Hormozgan Medical Journal

doi 10.5812/hmj.102953

Hormozgan Med J. 2021; 25(1):19-23

Research Article

Total Phenolic and Flavonoid Content and Antibacterial Properties of *Polygonatum orientale* Desf and *Tilia dasystyla*

Parichehr Hanachi[1*], Roshanak Zarringhalami[1], Ertugrul Kaya[2]

[1]Department of Biotechnology, Faculty of Biological Science, Alzahra University, Tehran, Iran
[2]Medical Pharmacology, Faculty of Medicine, Duzce University, Düzce, Turkey

***Correspondence to**
Parichehr Hanachi,
Email: p.hanachi@alzahra.ac.ir

Abstract

Background: Secondary metabolites of plants such as phenol and flavonoids have an extreme potential for neutralizing free radicals. The antioxidant and cytotoxic activities of plants are related to phenolic or flavonoids compounds. The occurrence of drug resistance to antimicrobial drugs has led to use of medicinal herbs in treatment of infections. Antibiotic resistant of *Staphylococcus aureus* has become a major problem in the treatment of diseases.

Objectives: The aim of this study was determination of total phenolic content (TPC) and total flavonoids content (TFC) of *Polygonatum orientale* Desf and *Tilia dasystyla* and evaluation of their anti-bacterial effects on *Staphylococcus aureus* bacterium. Investigation of TPC of *P. orientale* Desf and *T. dasystyla* has not been reported before.

Methods: Total phenolic and flavonoid content of *P. orientale* Desf and *T. dasystyla* extracts were determined using colorimetric methods of Folin-Ciocalteu and aluminum chloride. Antimicrobial activities of the extracts were evaluated by microdilution broth and disc diffusion methods to determine minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values.

Results: The results showed that TFC of *P. orientale* Desf with the value of 7.9 ± 0.040 mg/g DW extracted with diluted water solvent and boiling method and TPCs of *T. dasystyla* with the value of 62.13 ± 0.073 mg/g DW extracted with methanol solvent and boiling method were the highest amount. Methanol extract of *P. orientale* Desf had more antibacterial activities with the MBC and MIC values of 0.140 mg/mL and 8±0.4 mm zone of inhibition.

Conclusion: *Tilia dasystyla* and *P. orientale* Desf contain bioactive compounds such as phenolic and flavonoids that can be used as promising option in pharmacognostical studies for treatment of *S. aureus* infections.

Keywords: Antibacterial, Flavonoid, Phenol, *Polygonatum orientale* Desf, *Tilia dasystyla*

Received March 25, 2020, **Accepted**: October 6, 2020, **Published Online**: December 2, 2020

Background

Secondary metabolites in plants such as phenolic and flavonoid compounds have antioxidant properties. Since plants are one of the important sources of antioxidants, they can protect cells against oxidative damage. Natural antioxidants increase the potency of plasma antioxidants and reduce the risk of certain diseases such as cancer, heart disease and stroke (1-3).

The precise mechanism of the antibacterial activity of the plant extracts has not yet been fully investigated, and it appears that the phenolic compounds of the plants exert their antibacterial activity by altering the structure and function of the cell membrane. Studies show that phenolic compounds increase the permeability of the cell membrane and, as a result, the cell swells and dies. In gram-positive bacteria such as *Staphylococcus aureus*, antimicrobial agents as well as phenolic compounds can easily destroy the cell wall of the bacterium and its membrane, causing the bacterial material to leak out into the environment. Other antimicrobial activities of phenolic compounds are their ability to form soluble compounds with proteins, disrupting bacterial surface receptors and ultimately disrupting protein synthesis in bacteria (4,5).

Medicinal plants are more preferred than prescription medicine due to unwanted side effects of prescription medicine (6,7). Plants are one of the important sources of antioxidants. Antioxidant compounds from plants protect cells from oxidative damage (8). Secondary metabolites such as phenol and flavonoid compounds show antioxidant effect and are derived from whole parts of the plant, like leaves, fruits, roots and skin (9, 10).

Because of the high prevalence of chronic diseases, it is logical to use plants to provide the antioxidants needed by the body, particularly the plants with high levels of phenol and flavonoid. Antioxidants are substances that, are able to prevent oxidation caused by active oxygen species (11).

Staphylococcus aureus bacteria threatens public health through infectious diseases and food poisoning. On the other hand, *S. aureus* resists antibiotics quickly. In addition to rapid resistance, it has ability to simultaneously resist multiple antibiotics. These features have led to new methods and therapies for its treatment. The uncontrolled use of these antimicrobials has led to an increase in drug resistance to antibiotics in most bacteria. This is one of the reasons for the growing use of plants as low-risk, accessible and cost-effective natural substances for synthetic antibiotics in the treatment of bacterial infections. These reasons are due to the increasing global studies and the introduction of antibacterial effects of different plants in recent years. Among all the identified substances contained in effective compounds of plants, phenolic compounds or secondary compounds without nitrogen is the most important substance that has many biological effects, including antibacterial activity (12-14).

Polygonatum orientale Desf is belongs to the asparagus family that exist in the north of Iran. Its rhizome has some medical properties such as wounds healing, anti-diabetic and anti-bacterial effects, gynecological disorders, anti-gout and rheumatism, aphrodisiac, (15).

Tilia is a genus from the family of Tiliaceae with about thirty species of trees that are mostly native to the northern hemisphere. Flowers and leaves of *Tilia* species (linden) commonly are used for cold symptoms and also is a traditional medicine as mosaic and diuretic, anti-inflammatory, anti-diarrhea, anti-spasmodic, anti-hair loss, anti-anxiety and sedative (16, 17).

Objectives

Investigation of the total phenol and flavonoid content of *P. orientale* Desf and *T. dasystyla* using three different solvents and two methods of extraction and determination of antimicrobial effects of two plants on *S. aureus* species is the aim of this study.

Materials and Methods

Chemicals and Reagents

Methanol, ethanol, Folin-Ciocalteu reagent, sodium carbonate, gallic acid, aluminum chloride, Potassium acetate, quercetin, Mueller–Hinton broth, Mueller–Hinton agar and other chemicals were prepared from Merck (Germany).

Plant Material

Polygonatum orientale Desf and *T. dasystyla* species were provided from Bagh Firuze (Tehran, Iran) and were identified at Alzahra University herbarium. In this study, the leaf of *T. dasystyla* and rhizome of *P. orientale* Desf were used.

Sample Preparation for Total Phenolic and Flavonoid Assay

0.1 g of dried samples was added to each solvent including 10 mL of 80% aqueous methanol and 10 mL of 80 % aqueous ethanol and 10 of mL distilled water. Ultrasonic bath (ultrasonic cleaner set, Model: WUC-A0H) was used for extraction. Samples remained 20 minutes in ultrasonic bath then the extracts were centrifuged for 15 minutes at 3000 rpm (18).

0.1 g of dried sample was added to 10 mL 80% aqueous methanol and 10 mL 80% aqueous ethanol and 10 mL distilled water, this time water bath method was used for extraction. Samples were heated in water bath for 60 minutes at a temperature of 70°C then the extracts were centrifuged for 20 minutes at 2000 rpm (4).

Determination of the Total Phenolic Compounds

Phenolic contents of samples were determined by the Folin–Ciocalteu method. 200 µL extract sample was added to 1 mL of 1:10 diluted Folin–Ciocalteu reagent. 800 µL of saturated sodium carbonate (75 g/L) was added after 4 minutes. The absorbance at 765 nm was measured after 2 hours of incubation at room temperature. The results were expressed as gallic acid equivalent (GAE) mg/g dry weight of crude extract (2).

Determination of the Total Flavonoid Compounds

Extracts of samples (0.5 mL) were mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, 2.8 mL of distilled water and 0.1 mL of 1M potassium acetate. The absorbance was measured at 415 nm with spectrophotometer after incubation at room temperature for 30 minutes. Stock solution of quercetin was used to make serial dilution of concentrations (10-100 µg/mL) in methanol to make the calibration curve (19).

Antibacterial Activity

Sample Preparation for Antibacterial Assay

Samples (100 mg) were extracted with 80% methanol and ethanol and diluted water (5 mL) at 70°C temperature for 2 hours. The extracts were centrifuged at 3000 g for 15 minutes. The extracts were used to determine antibacterial properties (20).

Preparation of Microorganism

ATCC 25923 stock cultures were kept at 4°C on tubes of nutrient agar. Active cultures were prepared by transferring a loop full of bacteria from the stock cultures to test tubes of Mueller–Hinton broth (MHB) and incubated for 24 hours at 37°C. The cultures were diluted with fresh MHB to get densities corresponding to 2.0×10^6 colony forming units.

Antimicrobial Susceptibility Test

The disc diffusion method was used to investigate the antimicrobial activity. Fifteen milliliters of molten media was poured into sterile petri plates to prepare the MHA plates. After 5 minutes the plates were solidified. 0.1% microorganism suspension was spread and after 5 minutes it was dried. Thirty microliters of extracts was added on 8 mm sterile disc. The disc was located on the surface of medium, after 5 minutes the compounds was diffused, and for incubation the plates were kept at 37°C for 24 hours. Gentamicin discs was positive control. Inhibition zones were formed at the end of incubation, around the disc and were measured with a ruler in millimeters (21).

Minimum Inhibitory Concentration Determination

One milliliter of TSB medium was added to nine autoclaved tubes. One milliliter sanitizing agent was added to the first and the second tubes of the series; tube 2 was stirred and 1 mL of solvent from tube 2 was transferred to tube 3. The transfer of solvents was repeated until tube 8. Then 0.1 mL of microorganism was added to all flasks, except flask number 8. Then all tubes were incubated for 24 hours. Tubes 8 and 9 are positive and negative controls, concentration of extracts were 9 mg/mL, 4.5 mg/mL, 2.25 mg/mL, 1.125 mg/mL, 0.562 mg/mL, 0.281 mg/mL, 0.140 mg/mL respectively (22).

Statistical Analysis

All data are the average of three times analyses. ANOVA test by SPSS version 24 program was used to perform statistical analysis and P value < 0.05 was regarded as significant. Data are shown as mean ± standard deviation.

Results

The content of phenolic and flavonoids compounds (mg/g DW) in ethanol, methanol and diluted water extracts, was determined from regression analysis results.

Results indicate that total phenolic content (TPC) values are higher in water bath (boiling) method and methanol solvent (Figures 1 and 2). Methanol solvent had the higher amount of TPC. Phenolic compounds of *P. orientale* Desf methanol extract was 11.17±0.45 mg/g DW and *T. dasystyla* was 62.13±3.53 mg/g DW. Results show that *T. dasystyla* was richer source of phenolic compound than *P. orientale* Desf. Statistic results indicate that the two methods of extraction have significant difference ($P<0.05$) in TPC of *P. orientale* Desf which the most total flavonoids content (TFC) value in water bath method was about 11.17±0.054 mg/g DW; however, this amount in sonication method was about 9.97±0.54 mg/g DW. Additionally, there is a significant difference between solvents and TPC of *T. dasystyla*, which the highest TPC value is belonged to methanol solvent with amount of 62.13±3.53 mg/g DW and the lowest with amount of 28.97 ± 2.94 mg/g DW is belonged to ethanol solvent.

According to the Figures 3 and 4 diluted water solvent and water bath method is more efficient in extracting flavonoid compounds of *P. orientale Desf* with the value of 7.9±0.049 mg/g DW. Ethanol solvent and water bath (boiling) method are more efficient in extracting total flavonoid compounds of *T. dasystyla* with the value of 4.1±0.056 mg/g DW, it shows that *P. orientale Desf* is richer source of flavonoid compounds than *T. dasystyla*.

Table 1 indicates that the lowest concentration of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) in both plants belonged to methanol solvent with amount of 0.140 (mg/mL). Table 2 highlights that the widest ZOI in *P. orientale* Desf with the size of 8±0.4 belonged to 20 (mg/mL dry wt) concentration of methanol solvent.

Discussion

Statistic results showed that the two methods of extraction are different ($P<0.05$) in total flavonoid content of *T. dasystyla* and *P. orientale* Desf. In *T. dasystyla* the highest amount of TFC is 4.1±0.056 mg/g DW with water bath method, however; this amount is 1.6±0.22 mg/g DW with sonication method. Also in *P. orientale* Desf the highest amount of TFC is 7.9±0. 53 mg/g DW with water bath method and this amount is 1.7±0.21 mg/g DW with

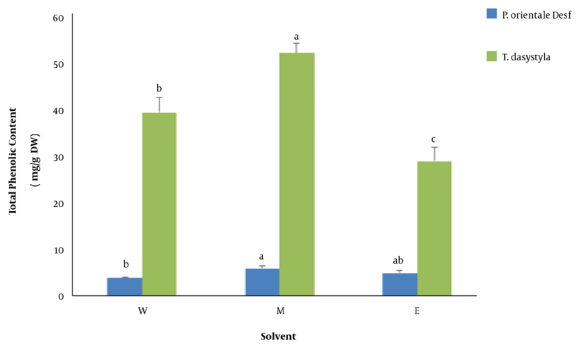

Figure 1. Total Phenolic Contents (mg/g DW) of *Polygonatum orientale* Desf and *Tilia dasystyla* by Sonication Method and 3 Solvents (W: water, M: methanol, E: ethanol). Values in each column with different letter are significantly different ($P<0.05$).

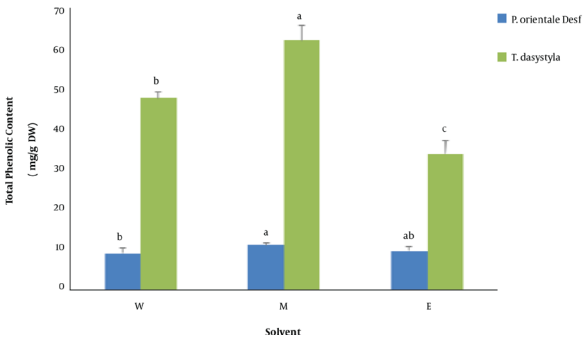

Figure 2. Total Phenolic Contents (mg/g DW) of *Polygonatum orientale* Desf and *Tilia dasystyla* by Water Bath Method and 3 Solvents (W: water, M: methanol, E: ethanol). Values in each column with different letter are significantly different ($P<0.05$).

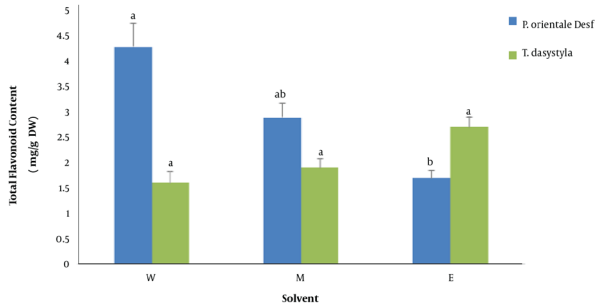

Figure 3. Total Flavonoid Contents (mg/g DW) of *Polygonatum orientale* Desf and *Tilia dasystyla* by Sonication Method and 3 Solvents (W: water, M: methanol, E: ethanol). Values in each column with different letter are significantly different ($P<0.05$).

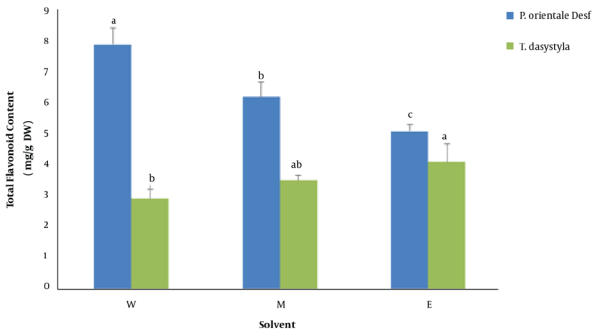

Figure 4. Total Flavonoid contents (mg/g DW) of *Polygonatum orientale* Desf and *Tilia dasystyla* by Water Bath Method and 3 Solvents (W: water, M: methanol, E: ethanol). Values in each column with different letter are significantly different ($P<0.05$).

sonication method.

Result showed that methanol was the best solvent for isolation of polyphenols, as the previous reports showed that aqueous methanol is the best solvent for polyphenols and it can be explained by the polarity of phenolic compound (23). The water bath method was more effective because of the high temperature. Temperature increases solubility of any compounds. Water bath method showed the highest amount of phenolic and flavonoid compounds in both plant species (24).

According to Hanachi et al study, methanol extracts followed by water extracts of *T. dasystyla* and *P. orientale* Desf showed more antioxidant properties (25). Therefore according to the Hanachi et al study, it can be concluded that there is a direct correlation between phenolic compounds and antioxidant properties in these two plants. Methanol solvent has been able to extract more phenolic compounds and showed more antioxidant properties in both plant species (25).

Results indicates that methanol extracts of these two plants showed the most antibacterial activities and it may be because of high amount of TPC and TFC in methanol solvent. Besides, *P. orientale* Desf had more antibacterial activities than *T. dasystyla* and there was a direct relationship between the amount of phenol and antibacterial properties. Based on the results, it could be concluded that the higher amount of phenol content lead to the more antibacterial activity.in some studies stated that the hydroxyl group in phenol compounds can lead to bacterial inhibition and damage to bacterial DNA (26).

Conclusion

Considering the results and increasing the resistance of bacteria to chemical antibiotics, it is suggested that with further studies on these plants, antibacterial compounds of them can be used to treat infectious diseases. We can conclude that the highest amount of total phenolic compound were belonged to *T. dasystyla* methanol extract by water bath method . However, purification of polyphenol extracts and in vivo evaluation should be further studied.

Authors' Contribution

Study concept and design: PH, and RZ. Acquisition of data: RZ. Analysis and interpretation ofdata: RZ, PH, EK. Drafting of the manuscript: RZ and PH. Critical revision of the manuscript for

Table 1. MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) of *Polygonatum orientale* Desf and *Tilia dasystyla* extracts against *Staphylococcus aureus*

Plant	Extracts	MBC (mg/mL)	MIC (mg/mL)
Polygonatum orientale Desf	Water	0.281	0.140
	Ethanol	0.562	0.281
	Methanol	0.281	0.140
Tilia dasystyla	Water	1.125	0.562
	Ethanol	0.281	0.140
	Methanol	0.281	0.140

Table 2. Zone of Inhibition (mm) of *P Polygonatum orientale* Desf and *Tilia dasystyla* Extracts Against *Staphylococcus aureus*

plant	Extracts	20 (mg/mL Dry wt)	10 (mg/mL Dry wt)	5 (mg/mL Dry wt)
Polygonatum orientale Desf	Water	_	_	_
	Ethanol	6±0.2	_	_
	Methanol	8±0.4	_	_
Tilia dasystyla	Water	_	_	_
	Ethanol	_	_	_
	Methanol	5±0.3	_	_

important intellectual content: EK, PH, and RZ. Statistical analysis: RZ. Administrative, technical, and material support: PH and RZ. Study supervision: EK and PH.

Conflict of interests

The authors declare that they have no conflicts of interests.

Ethical Approval

The experiment was performed under the approval of Motamed Cancer Institute-Academic Center for Education, Culture and Research, Tehran, Iran (Approval ID: IR.ACECR.REC.1398.006).

Funding/Support

The study was supported by Alzahra University, Iran.

References

1. Parker TD, Adams DA, Zhou K, Harris M, Yu L. Fatty acid composition and oxidative stability of cold-pressed edible seed oils. J Food Sci. 2003;68(4):1240-3. doi: 10.1111/j.1365-2621.2003.tb09632.x.
2. Zheng W, Wang SY. Antioxidant activity and phenolic compounds in selected herbs. J Agric Food Chem. 2001;49(11):5165-70. doi: 10.1021/jf010697n.
3. Kris-Etherton PM, Hecker KD, Bonanome A, Coval SM, Binkoski AE, Hilpert KF, et al. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. Am J Med. 2002;113 Suppl 9B:71S-88S. doi: 10.1016/s0002-9343(01)00995-0.
4. Morrissey JP, Osbourn AE. Fungal resistance to plant antibiotics as a mechanism of pathogenesis. Microbiol Mol Biol Rev. 1999;63(3):708-24.
5. Shokooh Saremi E, Habibi Najafi MB, Haddad Khodaparast MH, Bahreini M. Effect of extraction methods on phenolic content and antimicrobial properties of *Pimpinella affinis* leaf. Iranian Food Science and Technology Research Journal. 2018;14(1):59-68. doi: 10.22067/ifstrj.v1396i0.59302.
6. Cassileth BR, Deng G. Complementary and alternative therapies for cancer. Oncologist. 2004;9(1):80-9. doi: 10.1634/theoncologist.9-1-80.
7. Richardson MA, Sanders T, Palmer JL, Greisinger A, Singletary SE. Complementary/alternative medicine use in a comprehensive cancer center and the implications for oncology. J Clin Oncol. 2000;18(13):2505-14. doi: 10.1200/jco.2000.18.13.2505.
8. Kumaran A, Joel karunakaran R. Antioxidant and free radical scavenging activity of an aqueous extract of *Coleus aromaticus*. Food Chem. 2006;97(1):109-14. doi: 10.1016/j.foodchem.2005.03.032.
9. Rao YK, Geethangili M, Fang SH, Tzeng YM. Antioxidant and cytotoxic activities of naturally occurring phenolic and related compounds: a comparative study. Food Chem Toxicol. 2007;45(9):1770-6. doi: 10.1016/j.fct.2007.03.012.
10. Mathew S, Abraham TE. In vitro antioxidant activity and scavenging effects of *Cinnamomum verum* leaf extract assayed by different methodologies. Food Chem Toxicol. 2006;44(2):198-206. doi: 10.1016/j.fct.2005.06.013.
11. Halliwell B, Aeschbach R, Löliger J, Aruoma OI. The characterization of antioxidants. Food Chem Toxicol. 1995;33(7):601-17. doi: 10.1016/0278-6915(95)00024-v.
12. Normanno G, La Salandra G, Dambrosio A, Quaglia NC, Corrente M, Parisi A, et al. Occurrence, characterization and antimicrobial resistance of enterotoxigenic *Staphylococcus aureus* isolated from meat and dairy products. Int J Food Microbiol. 2007;115(3):290-6. doi: 10.1016/j.ijfoodmicro.2006.10.049.
13. Soltan Dallal MM, Salehipour Z, Eshraghi S, Fallah Mehrabadi J, Bakhtiari R. Occurrence and molecular characterization of *Staphylococcus aureus* strains isolated from meat and dairy products by PCR-RFLP. Ann Microbiol. 2010;60(2):189-96. doi: 10.1007/s13213-010-0025-4.
14. Weinstein RA. Controlling antimicrobial resistance in hospitals: infection control and use of antibiotics. Emerg Infect Dis. 2001;7(2):188-92. doi: 10.3201/eid0702.010206.
15. Sadati Lamardi SN, Ghambariyan S, Shams Ardekani MR, Amin G. Major phytosteroid from *Polygonatum orientale* Desf. Rhizome. J Pharm Health Sci. 2017;5(1):43-9.
16. Radoglou K, Dobrowolska D, Spyroglou G, Nicolescu VN. A review on the ecology and silviculture of limes (*Tilia cordata* Mill., *Tilia platyphyllos* Scop. and *Tilia tomentosa* Moench.) in Europe. Bodenkultur. 2009;60(3):9-19.
17. Delnavazi MR, Shahabi M, Yassa N. Flavonoids from the leaves of Iranian Linden; *Tilia rubra* subsp. *caucasica*. Res J Pharmacogn. 2015;2(3):17-22.
18. Chen Y, Xie MY, Gong XF. Microwave-assisted extraction used for the isolation of total triterpenoid saponins from *Ganoderma atrum*. J Food Eng. 2007;81(1):162-70. doi: 10.1016/j.jfoodeng.2006.10.018.
19. 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(3):178-82. doi: 10.38212/2224-6614.2748.
20. Nadernejad N, Ahmadimoghadam A, Hosseinifard SJ, Poorseyedi S. Evaluation of PAL activity, phenolic and flavonoid contents in three pistachio (*Pistacia vera* L.) cultivars grafted onto three different rootstocks. J Stress Physiol Biochem. 2013;9(3):84-97.
21. Chakraborty M, Mitra A. The antioxidant and antimicrobial properties of the methanolic extract from *Cocos nucifera* mesocarp. Food Chem. 2008;107(3):994-9. doi: 10.1016/j.foodchem.2007.08.083.
22. Mazzola PG, Jozala AF, de Lencastre Novaes LC, Moriel P, Penna TC. Minimal inhibitory concentration (MIC) determination of disinfectant and/or sterilizing agents. Braz J Pharm Sci. 2009;45(2):241-8. doi: 10.1590/s1984-82502009000200008.
23. Roby MHH, Sarhan MA, Selim KAH, Khalel KI. Evaluation of antioxidant activity, total phenols and phenolic compounds in thyme (*Thymus vulgaris* L.), sage (*Salvia officinalis* L.), and marjoram (*Origanum majorana* L.) extracts. Ind Crops Prod. 2013;43:827-31. doi: 10.1016/j.indcrop.2012.08.029.
24. Spigno G, De Faveri DM. Antioxidants from grape stalks and marc: Influence of extraction procedure on yield, purity and antioxidant power of the extracts. J Food Eng. 2007;78(3):793-801. doi: 10.1016/j.jfoodeng.2005.11.020.
25. Hanachi P, Zarringhalami R, Ramezani Tamijani R. Investigation of antioxidant properties of *Polygonatum orientale* Desf and *Tilia dasystyla* extracts by different methods and solvents. Hormozgan Med J. 2018;22(4):e86504. doi: 10.5812/hmj.86504.
26. López López LI, Nery Flores SD, Silva Belmares SY, Sáenz Galindo A. Naphthoquinones: biological properties and synthesis of lawsone and derivatives-a structured review. Vitae. 2014;21(3):248-58.