International Journal of Natural Sciences: Current and Future Research Trends (IJNSCFRT)
ISSN (Print) , ISSN (Online)

https://ijnscfrtjournal.isrra.org/index.php/Natural_Sciences_Journal/index

Evaluation of Antioxidant and Antibacterial Activities of Different Solvent Extracts of Quince Fruit and Leaf

Gulnur Arabaci[a]*, Inam Muayad Abdulmaged Al-Sabbagh[b]

[a,b]*Department of Chemistry, Faculty of Science and Arts, Sakarya University, Serdivan-Sakarya 54187, Turkey*
[a]*Email: garabaci@sakarya.edu.tr*

Abstract

This study aims to determine the antioxidant and antibacterial effects of quince (Cydonia oblonga) and quince leaf extracts grown in Sakarya and used in the treatment of various diseases with various solvents. Dried quince and leaves were extracted with ethanol, acetone, and ethyl acetate solvents, and their antioxidant activity was determined with three different methods. These methods are; DPPH free radical removal activity, iron ion chelating, and reduction capacity methods. As a result of the experiments; among the tested substances, the highest DPPH activity in ethanol was determined as 95.282% (±0.01) with quince fruit substance. The highest activity of quince fruit substance in acetone with a value of 68.250% (±0.01) in its capacity to chelate iron (II) Ions. The highest reducing capacity ratio among ethyl acetate extracts was determined as 3.91 mg/mL of quince leaf extract. In addition, the antibacterial properties of the quince and leaves were determined by the agar well diffusion method against E.coli, B. Cereus and S. aureus bacteria. According to the results obtained, the material of the quince fruit showed effective antibacterial properties against all bacteria, the diameters of which ranged from 10 to 20 mm, and the material of the quince leaf, the diameters of which ranged from 18 dec19 mm. The highest quince fruit ratio in acetone solution is 16 mm zone diameter found against B. Cereus, while its highest ratio is 20 mm zoned against E.coli.

Keywords: Quince antioxidant; DPPH; ferrous ion chelating; reducing power; antibacterial.

1. Introduction

Oxygen, which is the basic requirement for aerobic respiration, can cause significant damage to the cell during oxidation. While oxygen entering the body produces energy through oxidative phosphorylation, it can also form reactive oxygen species (ROS), which are sources of free radicals, and these species can have a toxic effect for living systems [1]. Free radicals are highly reactive, short-lived compounds that have the ability to interact with organic and inorganic molecules that carry unpaired electrons in their outer orbits [2, 3].

---

* Corresponding author.

The most important free radicals are the superoxide radical ($O_2^{\bullet -}$), the hydroxyl radical ($^{\bullet}OH$), singlet oxygen ($^1O_2$), and the nonradical addition of hydrogen peroxide (H_2O_2) and peroksinitrite ($ONOO^-$), as well as "reactive oxygen species (ROS). ROTS can easily react with biological molecules such as lipids, nucleic acids, proteins and carbohydrates in the living organism and damage them. Thus they can cause many diseases such as cancer, cardiovascular diseases, immune system diseases, cataracts, diabetes, kidney and liver diseases [4, 5, 6]. Antioxidant defense systems are available to eliminate or slow down the negative effects of free radicals in living organisms. The antioxidants stop the chain reaction that they start by interacting with free radicals that form for various reasons in the cell and damage the cell, or slow down its progression, and therefore they are essential in the body. They are defined as molecules that prevent component damage. There is an antioxidant defense system against various oxidants that cause oxidative stress in the organism. This antioxidant defense system acts by preventing the overproduction of free radicals, reducing the effect of free radicals that do occur, or either reducing or repairing the oxidative damage that does occur. These systems include endogenous antioxidant enzymes such as SOD, CAT and GPX, metal binding proteins such as GSH, ceruloplasmin and transferrin, some antioxidant elements such as Zn and Fr and antioxidant vitamins such as A, C and E [7]. The increase in the damage caused by free radicals in recent years has led to an increased interest in substances with antioxidant properties. At the same time, interest in plant-derived antioxidants has increased due to the side effects of synthetic antioxidants used today [8, 9]. Another factor that threatens human health is harmful microorganisms that arise due to environmental pollution and neglect of diet. One of the factors that inhibit the growth and reproduction of these harmful microorganisms are antibacterial agents and antibiotics. Currently, diseases caused by bacteria microorganisms have developed resistance to these antibiotics due to their excessive use and abuse of existing antibiotics used in treatment. Increasing the resistance of bacteria to existing antibacterial agents leads researchers to develop new antibacterial agents [10]. Antibacterial agents are also known today as the most common agents for disinfecting surfaces and destroying potentially harmful bacteria. Antibacterial agents have been used for many years and continue to be effective agents in the control of pathological organisms in a variety of healthy and home settings. Antibacterial agents have proven effective in controlling bacterial and fungal infections in clinical settings such as hospitals, nursing homes, neonatal care rooms, and other healthcare facilities where the risk of infection may be high [10]. It has been reported that there is an important link between the increasing consumption of fruits and vegetables and its importance for many diseases, especially cancer and cardiovascular disease. This association is believed to be due to phenolic compounds with antioxidant properties, vitamin E, carotenoids and ascorbic acid found in fruits and vegetables [11]. It has been reported that a diet rich in fruits reduces oxidative damage to DNA and therefore plays an important role in cancer prevention by preventing oxidative stress [12]. Quince (*Cydonia oblonga* Miller) is one of the oldest fruit known as a plant from the Rosaceae family and has been cultivated since the time of the ancient Greeks and Romans. It is considered to be a good and cheap natural source for antioxidants including phenolic acids and flavonoids [13]. The quince has a refreshing taste and aroma. It is one of the fruits with a high pectin content and can get very well due to bacterial substances. Quince fruit can have a bitter taste due to its high tannin content, which can have a negative effect on the consumption of the fresh fruit [13]. Quince is a nutritious fruit rich in vitamins, minerals and sugars. It is also said to be a good source of fiber, potassium, and vitamin C [14]. In the present study, quince fruit and leaves (*Cydonia oblonga*) were collected from the Sakarya region of Turkey and extracted with ethanol, acetone and ethyl acetate. Then the extracts were investigated for

their antioxidant activities by using most common three different antioxidant methods: DPPH free radical scavenging activity, chelating activity of iron ions and reducing power activity methods. In addition, their antibacterial activities were determined by using Agar well diffusion method.

2. Material and Method

2.1. Materials

Quince fruit and leaves (*Cydonia oblonga*) were collected from the Sakarya region and they were immediately cleaned, dried, and then started the analysis. The chemicals used were obtained from Sigma-Aldrich, Merck companies. Absorbance activity studies were performed with a Shimatzu UV-2401 PC UV-VIS model UV-Vis spectrophotometer.

2.2. Preparation of the Plant Extracts

Quince fruits and leaves were dried in an oven at an average temperature of 40ºC, then ground and crushed to coarse powder using a grinder. Solvents were added and extracted for 8 hours at 250 rpm in a turbulent water bath. Ethanol, acetone and ethyl acetate were used as solvents. At the end of the time, the solution was filtered through Whatman filter paper. The solvents of the filters were evaporated at 50ºC in an evaporator and stock solutions were prepared by weighing the remaining solid. These stock solutions were centrifuged at 5000 rpm for 15 minutes, separated from the precipitates and stored in the freezer. Preparation of stock solutions: After evaporation of the solvents in the evaporator, the remaining solid for each plant was prepared by dissolving them in three different solutions from which they were extracted. Ethyl acetate, acetone and ethanol solutions were used to dilute these stock solutions to the desired concentrations.

2.3. Antioxidant Activities

2.3.1. Determination of chelating activity of iron (II) ions

The chelating activity of Fe^{+2} ions at different concentrations (50-1000 µg/ml) of plant extracts was studied. The method is based on the competition of ferrosin reagent, a strong iron chelator, and metal-binding compounds in the environment to bind Fe2+ ions. .If the chelating power is high; the formation of a red Fe^{+2} ferrosin complex is prevented [15]. 3.7 mL of deionized water and 100 µL of a 2 mM FeCl2 solution were added to 1 mL of the sample. After incubation for 30 minutes at room conditions, 200 µL of a 5 mM ferrozine solution was added and vortexed. After 10 minutes, the absorbance values of the mixtures were measured at 562 nm. Control was performed using 1 mL of deionized water instead of the sample. EDTA was used as the standard. Water was used as a blank. The percentage inhibition of the ferrozine-Fe^{+2} complex was calculated using the following formula. % Chelating activity = 1 - (Sample Absorbtion at 562 nm)/(Control Absorbtion at 562 nm) × 100

2.3.2. Determination of DPPH Free Radical Scavenging Activity

The activities of the plant extracts and the standard antioxidant DPPH radical scavenger were determined

according to the method of Brand-Williams and his colleagues [16]. 4 mg of DPPH solution was prepared by dissolving in 100 mL of ethanol. 4 mL of this solution was taken and 1 mL of the plant extracts prepared at different concentrations (50-1000 µg/mL) was added and the absorbance value was measured after 30 min at room temperature at 517 nm using a spectrophotometer. The absorbance values of the samples were evaluated in comparison with the control. Troloks and BHT were used as standards. 1 mL of distilled water and 4 mL of DPPH solution were used as control. Only ethanol was used as blank sample. The radical scavenging activity was calculated using the following equation: DPPH radical scavenging activity (%) = (1-[Absorbace of sample/Absorbance of control])x100

2.3.3. Determination of reducing power

The determination of the reducing power can give information about the antioxidant capacity depending on the concentration. The reducing power was determined as the reduction of $Fe[(CN)_6]^{+3}$ to $Fe[(CN)_6]^{+2}$. The strength of reduction was determined by the method of Oyaizu [17]. 1 mL of plant extracts at different concentrations (50-1000 µg/mL) and in aqueous solutions of chemical substances, 2.5 mL of 0.2 M phosphate buffer (pH=6.6) and 2.5 mL of 1% potassium ferricyanide ($K_4[Fe(CN)_6].3H_2O$) solution were added and mixed. The prepared solutions were incubated in a water bath at 50°C for 30 min. 2.5 mL of 10% trichloroacetate (TCA) solution was added to the solutions and centrifuged at 3000 rpm for 10 minutes. After centrifugation, 2.5 mL of the top of the solution was removed. 2.5 mL of water and 0.5 mL of a 0.1% solution of $FeCL_3$ were added. Distilled water was used as a blank solution. Vitamin C (ascorbic acid) and BHT were used as standards and the absorbance of the solutions were measured at 700 nm against the blank. The result shows that increase in absorbance of the reaction mixture shows increase in reducing power.

2.4. Antibacterial activity

Three bacterial strains were used to determine the antibacterial activity. These are two gram positive *Staphylococcus aureus* (*S. aureus*) (ATCC 29213) and *Bacillus cereus* (*B. Cereus*) (ATCC SBT8) and one gram negative *Escherichia coli* (*E. Coli*) (ATCC 25922) bacterial strains.

2.4.1. Preparation of the medium

The Mueller-Hinton agar method was used to prepare the medium. It is taken from 34 grams of MHA in 1 liter. It is soluble in pure water. The opening of the bottle is sealed with aluminum foil. It is placed in an autoclave. The standard program is selected. The instrument is switched on, after closing the lid, the bottle is thrown into the autoclave and cooled slightly when it comes out of the autoclave. 20 mL was poured into petri dishes, the petri dishes were sealed and wrapped in aluminum foil and placed in the refrigerator to cool. The environment for all bacteria is prepared in the same way.

2.4.2. Preparation of Agar Medium

Mueller Hinton Agar (MHA) was used for agar well diffusion method to determine the antibacterial effects of the different solvent extracts of quince and quince leaf [18]. Sterilized flasks were used to pour the medium into

Petri dishes. For the agar well diffusion method [19], a 4 mm thick MH agar was used. Then the agar plate is inoculated by spreading with bacteria solution over the entire agar surface. Then, a hole with a diameter of 6 mm is punched with a sterile cork borer and a volume (20–100 µL) of the extract solution was added into the well. Then, agar plates were incubated at 37°C for 24 h. Antibacterial activity was determined by measuring the zone of inhibition (including the wells diameter) appeared after the incubation period. All used solvents at a concentration of 10% were also employed as negative controls.

3. Results and Discussion

3.1 Antioxidant activity

In the present study, quince and quince leaves were extracted with ethanol, ethyl acetate and acetone and their antioxidant properties were determined by three most commonly used antioxidant methods. These are DPPH radical scavenging activity, ferrous ion chelating activity and reducing power activity methods. The radical scavenging activity of DPPH was determined at a concentration of 50-1000 µg/mL and the activity was compared with BHT and Trolox which were used as standards. The results were presented in Figure 1.

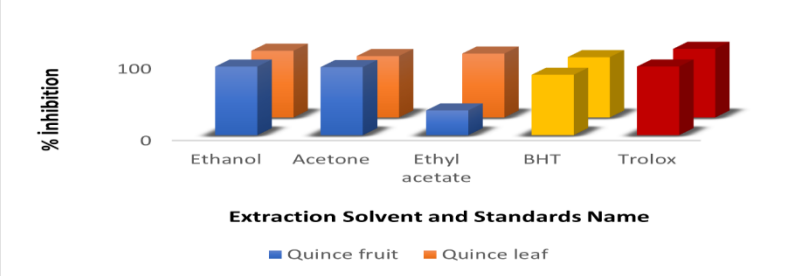

Figure 1: % Inhibition values of DPPH free radical scavenging activity of the quince extracts and standards.

According to the results, the substance Troloks showed the highest DPPH removal activity (95.40 %). The second ranked ethanol extract of quince fruit showed higher activity than the BHT standard with a value of 95.28 % and ethanol extract of quince leaves showed an activity of 92.70 % . As shown in Figure 1, it was found that all the solvent extracts of quince fruits and quince leaves showed high DPPH removal activity and the results varied according to the solvent difference as followed ethanol extract > acetone extract > ethyl acetate extract of quince fruit, as well as ethanol extract > ethyl acetate extract > acetone extract were listed according to the solvent difference for quince leaves. Among the all extracts, the ethyl acetate extract of quince fruit showed the lowest DPPH radical scavenging activity. The extracts were also examined for their reduction capacities at a concentration of 50-1000 µg/mL and activity comparisons were made with BHT and Ascorbic acid as standards. The reduction power of the extracts was determined by measuring the absorbance of the solutions at 700 nm. The results were presented in Figure 2. The reduction capacity of the extracts used in the study increases in direct proportion to the increase in concentration. According to the results, quince leaf ethyl acetate extract (3.912) and quince fruit ethanol extract (3.88) had the highest reduction power capacity. The order of the reduction capacity activity according to the solvent difference; for quince fruit: ethanol extract >

ethyl acetate extract > acetone extract and for quince leaf: ethyl acetate extract > ethanol extract > acetone extract.

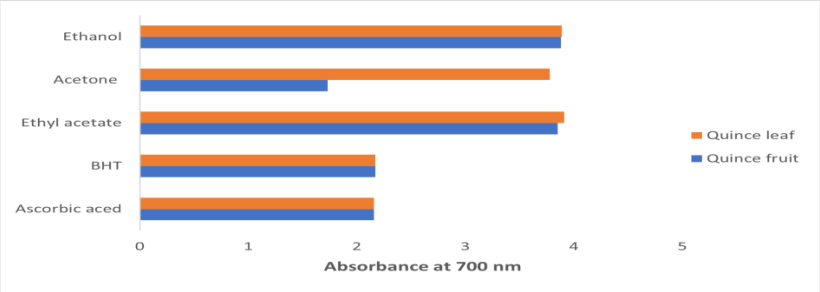

Figure 2: The reducing power activities of quince fruit and quince leaf extracts in solutions of ethanol, acetone, and ethyl acetate and BHT and Ascorbic acid as standards.

The third method for the determination of the antioxidant activity of the plant extract was iron ion chelating capacity method. The chelating activity of iron ion was studied in different concentrates of the extracts by comparing it with the EDTA standard used. The results were shown in Figure 3.

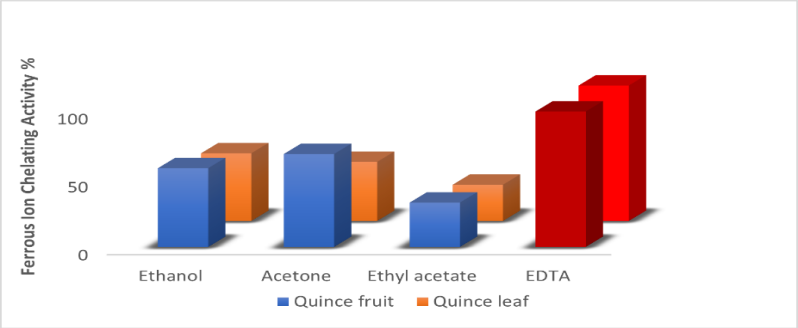

Figure 3: % Ferrous ion chelating activity of quince fruit and quince leaf extracts and EDTA standard.

According to the result, all tested extracts had reducing power activity. However the reducing power activity values of quince fruit and leaves were lower than the one of EDTA as a standard. Among the tested extracts quince fruit acetone extract (68.25) had the highest iron chelating activity, while quince leaf ethanol extract (49.69) also had the good iron chelating activity. Ranking of iron chelating activity according to solvent difference; for quince fruit: acetone extract > ethanol extract >ethyl acetate extract and for quince leaf: ethanol extract > acetone extract > ethyl acetate extract. In this study, the antioxidant activities of ethanol, acetone and ethyl acetate extracts of different parts of quince were investigated with the three common methods (DPPH, reducing power and ferrous ion chelating activity). The overall results showed the highest antioxidant potential for ethanolic and acetonic extracts of quince leaves. Our results are similar to literature works as followed. Muzykiewiczer et a [20]. was evaluated the antioxidant activity of methanolic, ethanolic and acetonic extracts treated with ultrasound of different parts of quince using the four applied methods (DPPH, FRAP, F-C and ABTS) and their results showed the highest antioxidant potential for methanolic extracts of quince leaves treated with ultrasound for 60 minutes [20]. Wojdyło and his colleagues [21] also investigated the properties of quince

fruit using the DPPH, ABTS and FRAP methods. They investigated the samples from frozen and dried raw material of 13 different plants. They found that The antioxidant activity depended on the plant and ranged between 0.9 and 2.4 μmol trolox/g dry matter for ABTS, 0.9 and 2.5 for DPPH as well as 0.4 and 1.5 for the FRAP method. Using the Folin-Ciocalteu method, the highest total polyphenol concentrations were found for methanolic and acetonic leaf extracts. The extracts from quince fruit showed lower antioxidant potential compared to the leaves [21].

3.2 Antibacterial activity

The antibacterial activities of ethanol, ethyl acetate and acetone solvent extracts of quince fruit and leaves were tested against one gram negative (Escherichia coli) and two gram positive (Bacillus cereus and Staphylococcus aureus) bacteria. The results were given in Table 1 as a unit of millimeter for bacteria inhibition zones. According to the results, the highest inhibition value for Escherichia coli bacteria in the acetone extract of quince fruit was 20 mm and the ethyl acetate extract of quince leaf was 18 mm. The inhibition zones for Staphylococcus aureus was 17 mm. The tested extracts of quince leaf had no antibacterial activity against Staphylococcus aureus. Over all three different solvent extracts of quince fruit had much better antibacterial activity than the ones of quince leaf against all tree tested bacteria strands.

Table 4: The diameters of the inhibition zone in mm of the quince fruit (QF) and Quince leaf (QL) in different solvent extracts and AMP (ampicillin) standard antibiotic.

Bacteria	QF Acetone	QF Ethanol	QF Ethyl Acetate	QL Acetone	QF Ethanol	QF Ethyl Acetate	AMP
Bacillus cereus (SBT-8)	16	15	_	11	15	_	10
Escherichia coli (25922)	20	11	10	_	12	18	22
Staphylococcus aureus (29213)	10	17	11	_	_	_	48

4. Conclusion

In this study, the antioxidant and antibacterial activities of quince fruit and leaves were investigated using different solvents such as ethanol, ethyl acetate and acetone. The antioxidant activities were highlighted using three different antioxidant analytical methods. These methods are: DPPH free radical scavenging activity, iron ion chelating activity and reducing capacity. Acetone and ethanol extracts of quince fruit and leaf had good much better DPPH radical scavenging activity, iron ion chelating activity and reducing power activity. In addition, antibacterial activity against three different bacteria was determined in terms of their antibacterial properties using agar well diffusion method. Acetone and ethanol solvent extracts of quince fruit had reasonable better antibacterial activity than the other extracts. In conclusion, it can be said that the compounds have reasonable good and positive antioxidant activities due to the effective solvent extraction and the concentrations of the extracts. The acetone and ethanol extract of quince fruit have very good antibacterial activity. Therefore, it is believed that this work can shed light on the use of natural antioxidant and antibacterial sources in some food and pharmacological industries.

References

[1]. Mandal S., Yadav S., Yadav S. ve Nema, R.K., 2009. Antioxidants: A Review, Journal of Chemical and Pharmaceutical Research, 1, 1, 102-104.

[2]. Lushchak V., 2014. Free radicals, reactive oxygen species, oxidative stress and its classification, Chemico- Biological Interactions., 224, 164–175.

[3]. Commoner B., Townsend J., Pake G. E., 1954. Free radicals in biological materials. Nature., 174(4432):689–691.

[4] Halliwell B., Gutterıdge JMC., "Role Of FreeRadicals and Catalytic Metal Ions in Human Disease: AnOverview." In: Methods inEnzymology,186, 1-85, 1990.

[5]. Tsao R. ve Deng Z., 2004. Separation Procedures For Naturally Occurring Antioxidant Phytochemicals, Journal of Chromatography B, 812, 85-99

[6]. Halliwell B., Cross C.E. ve Gutteridge J.M.C., 2002. Free Radicals, Antioxidants, and Human Disease. Where are We Now?, Journal of Laboratory and Clinical Medicine,119, 598-620

[7]. Pillai C.K., Pillai K.S., 2002 Antioxidants in health. Indian J Physiol Pharmacol.,46(1):1-5.

[8]. Koleva I.I., van Beek T.A., Linnsen J.P.H., de Groot A. ve Evstatieva, L.N., 2002. Screening of Plant Extracts for Antioxidant Activity: A Comparative Study of Three Testing Methods, Phytochemical Analysis. 13, 8-17.

[9]. Wettasinghe M. ve Shahidi F., 1999. Antioxidant and Free Radical-Scavenging Properties of Ethanolic

Extracts of Defatted Borage (Borago officinalis L.) Seeds, Food Chemistry, 67,4, 399-414.

[10]. Janovska D. Kubikova K, Kokoska L 2003. Screening for Antimicrobial Activity of Some Medicinal Plants Species of Traditional Chinese Medicine. Czech J. Food Sci. 21: 107-110.

[11]. Oliveira, P. A., Pereira, J. A., Andrade, P. B., Valenta, P., Seabra, M. R. and Silva, B. M. 2008. Organic acids composition of Cydonia Oblonga Miller leaf. Food Chem., 111,393-399.

[12]. Vinson, J. A., Su, X., Zubik, L. and Bose, P. 2001. Phenol antioxidant quantity and quality in foods: fruits. J. Agric. Food Chem., 49, 5315-5321.

[13]. Vaez, H., Hamidi, S., and Arami, S. (2014). Potential of Cydonia oblonga leaves in cardiovascular disease. Hypothesis 12, 1–10.

[14]. Silva, B. M., Andrade, P. B., Ferreres, F., Domingues, A. L., Seabra, R. M. and Ferreira, M. A. 2002a. Phenolic profile quince fruit (Cydonia oblonga Miller) (Pulp and Peel). J.Agric. Food Chem., 50, 4615-4618.

[15]. Moreira, R., Chenlo, F., Torres, M.D. and Vallejo, N. 2008. Thermodynamic analysis of experimental sorption isotherms of loquat and quince fruit. J. of Food Eng., 88,514-521.

[16]. Brand-Williams, W., Cuvelier, M. E., Berset, C., 1995. Use of a free radical method to evaluate antioxidant acitivity, Lebensmittel-Wissenschaft undTechnologie/Food Science and Technology, 28, 25-30.

[17]. Oyaizu M., 1986. Studies on product of browning reaction prepared from glucose amine, Japan Journal of Nutrition, 44, 307- 315.

[18]. Valgas C., De Souza S.M., Smânia E.F.A., 2007. Screening methods to determine antibacterial activity of natural products. Braz. J. Microbiol. 2007;38:369–380.

[19]. Balouiri M, Sadiki M, Ibnsouda S.K. 2016. Methods for in vitro evaluating antimicrobial activity: A review. JPA. 6: 71-79.

[20]. Muzykiewicz A., Zielonka-Brzezicka J., Klimowicz A., 2018. Quince (*Cydonia oblonga* Mill.) as a useful source of antioxidants – antioxidant activity evaluation, Herba Polonica, 64, 4, 23-33.

[21]. Wojdyło A., Oszmiański J., Bielicki P., 2013. Polyphenolic composition, antioxidant activity, and polyphenol oxidase (PPO) activity of quince (Cydonia oblonga Miller) varieties. J Agric Food Chem; 61:2762-72.