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Investigation of Antioxidant and Antibacterial Characteristics in Cydonia Leaves Extract

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ABSTRACT

Aim and background: *Due to increasing antibiotic resistance, it is important to identify the antimicrobial and antioxidant activity of herbs. The aim of this study was to evaluate the antioxidant and antibacterial activity of the leaf extract in Cydonia oblonga plants.*
Material and method: *The leaf extracts of Cydonia oblonga was collected from Dohezar, Tonekabon city. The antioxidant properties of these extracts was determined by DPPH method. In addition, the amounts of phenol and flavonoid was assayed by Folin-ciocalteu and Aluminum chloride methods, respectively. The antibacterial activity of leaf extracts of Cydonia oblonga versus Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa was determined by disc and agar well diffusion methods.*

Results: *S. aureus and B. subtilis showed higher susceptibility to Cydonia oblonga extracts compared to E. coli and P. Aeruginosa in both of antibacterial susceptibility testing. The leaf extracts of Cydonia oblonga in concentration of 250 mg/ml had the highest scavenging percentage of free radical (DPPH =28/96%). The leaf extracts of Cydonia oblonga had the highest amount of phenol and flavonoid, 4233/235 (mgGAE/g) and 267/351 (mgQ/g), respectively.*
Conclusion: *The results of present study showed that the leaf extracts of Cydonia oblonga, showed antibacterial ability. Since the phenolic and flavonoids compounds have antibacterial and anti-oxidant properties, the property showed in this study can be because of the presence of these compounds in the tissues studied.*

Key Words*: Cydonia oblonga, antibacterial activity, antioxidant property*

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INTRODUCTION

Today, several countries are seeking important compounds and plants that are helpful in the treatment of disease , or that can be used in various industries as a natural substance. Our country is rich in important plant species (Palaniappan K, Holley RA, 2010). Despite, investigations on medicinal and aromatic species in country, there are many fields to be studied regarding recognition of important compounds or species. In addition to microorganism's growth inhibition compounds, plants contain other valuable compounds, too; known as antioxidant (Khan R, Islam B, Akram M, Shakil S, Ahmad AA, Ali SM, 2009). The antioxidants are those type of compounds neutralize metabolic active products chemically, like free radicals that can hurt body. Due to free radical's potential activity as antioxidants, they can play an important role preventing from cancer and cardiovascular and neurological disease (Chun S-S, Vattem DA, Lin Y-T, Shetty K ,2005). The significance of new antibacterial medicine exploration can be indicated when various bacterial species are always being changed and mutated genetically leading to emergence of bacterial resistant strains, being resistant to most of common antibiotics, sometimes (Preethi R, Devanathan VV, Loganathan M ,2010). Applying botanic compounds is an ancient approach in while regions of world particularly in developed countries, to treat the infections. Focus on medicinal plants with their antibacterial properties could rehabilitate the common problems in using the antibiotics. The plants extracts are reported as significant factors of naturally antibacterial (Lai P, Roy J,2004; Oliveira AP, Pereira JA, Andrade PB, Valentão P, Seabra RM, Silva BM ,2007). Among dark plant extracts, Rosaceae are indentified as antibacterial extracts along with definite chemical compounds. There are antibacterial and antioxidant compounds in cydonia fruit extract that are proved in many cases to be helpful and the extracts also contain antibacterial compounds like phenolic compounds in abundance. Cydonia contains an average of 10% of sugar Amygdalin, pectin, Malic acid that is an ether with a strong smell up to 0/09 per each kilogram of Tannin (Oszmianski J, Wojdylo A, Lamer-Zarawska E, Swiader K,2007; Minaiyan M, Ghannadi A, Etemad M, Mahzouni P. A ,2012). The fruit cydonia, contains Vitamins B,C, PP and provitamin A. cydonia leave also contains a glycoside producing cyanidric acid in a relatively low value. In pharmacology, Cydonia has a tonic effect in stomach's performance and stops simple and dysentery diarrhea (Ivanova D, Gerova D, Chervenkov T, Yankova T ,2005; Wojdyło A, Oszmiański J, Bielicki P,2013). Its helpful effects are emerged in acute inflammation of intestines, blood clots, uterine bleeding and hemorrhoids and vaginal transpiration. It stops vomiting and reduces saliva secretion. Because of having a little of glycoside of cyanidric acid production, it is analgesics and sedative, and it is usually consumed as brewed. It is effective in healing whooping cough and insomnia (Shulaev V, Korban SS, Sosinski B, Abbott AG, Aldwinckle HS, Folta KM,2008; Alamhulu

M, Nazeri S,2015). The present study aims to investigate antibacterial and antioxidant properties in cydonia oblonga extract.

MATERIALS AND METHOD

Plant collection:
Cydonia Oblonga was collected in the region of Ghale Gadan and Dow Hezar in Tonekabon in mid November, in 2012. The region of Ghale Garden was measured to be 80 m higher from the sea, 36°, 45′, 25″ of northern latitude and 50°, 50′, 50″ of eastern longitudes and Dow Hezar was measured to be in 524 m higher from the sea and in 36°, 39′, 31″ of northern latitude and 50°, 48′, 13″ of eastern longitudes by the device GPS Map 78s. The leaves were exposed to dry air in the shade, they were powdered then and were used to produce extract.

The used extract in non-enzymatic antioxidant tests:
The device suckcele was used for extraction. To do it, an amount of 30 to 40 gr of the plant was wrapped in filter paper and was put in the middle of the device. The solvent used here is Methanol, poured in balloon separately, and then it was put on heater with mild temperature after making inlet and outlet water flow. Extraction took about 3 to 4 hours. The coming extract was put at 35 to 40°C for an hour to remove solvent. Ultimately, a viscous extract was made. It was in fridge at 4°C until the time it is used. The product extract is prepared for related tests to non-enzymatic antioxidant activity (phenol and flavonoid) (Alamhulu M, Nazeri S , 2015).

ANTIOXIDANT METHODS

Determination of flavonoid content:
In order to measured flavonoid, 0/5 g of powdered plant was put in 3 tests tuber for 3 times. An amount of 10cc methanol was added in each tube, and the tubes were placed for centrifuge after 24 hours, at 400rpm for 20 minutes. After centrifuge, the surface liquid was removed and moved to another container. An amount of 10cc of methanol was added into the remained extract in the tube and centrifuge was done once more. Next, the surface liquid was added into the previous solution. An amount of 0/5ml from the main solution was taken and 1/5ml of Methanol and 0/1ml of 10% Aluminum chloride and 0/1ml of 1M Potassium acetate and ultimately, 2/8ml of distilled water were added to the main solution. After mixing, they were put at room temperature for 30 minutes. In the last phase, the intended compound's absorption amount was reported by spectrophotometer in the wavelength of 415 NM. Quercetin was used to draw the quercetin calibration curve as a standard. Amount of flavonoid was reported based on the amount of quercetin mg per extract mg. the tests were repeated 3 times and the average was announced (Alamhulu M, Nazeri S , 2015).

Phenol Measurement:
The content of phenol compounds was investigated by the method Folin-cioCalteu. A density of 10mg/ml of each extract was made and then 0/5ml of phenol was mixed with 2/5ml of extract. The absorption in samples was measured in wavelength of 765Nm against (Methanol). The result were announced as equivalent values using the standard Gallic acid. In the equation, the drawn line in curve drawing is Gallic acid. The result was reported as the content of total phenol in extract based on equivalent value of Gallic acid mg per extract mg (Alamhulu M, Nazeri S , 2015).

Investigation of antioxidant properties and concentration method:
Antioxidant property or trapping free radical activity was studied based on the method of Brand et al. in this method, first, 0/5 g of intended plant power (leaf) was poured in test tube in 3 repetitions. An amount of 10cc of Methanol was added into each, and after 24 hours, the three tubes were put on centrifuge for 20 minutes. After centrifuge at 4000 rpm, the surface liquid was removed from tubes and poured into glass containers that were already measured for weight. Next, an amount of 10cc of Methanol was added to the remained extract in the test tube and it was put in centrifuge once more for 20 minutes, the surface liquid was poured into already weighted tubes the same containing primary solution. Then, they were put in oven for the Methanol to skip and a dry extract to remain. Afterwards, the containers of dry extract were measured for weight again and the final weight of extract was gained through the following formula:
The final extract weight = weight of empty tube - weight of extract holding tube = extract weight
In following, the final weight of extract gained by above formula was put in following formula for concentration in four concentrations (150,100,200,250):
Final weight of extract X-10 ^{x} (250,200,150,100) - the value of each concentration ÷ 1000 = the final number
The final number that is gained through above formula, was taken out of the Mother solution and was put into tubes in intended concentration values. All were mixed by 10cc of Methanol and next, 0/1cc of the final solution was removed and mixed with 3/9cc of DPPH solution (25ppm), the tube was wrapped with foil to prevent the solution from entering dark phase. After some time, the extract absorption was studied in the wavelength of 517NM by the device spectrophotometer. After taking out the tubes from the dark environment, the solution would possess antioxidant properties if the color is changing from purple to yellow. Filially, the values that are created were put in below formula to reach a percentage of trapping free radicals:
$(A_{\circ} - As) / A_{\circ}$ 100X – (1%)
$A_{\circ}$ is the control absorption, As is tested extract absorption and AIC50 is the concentration of the extract, in which 50% of the free radical trapping occurs in it. Methanol was used as blank (Alamhulu M, Nazeri S , 2015).

Determination of bacterial sensitively to extract using the method disk-diffusion:
In order to make Methanol extract the method suckcele was used. The solvent was dropped out using oven. These extract were assumed as the pure ones. In this method, disks containing 10, 20, 30, 40 and 50µl of pure extract were put on Mueller-Hinton agar medium using swab after bacteria's culture (bacteria concentration equaling to half MC Farland 1/5 ^{x} 10^{8} CFU/ml). Afterwards, plates were incubated at 37°C for 24 hours. Sensitively or resistance of intended bacteria to extract was determined by measuring clear zone around the disks for 24 hours. To measured sensitivity, the following microbial isolates were used: staphylococcus aureus PTCC 112, E.coli PTCC1330, Pseudomonas aeruginosa PTCC 13/0 and Bacillus subtilis PTCC 5027 (Alamhulu M, Nazeri S , 2015).

Determination of bacteria sensitivity to extract using wells:
A number of 5 wells were provided in related inoculated mediums in equal and definite intervals, an amount of 60,70,80,90 and 100µl of the extract were poured in the wells, and after a while, extract penetrated into medium. The time and the temperature of bacteria incubation were 24 and 37, respectively. After incubation, growth or lack of growth around

the wells was investigated (Quiroga EN, Sampietro AR, Vattuone MA,2001).

Statistical analysis:
For statistical analysis and comparison of averages in the independent communities, the test ANOVA and for comparison of two communities, the test Duncan was used. Data description and diagram drawings were done using the software SPSS.

Results:
Analysis of variance and comparison of plant antioxidant property averages:
DPPH free radical scavenging activity in analysis of variance in the concentrations of 100, 150, 200, 250µl indicated that the effect of factor of types of leaves (P<0/01) on DPPH free radical scavenging was on the level of 1% and it was meaningful probably up to 99 percent. (Figure 1).
The total phenol and flavonoid in plant:
The findings from the present study suggest that the types of leaves (P< 0/05) had a meaningful effect of 0/05 of the level on the total amount of phenol and flavonoid. In other words, the total phenol and flavonoid in the leaves of trees under study had a meaningful difference (figure 2 and 3).
The results of determining antibacterial sensitivity of Cydonia plant by methods disk diffusion and well diffusion:
The bacteria under study were divided into two groups by comparing the average of data using the method Duncan. It means that each group of bacteria show a different reaction to the used extract, and the groups have a meaningful difference in this regard. Accordingly, the maximum diameter of growth zone for staphylococcus aureus and Baciluus subtilis was averaged by 10/53 and 9/93mm, respectivly and the minimum diameter of non-growing zone for E.coli and Pseudomonas aeruginosa were recorded to have no value (no zone). The results of extract sensitively in cydonia on the studied bacteria using the wells indicated that the maximum diameter of non-growing zone for staphylococcus aureus, Bacillus subtilis, Pseudomonas auruginosa was 18/40, 18/13, and 11/20mm, respectively and the minimum diameter of non-growing zone for E.coli was recorded as an average of 1/53mm. (Table 1 and figure 3).

Discussion:
The present study aimed to investigate antioxidant and antibacterial properties in cydonya oblonga extract. The results from the present study indicated that cydonia oblonga had 235/1mg of phenol and 235/42mg/mg of flavonoid. Cydonia extract is able to restrain 96/28 percent of DPPH free radical that is the maximum amount recorded in the concentration of 250mg/l in the related extract from the region Dow Hezar in Tonekabon. The studies exhibit that high amounts of phenolic compounds are the main cause high antioxidant activity in some extracts including Methanol and Ethanol ones. Because there is a positive relation between phenolic compounds and plant antioxidant strength, based on evidence. Moreover, Phenolic compounds act as hydrogen provider more effectively; thus, they act as an effective antioxidant (Wang SY, Lin H-S,2000). Gawrom-Gzella et al. (2012) studied the DPPH free radical scavenging power and phenolic compounds in three types of leaf extract in 3 species of Polish raspberry from leaf extract in 3 species of Polish raspberry from the plants (R. fabrimontanus (Sprib.) Sprib. and R. capitulatus Utsch. Rubus kuleszae Ziel belonging to the family Rosaceae and to the family of the plant under study. All the extract studied, including Methanol extract, showed a high inhibiting strength of DPPH. Among them, the species Rubus Kuleszae had the maximum strength in inhibition. The total phenol and phenolic compounds were investigated by the method spectrophotometer. The results indicated that antioxidant activity and amount of phenolic compounds have a positive correlation (Gawron-Gzella A, Dudek-Makuch M, Matławska I , 2012). Wang and Lin (2000) studied antioxidant activity in raspberry leaves and some other plants belonging to Rosaceae family and relative of cydonia, in different phases of development. The extract from these plant leaves were assessed regarding the total extent of phenol. The results suggested that the younger leaves contain more total phenol and there is also a positive correlation between the total amount of phenol and antioxidant capacity of extract Wang and Lin , 2000). Gudej and Tomczyk (2004) measured extent of flavonoid in leaf extract of the species raspberry and blackberry from the family Rosaceae and relation of cydonia. The results indicated that the amount of measured flavonoid in blackberry was observed to be higher than raspberry(Gudej J, Tomczyk M,2004). Based on a study done by Costa et al. (2009) on the ability of DPPH free radical inhibition by cydonia oblong, leaf extract this fruit was able to inhibit DPPH free radical up to 21/6 ± 3/5 mg/ml. Brancka et al. (2004) tested the antioxidant properties. In the fore-mentioned study, the extent DPPH free radical inhibition was studied by Methanol extract of the fruit cydonia (Costa RM, Magalhães AS, Pereira JA, Andrade PB, Valentão P, Carvalho M, et al,2009). Similar to findings of these researchers, Methanol extract of the fruit cydonia peel, Methanol extract of the fruit cydonia flesh and cydonia stone, could inhibit DPPH free radical. The results of previous studies are consistent with the present study, meaning that leaf extract of plants from Rosaceae family possess an antioxidant property. In addition, the findings from the present study suggest that cydonia plant extract resulting from samples collected from the region Dow Hezar in Tonekabon had the most impact on staphylococcus aureus and the least impact on Psedumonas auruginosa. Less impact of plant extract on the bacteria Psedumonas auruginosa may be due to existence of lipo polysaccharide in external membrane of gram-negative bacteria, who makes them resistant to external factors naturally, like antibiotics, detergents and hydrophilic colors (Khan R, Islam B, Akram M, Shakil S, Ahmad AA, Ali SM, et al,2009; Nascimento GG, Locatelli J, Freitas PC, Silva GL,2000). Silvia Oliveira (2013) studied antibacterial activity. The effect of fruit and leaf extract in different concentrations was studied on 6 bacteria like staphylococcus aureus, E.coli and Psedumonas auruginosa. The researcher reports that all three mentioned bacteria were resistant regarding antibacterial activity of extract and from among them, only cydonia Oblonga extract affected on staphylococcus Galaktya and led to emergence of an insignificant zone diameter against these bacteria (Silva F, Oliveira G ,2013). Due to results from above-mentioned studies and researches, it can be announced that the plants from the family Rosaceae possess antibacterial and antioxidant properties against some bacteria of gram-positive pathogen.

CONCLUSION

The results from the present study indicate that cydonia Oblonga extract possesses a high capability regarding antioxidant features, due to 96/28% inhibition of DPPH free radical. Besides, the results from the present study exhibit that cydonia Oblonga extract was more effective in Gram-positive bacteria comparing to Gram-negative ones.

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Figure 1) The extent of free radical inhibition by Cydonia Oblonga extract in the concentration of 250.
Figure 2) The value of total phenol in Cydonia Oblonga extract.
Figure 3) The amount of flavonoid compounds in cydonia 6-leaf extract.
Figure 4) The results of determining cydonia Oblonga sensitivity on various bacteria by two methods of disk and well diffusion A) Staphylococcus aureus B) Bacillus subtilis C) Psedumonas auruginosa and D) E.coli

Table 1) The results of average comparison of non-growing zone diameter caused by studied bacteria in the extract related to samples in the region Dow-Hezar, using the method Disk Diffusion.

Bacteria	Disk diffusion	Well Diffusion
	Non-growing zone diameter (mm)	Non-growing zone diameter (mm)
E.coli PTCC1330	0/0	1/53
Staphylococcus aureus PTCC 112	16/87	18/40
Bacillus subtilis PTCC 5027	12/13	18/13
Psedumonas auruginosa PTCC 1310	1/07	11/20