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ORIGINAL ARTICLE

Phytochemical Content and Antioxidant Activities of Pomelo Peel Extract

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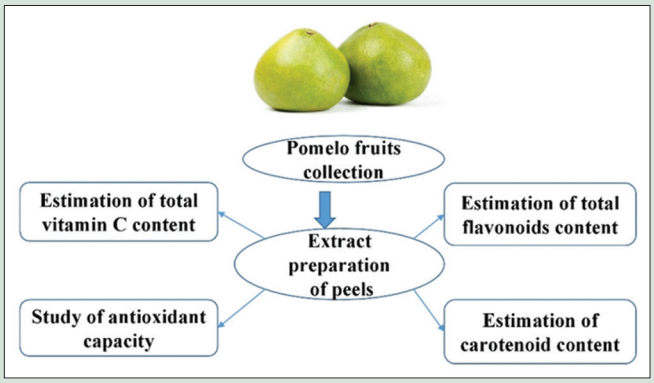
ABSTRACT

Background: There is an increasing interest in the antioxidant measurement of some plant constituents. Plant component, especially fruit, has antioxidant components which are safe, economic, and powerful to reduce oxidative stress and replace synthetic ones. **Objective:** To quantitatively measure the flavonoids, Vitamin C, and carotenoid content of hydroalcoholic extract and to estimate the antioxidant activity of pomelo peel extract (PPE) and minimum inhibitory concentration 50% (IC_{50}) of the extract using 2,2-diphenyl-1-picrylhydrazyl (DPPH) ethanolic solution. **Materials and Methods:** Hydroalcoholic PPE was prepared; total flavonoids using aluminum chloride method, Vitamin C using thiourea and 2,4-dinitrophenylhydrazine method, and carotenoid content of the extract were estimated using 95% ethanol solution. All measurements were estimated using ultraviolet–visible spectrophotometer. Antioxidant activity and IC_{50} of PPE were measured using DPPH method. **Results:** Flavonoids, Vitamin C, and carotenoid content were measured as 21.20 mg/100 g, 15.13 mg/100 g, and 62.98 μg/g, respectively, of dry PPE. The DPPH radical scavenging activity exhibits a significant dose-dependent inhibition of DPPH radical color, with IC_{50} being at a concentration of 68.55 μg/ml of the extract, comparable to 55.87 μg/ml IC_{50} of Vitamin C standard antioxidant. **Conclusion:** From the study results, we could conclude that pomelo peel possesses high antioxidant properties and it is potentially rich sources of natural antioxidants.

Key words: 2,2-diphenyl-1-picrylhydrazyl, flavonoids, antioxidant capacity, ascorbic acid, *Citrus maxima*

SUMMARY

- *Citrus maxima* fruits were collected from the local market of Amman, Jordan
- An aqueous ethanolic pomelo peel extract (PPE) was prepared
- Estimation of total flavonoids, Vitamin C, and carotenoid content in PPE
- Measuring antioxidant activity of the extract using 2,2-diphenyl-1-picrylhydrazyl (DPPH), with inhibitory concentration 50% of DPPH antioxidant activities values.



Abbreviations Used: DPPH: 2,2-diphenyl-1-picrylhydrazyl; IC_{50}: The half maximal inhibitory concentration; PPE: Pomelo peel extract.

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INTRODUCTION

Oxidative stress is the imbalance between the generation of reactive oxygen species (ROS) and body defense mechanisms, characterized by the emergence of free radicals.[1] Under oxidative stress conditions, excess generation of these oxygen radicals as superoxide anions (O^{2-}), hydroxyl radical, and peroxyl radicals leads to pathological consequences resulting from the damage of different biological components of healthy human cells including proteins, lipids, and DNA.[2] Environmental pollutants; toxic habits such as drugs, smoking, and/or alcohol; inadequate nutrition; excess solar radiation; large exposure to toxic substances; drug side effects; and a high physical stress are the most common exogenous factors originating ROS in the human body.[3]

Many epidemiological studies have indicated the relationship between the plant antioxidants and reduction of chronic diseases.[4,5] Therefore, in recent years, it is considered to be an important task in evaluating plant antioxidant activities and their free radical quenching ability. There has been an increased interest in the therapeutic potential of medicinal plants as antioxidants properties in reducing such free radical-induced damages, rather than looking for synthetic form.[6] Natural antioxidants are an interesting alternative in view of their variety of structures and chemical interactions, as well as the numerous biological activities they can perform. Intensive research activities are currently being carried out on plant antioxidants to meet this challenge.

Citrus genus that belongs to *Rutaceae* family includes some of the most widely cultivated crops in the world because of their many nutritional and health benefits. It is an important economically but the attention to leaves and seeds have not given importance in comparison fruits despite the presence of phenols quantity that varies among species.[7] Citrus fruits are among the most powerful antioxidants because they have ascorbic acid, polyphenols, flavonoids, and carotenoid compounds.[7,8]

The pomelo (*Citrus maxima* [Burm.] Merr.) is one of the most common citrus fruits growing broadly in tropical and subtropical southern regions

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of Asia, which is thought to be the local version of grapefruit and has high antioxidants including polyphenols, carotenoids, and vitamins that impart health benefits beyond basic nutrition.[9]

The main chemical compositions of pomelo peel are Vitamin C, flavonoids, and carotenoids, which are strongly associated with the broad spectrum of biological activities, such as antioxidant, antiatherogenic, anti-inflammatory, antimicrobial, anticancer, appetizer, stomach tonic, cardiac stimulant, antiepileptic, and anticough.[10,11]

Moreover, previous studies reported that the peel of pomelo fruit contained a higher amount of antioxidant content and antioxidant capacity as compared to its pulp.[12-14]

A recent study revealed that all organs' extracts of pomelo (except n-hexane peel extract) contained flavonoids which were classified as the major contributor in antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) method.[15]

In addition, pomelo essential oils have been reported to possess high antioxidant.[16]

A number of studies on pomelo extract showed the antioxidant properties through free radical scavenging effects *in vitro*.[17-20] However, phytochemical content and antioxidant activities of pomelo peel in Jordan were insufficiently explored. The objectives of this study were to determine the phytochemical composition and the possible antioxidant activities of pomelo peel extract (PPE), as an *in vitro* study.

MATERIALS AND METHODS

Plants collection

The fruits of pomelo, *C. maxima*, were planted in Jordan and collected from the local market of Amman, Jordan. After washing 2–3 times with running tap water, the skin of the fruit was scored into quarters with a sharp knife and then peeled off by the fingers. The removed peels of pomelo were shade dried for about 1 month. All the peel samples were ground into powder (<90 μm). The powders of the samples were kept in air-tight bottles after sieving.

Extract preparation

An aqueous ethanolic PPE was prepared according to the method reported by Abeysinghe *et al.* in 2007 with slight modifications, by soaking 10 g of the dry powdered *C. maxima* peel in 100 ml of 80% ethanol. The mixture was poured into test tubes, covered with the aluminum foils, and placed in a water bath for extraction at 65°C for 3 h, and the whole solution was filtered and the filtrate was allowed to evaporate into dryness in an oven at 60°C. The process was repeated several times and the yield was noted. The extract was refrigerated at 4°C for future use in experimental studies. Furthermore, the required extract was frequently prepared every 48 h and used fresh for each experiment.[21] Obtained extract is a powder with light yellowish–white color and specific pomelo odor. Preliminary phytochemical analysis revealed the presence of alkaloids, flavonoids, ascorbic acid, carotenoid, volatile oils, and saponins.[22]

Estimation of total flavonoid content in pomelo peel extract

Total flavonoids were quantified using aluminum chloride ($AlCl_3$) colorimetric method used by Chang *et al.* in 2002, which involve the reaction mixture containing 0.1 ml of 10% $AlCl_3$ in ethanol, 2.8 ml distilled water and 0.1 ml of 1 M potassium acetate (120 mM) was added to 0.5 ml of 1 g dried 80% ethanol-water extract of pomelo peel previously prepared, incubated at room temperature for 30 min. The absorbance was read at 415 nm with ultraviolet (UV)–visible spectrophotometer (Shimadzu, Japan), the amount of 10% $AlCl_3$ was substituted by the same amount of distilled water in blank, and the calibration curve was obtained using 80% alcoholic solution containing 10 mg of quercetin; then, the final solution was diluted to 25, 50, and 100 μg/ml standard solutions to determine the flavonoid content of the sample extract.[23] All samples were analyzed in triplicate.

Estimation of total Vitamin C content in pomelo peel extract

Ascorbic acid (Vitamin C) content of PPE made was estimated according to the method used by Mohammed *et al.* in 2009, in which 0.01 g of dried extract sample was homogenized with acetic acid solution and transferred into a 100 ml volumetric flask and was shaken gently until a homogeneous dispersion was obtained. Then, it was diluted up to the mark by acetic acid solution. Then, the solution was filtered, and a few drops of bromine water was added to the filtrated sample solution until the solution became colored. A fresh 100 μg/ml stock solution of ascorbic acid was prepared and diluted to get varying concentrations of 1, 2, 3, 4, 5, 6, and 7 μg/ml standard solutions. Then, a few drops of thiourea solution and 2,4-dinitrophenylhydrazine solution were added in both standard and sample. The absorbance was measured with UV–visible spectrophotometer (Shimadzu, Japan) at 280 nm. Then, a standard curve of absorbance against concentration was plotted. The total Vitamin C was expressed in mg/100 g.[24] All samples were analyzed in triplicate.

Estimation of carotenoid content in pomelo peel extract

According to the method used by Tao *et al.* in 2010,[25] about 5 g of *C. maxima* peel powder was extracted with 10 ml of 95% ethanol and incubated at 50°C for 60 min until the extraction phase was colorless. The final volume of the carotenoid extract was adjusted to 75 ml by adding 95% ethanol. The optical density value of the carotenoid extract was determined by UV–visible spectrophotometer (Shimadzu, Japan) at 450 nm.

The total carotenoid yield (μg/g dry weight) was calculated according to the formula by Tao *et al.* (2010) as follows:

$$\text{Carotenoid yield} = \frac{V(A-0.0051)}{0.175\,W}$$

Where,

A: The absorbance value of the diluted extraction at 450 nm

V: The final volume of the extract (ml)

W: The weight of dried powder (g)

0.175: The extinction coefficient of carotenoids.

Free radical scavenging activity of pomelo peel extract for 2,2-diphenyl-1-picrylhydrazyl radical

Antioxidant capacity of the PPE was measured using DPPH assay as described by Tippani *et al.*[26] with minor modification. Briefly, various concentrations of PPE starting from 1000 to 62.5 μg/ml were made by serial dilutions from previously prepared ethanolic stock solution (10^5 μg/ml). Equal volumes of each extract were pipetted into 0.2 mM ethanolic solution of the DPPH to initiate the reaction for creating a calibration curve. After shaking, the mixture was incubated in the dark for 30 min. Ascorbic acid (Vitamin C), widely studied antioxidant, was used for comparison or as a positive control. The DPPH solution in the absence of PPE was used as a control, and the 80% ethanol was used as blank discolorations which were measured at 517 nm using UV spectrophotometer (HITACHI U-1900). Measurement was performed at least in triplicate. The percentage of the DPPH free radical was calculated using the following equation:

DPPH scavenging effect (%) = $([A_0 - A_1]/A_0) \times 100$.

Where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the PPE. The actual decrease in absorption induced by the test was compared with the positive controls.

The inhibitory concentration 50% (IC_{50}) (concentration providing 50% inhibition) values were calculated using the dose inhibition curve in linear range by plotting the extract concentration versus the corresponding scavenging effect.

Statistical analysis

The results were expressed as the mean of three measurements ± standard error. Descriptive statistical analysis was performed using Microsoft Excel 2010 version 14 (American Multinational Technology Company, Redmond, Washington).

RESULTS AND DISCUSSION

Total flavonoids, Vitamin C, and carotenoids in an aqueous alcoholic extract of pomelo peel are shown in Table 1; the total flavonoids content was found to be 21.20 mg/100 g, the mean Vitamin C concentration of the extract was 15.13 mg/100 g, and carotenoid content was 62.98 µg/g of dry extract of pomelo peels which was prepared previously. These results are comparable with some previous studies that estimated the phytochemical and antioxidant content of fruit juice and peel of different pomelo species and showed that these natural products synthesized by plants have been reported to be responsible for the plant antioxidant capacity and correlated with total antioxidant potential.[27]

Free radical scavenging activity of pomelo peel extract for 2,2-diphenyl-1-picrylhydrazyl radical

DPPH radical is commonly used as a substrate for the fast evaluation of antioxidant activity because of its stability in the radical form and simplicity of the assay.[28] Figure 1 describes the effect of different PPE concentrations on the time course of DPPH oxidation compared to the control sample that was obtained without the presence of ethanolic PPE in the reaction mixture. In the present study, PPE was screened for DPPH radical scavenging activity according to the method previously described based on the color change of DPPH solution from purple to yellow as the radical is quenched by the antioxidant, which was measured quantitatively by a spectrophotometer at 517 nm. The possible explanation is that, in the presence of ethanolic PPE, the oxidation process was delayed in a dose-dependent manner related to reduced levels of formed free radical from oxidized DPPH in control test samples.[29] The DPPH radical scavenging activity and the concentration of the extract required to inhibit 50% of the initial DPPH free radicals (IC_{50}) are shown in Figure 2. In the current study, the DPPH free radical scavenging activity of the PPE increased with increasing concentration of the test ethanolic extract, and compared to that of ascorbic acid (Vitamin C) as standard antioxidant, such phenomena suggest that the PPE may act as free radical scavenger and may react with radicals to convert them to more stable products and terminate radical chain reaction. The possible explanation according to Silva CG *et al.* in 2005 that DPPH picks up one electron in the presence of a free radical scavenger, the absorbance decreases and the resulting discoloration related to the number of electrons gained.[30] Apart from that, the study results showed that the ethanolic PPE exhibits a significant dose-dependent inhibition of DPPH activity with 50% of inhibition (IC_{50}) at concentration of 68.55 µg/ml compared to IC_{50} value of Vitamin C which was 55.87 µg/ml [Figure 2]. Basically, a higher DPPH radical scavenging activity is associated with a lower IC_{50} value. Although PPE extract shows lower reducing power activity than Vitamin C for the concentrations tested, previous studies showed that samples which had IC_{50} lower than 50 µg/ml were very strong antioxidant, 50–100 µg/ml were strong antioxidant, and 101–150 µg/ml were medium antioxidant, whereas a weak antioxidant with IC_{50} >150 µg/ml.[31] Therefore, the PPE of *C. maxima* can be categories as very strong free radical scavenger. The current study results were comparable to previous study which represented that ethanolic peel extract of pomelo (*C. maxima*) from Subang (West Java) had IC_{50} of DPPH 44.96 µg/ml;[32] moreover, Ghasemi *et al.* reported that the IC_{50} values of DPPH radical scavenging activity of methanolic extract from 13 different types of citrus peels were found to fall between 0.6 and 2.9 mg/ml.[33] Therefore, our results indicated that PPE exhibited great efficiency in scavenging DPPH and reactive oxygen radicals.

Table 1: Average total flavonoids, Vitamin C, and carotenoids in pomelo peel extract

Constituent	Concentration, mean±SE
Total flavonoids (mg/100 g)	21.20±2.62
Vitamin C (mg/100 g)	15.13±0.74
Carotenoids (µg/g)	62.98±1.70

Values are expressed as mean±SE of triplicate experiments. SE: Standard error

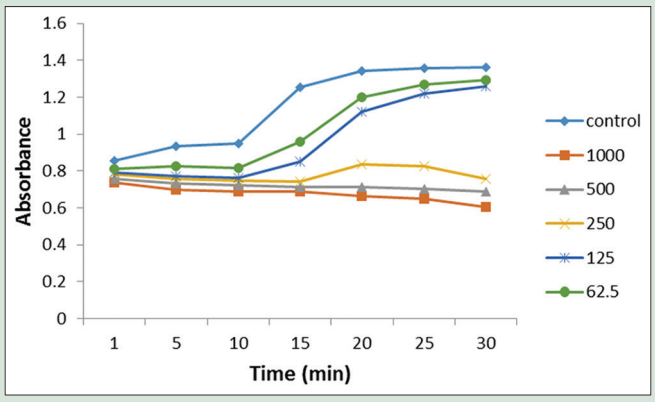

Figure 1: Effects of different ethanolic pomelo peel extract concentrations on the time course of 2,2-diphenyl-1-picrylhydrazyl-induced oxidation

CONCLUSION

Chemical compositions, the DPPH radical scavenging activity, and the concentration of the extract required to inhibit 50% of the initial DPPH free radicals (IC_{50}) compared to Vitamin C as standard were investigated in this study. The results indicate that the PPE contains high antioxidant

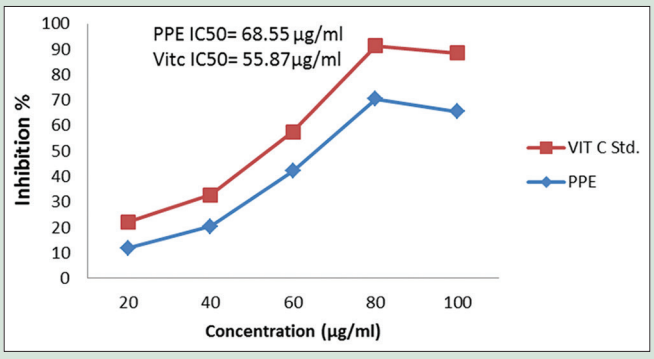

Figure 2: 2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity and the inhibitory concentration 50% values of ethanolic pomelo peel extract compared to Vitamin C as standard antioxidant

content including Vitamin C, flavonoids, and carotenoids that exhibit an excellent scavenging ability for different forms of free radicals. Therefore, pomelo (*C. maxima)* is one of the nutritious fruits and can be used as an excellent source of antioxidants for human consumption.

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Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Nerdy, Putra ED, Haro G, Harahap U. *In silico* screening of hesperetin and naringenin ester derivatives as anticancer against phosphoinositide 3-kinase. Int J Pharm Tech Res 2016;9:388-94.
2. Halliwell B, Gutteridge JM. Free Radicals in Biology and Medicine. 3th ed. Oxford: Oxford University Press; 1999.
3. Alberto JN. Antioxidant therapy: Myth or reality? J Braz Chem Soc 2005;16:699-710.
4. Halliwell B. Free radicals and antioxidants: A personal review. Nutr Rev 1997;52:253-65.
5. Liu RH. Health benefits of fruits and vegetables are from additive and synergestic combination of phytochemicals. Am J Clin Nutr 2003;78:517-20.
6. Stevanovic T, Diouf N, Garcia-Perez M. Bioactive polyphenols from healthy diets and forest biomass. Curr Nutr Food Sci 2009;5:264-95.
7. AL-Anbari AK, Al-Khesraji TA, Al-Mashhadani A, Aljewari H. Determination of some chemical compounds in *Citrus* species in Iraq. Int J Adv Res 2013;1:555-62.
8. Munwar S, Roy H, Rahaman SA. Antioxidant and free radical scavenging activity of *Citrus medica*. Int J Pharm Res Health Sci 2015;3:810-6.
9. Barrion AS, Hurtada WA, Papa IA, Zulayvar TO, Yee MG. Phytochemical Composition, Antioxidant and Antibacterial Properties of Pummelo (*Citrus maxima* (Burm.)) Merr. against Escherichia coli and Salmonella typhimurium. Food Nutr Sci 2014;5:749-58.
10. Ajeet S, Navneet. *Citrus maxima* (Burm.) Merr. A traditional medicine: its antimicrobial potential and pharmacological update for commercial exploitation in herbal drugs – A Review. Int J Chem Tech Res 2017;10:642-51.
11. Ahmad AA, Al Khalifa II, Abudayeh ZH. The role of pomelo peel extract for experimentally induced wound in diabetic rats. Pharmacogn J 2018;10:885-91.
12. Guo CJ, Yang JJ, Wei JY, Li YF, Xu J, Jiang YG. Antioxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay. Nutr Res 2003;23:1719-26.
13. Kulkarni AP, Mahal HS, Kapoor S, Aradhya SM. *In vitro* studies on the binding, antioxidant, and cytotoxic actions of punicalagin. J Agric Food Chem 2007;55:1491-500.
14. Toh JJ, Khoo HE, Azrina A. Comparison of antioxidant properties of pomelo [*Citrus grandis* (L) Osbeck] varieties. Int Food Res J 2013;20:1661-8.
15. Irda F, Elvira S, Komar R. Phytochemical content and antioxidant activities in different organs of pomelo (*Citrus maxima* [Burm.] Merr.) using 2,2-diphenyl-1-picrylhydrazyl and phosphomolybdenum assays. Asian J Pharm Clin Res 2016;9:185-90.
16. Phi NT, Vy TT. Chemical composition, antioxidant and antibacterial activities of peels. Lan- essential oils of different pomelo varieties in the South of Vietnam international. Food Res J 2015;22:2426-31.
17. Caengprasath N, Ngamukote S, Mäkynen K, Adisakwattana S. The protective effects of pomelo extract (*Citrus grandis* L. Osbeck) against fructose-mediated protein oxidation and glycation. EXCLI J 2013;12:491-502.
18. Chung SK, Kim SH, Choi YH, Song EY, Kim SH. Status of citrus fruit production and view of utilization in Cheju. Food Ind Nutr 2000;5:45-52.
19. Jayaprakasha GK, Girennavar B, Patil BS. Antioxidant capacity of pummelo and navel oranges: Extraction efficiency of solvents in sequence. LWT Food Sci Technol 2008;41:376-84.
20. Lim HK, Yoo ES, Moon JY, Jeon YJ, Cho SK. Antioxidant activity of extracts from Dangyuja (*Citrus grandis* Osbeck) fruits produced in Jeju Island. Food Sci Biotech 2006;15:312-6.
21. Abeysinghe DC, Xian Li, Sun C, Zhang WS, Zhou CH, Chen SK. Bioactive compounds and antioxidant capacities in different edible tissues of *Citrus* fruit of four species. Food Chem 2007;104:1338-44.
22. Trease GE, Evans WC. Textbook of Pharmacognosy. 16th ed. London: Balliere-Tindal; 2009.
23. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal 2002;10:178-82.
24. Mohammed QY, Hamad WM, Mohammed EK. Spectrophotometric determination of total Vitamin C in some fruits and vegetables at Koya area-Kurdistan region/Iraq. J Kirkuk Univ Sci Stud 2009;4:46-54.
25. Tao NG, Gao YM, Liu Y, Ge F. Carotenoids from the peel of Shatian pummel (*Citrus grandis* Osbeck) and its antimicrobial activity. Am Euras J Agric Environ Sci 2010;7:110-5.
26. Tippani R, Porika M, Allenki V, Anreddy R, Yellu NR, Krishna D, *et al*. Antioxidant and analgesic activities of *Pterocarpus marsupium* Roxb. J Herbs Spices Med Plants 2010;16:63-8.
27. Tsai HL, Chang SK, Chang SJ. Antioxidant content and free radical scavenging ability of fresh red pummelo [*Citrus grandis* (L.) Osbeck] juice and freeze-dried products. J Agric Food Chem 2007;55:2867-72.
28. Bozin B, Mimica-Dukic N, Samojlik I, Goran A, Igic R. Phenolics as antioxidants in garlic (*Allium sativum* L., *Alliaceae*). Food Chem 2008;111:925-9.
29. Karagözler AA, Erdağ B, Emek YÇ, Uygun DA. Antioxidant activity and proline content of leaf extracts from *Dorystoechas hastata*. Food Chem 2008;111:400-7.
30. Silva CG, Herdeiro RS, Mathias CJ, Panek AD, Silveira CS, Rodrigues VP, *et al*. Evaluation of antioxidant activity of Brazilian plants. Pharmacol Res 2005;52:229-33.
31. Blois MS. Antioxidant determination by the use of stable free radicals. Nature 1958;181:1199-200.
32. Fidrianny I, Sari E, Ruslan K. Phytochemical content and antioxidant activities in different organs of pomelo (*Citrus maxima* [Burm.] Merr.) using 2,2-diphenyl-1-picrylhydrazyl and phosphomolybdenum assay. Assian J Pharm Clin Res 2016;9:185-90.
33. Ghasemi K, Ghasemi Y, Ebrahimzadeh MA. Antioxidant activity, phenol and flavonoid contents of 13 *Citrus* species peels and tissues. Pak J Pharm Sci 2009;22:277-81.