured traits.

Our results showed that the IBA affect the growth and development of dog rose. These effects can be used in the dog rose propagation industry in order to economic goals. Based on the results, by increasing the IBA concentrations, the rooting percentage, number of roots per cutting and number of leaves per cutting increased in three regions. The maximum rooting percentage, number of roots per cutting and number of leaves per cutting were founded in 4000ppm IBA concentration. The other researchers showed that different concentration of plant promotion hormone had different effect on rooting of plant spices [11, 20]. Among all auxins forms, IBA was the most common form used in the propagation and rooting of floricultural crops such as roses. As indicated in this study, the concentration ranges from 200 to 4000 ppm had a positive effect on the rooting, growth and development of both above-and underground parameters of dog rose cuttings. However the best rooting percentage, number of roots per cutting and number of leaves per cutting were obtained when rose cuttings were treated with 4000 ppm of IBA. These results confirmed by Younis [21] results that found increasing of IBA concentration gave the best result in the propagation of rose plants by cutting.

The results of present study clearly showed that treating of dog rose cuttings with 4000 ppm of IBA increased the rooting capacity and thus the growth and development of rose cuttings in three experimental regions Abestan, Kakareza and Shul-Abad. The untreated stem cuttings have shown the least rooting percentage, number of roots per cutting and number of leaves per cutting. The plant growth regulator such as IBA can stimulate the formation of adventitious root and increasing of roots and leaves per cutting on Canina rose and Dumalis rose [9], that in line with results of this experimental study.

Biochemical study

The results of analysis of variance showed that the effect of location and fruit section treatments were significant on phenolic content, flavonoid content, condenced tannins and DPPH but their interaction was not significant on all above treats (Table 3).

Table 3 ANOVA table for biochemical properties for dog rose fruit cultivated in different regions

S.O.V	Phenolic	Flavonoid	Condensed tannins	IC 50 DPPH
R	1.1	0.0006	0.0056	9.1
Location (a)	3.4^{*}	0.005^{*}	0.0091^{**}	41.2^{**}
fruit section (b)	2.5^{**}	0.0045^{*}	0.0082^{*}	52^{**}
a*b	1.59	0.0011	0.0045	21.1
Error	0.81	0.000011	0.00025	2.52
CV	7.5	8.33	5.3	3.6

The results showed that in Tajere-Sadat there was no fruit set for dog rose due to high warm conditions. The presence of phytochemical constituents was observed in fruit, fruit skin and seeds of dog rose cultivated at two regions of Ab-Sefid and Kuh-Kela.

The results of the present study showed that in Ab-Sefid region the maximum phenolic content was founded in fruit skin in rate of 17.12% and it difference was significant compared to fruit (14.5%) and seed (14.41%). The result for Kuh-Kela region shows that maximum phenolic content was recorded in fruit skin (16.72%) and minimum of it was recorded in seed (12.98%) (Table 4).

Table 4 Biochemical properties for dog rose fruit cultivated in different regions

Sample properties		Phenolic (%)	Flavonoid (%)	Condensed tannins (%)	IC 50 (µg/mL) DPPH
Ab-Sefid In nature	Fruit	14.50 ± 0.80 b	0.64 ± 0.27 b	0.60 ± 0.08 b	362.71 a
	Fruit skin	17.12± 0.55 a	3.23 ± 3.19 a	0.76 ± 0.12 b	253.67 c
	Seed	14.41± 1.23 b	0.54 ± 0.75 b	1.06 ± 0.16 a	328.27 b
Kuh-Kela In farm	fruit	13.48± 1.01 b	0.61 ± 0.44 c	0.89 ± 0.07 a	330.65 b
	Fruit kin	16.72± 0.60 a	2.29 ± 0.89 a	0.68 ± 0.02 b	286.08 c
	Seed	12.68± 3.05 b	1.69 ± 2.07 b	0.65 ± 0.11 b	454.72 a
Tajere-Sadat In farm	Fruit	0	0	0	0
	Fruit skin	0	0	0	0
	Seed	0	0	0	0

There is no significant difference for means with same letters

In dog rose fruit flavonoid content was measured in skin and seeds. The results showed that fruit skin had the highest flavonoid content in rate of 3.23% and 2.29% for Ab-Sefid and Kuh-Kela regions, respectively. Among the all integrated treatments of region and fruit parts, the lowest flavonoid content was recorded in Ab-Sefid region at seed part of fruit (0.54%). In our research condensed tannins percentage was determined in three parts of dog rose fruit at Ab-Sefid and Kuh-Kela regions. Based on the results, in Ab-Sefid region, the maximum condensed tannins percentage (1.06%) was recorded in seeds and the minimum amount of condensed tannins percentage was founded in fruit of dog rose (Table 4). However, in Kuh-Kela region, the maximum condensed tannins percentage was recorded in fruit (0.89%). These results revealed that condensed tannins content is depend on environmental conditions.

In the present study, the free radical scavenging activity of dog rose was studied by its ability to reduce the DPPH, a stable free radical. DPPD free radical is the main index for assessing of antioxidant defense system in live organisms. In the present study, the maximum DPPH was recorded in fruit (362.71 µg/mL) and seed (454.72 µg/mL) in Ab-sefid and Kuh-Kela, respectively. These results shows that DPPH antioxidant properties in depend of region with different environmental conditions. In both Ab-Sefid and Kuh-Kela regions, the maximum phenolic, flavonoid and condensed tannins was recorded in fruit skin. These results showed that DPPH antioxidant activity had more depended on other antioxidants such as enzymatic antioxidants, which was not measured in the present study.

We concluded that condensed tannins was present in scarce concentration but and phenolic and flavonoid were found to be present in high concentration in fruit skin in both Ab-Sefid and Kuh-Kela regions. In two different regions, thefruit skin found to possess maximum amounts of phenolic and flavonoids, while the maximum condensed tannins was found in seed and fruits at Ab-Sefid and Kuh-Kela regions, respectively. However, based on the obtained results, there were significant differences between different parts of fruits in dog rose for DPPH antioxidant activity. However, the amount of total phenolic content varies were between 14.41-17.12% and 12.68-16.72% for Ab-Sefid and Kuh-Kela regions, respectively and the total flavonoid amount is 20.54-3.23 and 0.61-2.29% for them respectively in dog rose. However, with regard to this that condensed tannins varies between 0.6-1.06% and 0.65-0.89% for Ab-Sefid and Kuh-Kela regions respectively, DPPH was varies between 253-362 µg/mL and 286-454 µg/mL respectively for those regions. In a same experiment, total phenolic and flavonoid content of *Rosa* × *damascena Herrm.* analyzed using type plant materials and founded same results and they told that the waste byproducts of rose include of fat rose can be used for natural antioxidant resources [1]. However, these findings are in line with results of Dilay and Özlem [22] on dry rose tea and their evaluation of antioxidant property of this medicinal and ornamental plant species. Also Sener [23] told that the fresh rose contains high levels of phenolic material that can use for medicinal goals. Ercisli [24] founded that in 6 different rose species phenolic substance values was varies between 73-96 mg GAE/g dry substance and *Rosa canina* L. had the highest value. However, in the present study on *Rosa canina* the highest phenolic content was in fruit skin for both regions and it difference was significant in comparison with other fruit parts.

Conclusion

The result of present study indicated that fruit set process in dog rose was blocked under high temperature condition. Based on the results, increasing of IBA result to increase of dog rose rooting in rates of 61, 59 and 60.5% for Abestan, Kakareza and Shul-Abad regions

respectively. Also results shows that highest phenolic and flavonoid was founded in fruit skin by 17.12 and 3.23% for Ab-Sefid and 16.72 and 2.29% for Kuh-Kela regions respectively. Also lowest condensed tannins and highest DPPH activity was founded in dog rose fruit and seed at Ab-Sefid and Kuh-Kela regions respectively. The present study revealed that by cultivation of dog rose in coold regions can achieve to higher rooting and antioxidant property to extract it for medicinal purposes.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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