Pharmaceutical Sciences
December 2017, 23, 308-315
doi: 10.15171/PS.2017.45
http://journals.tbzmed.ac.ir/PHARM

Research Article

Antibacterial and Antioxidant Properties of Methanolic Extracts of Apple (*Malus pumila*), Grape (*Vitis vinifera*), Pomegranate (*Punica granatum* L.) and Common Fig (*Ficus carica* L.) Fruits

Yasser Shahbazi[1*]

[1]*Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran*.

Article Info

Article History:
Received: 16 December 2016
Accepted: 17 August 2017
ePublished: 30 December 2017

Keywords:
-Antibacterial
-Antioxidant
-Malus pumila
-Vitis vinifera
-Punica granatum
-Ficus carica

ABSTRACT

Background: As people demand to consume natural foods due to health concerns, the investigation of natural compounds for the discovery of new constituents with antibacterial and antioxidant properties is increasing. The aim of the present study was to evaluate antioxidant and antibacterial activities of some fruits including apple (*Malus pumila*), grape (*Vitis vinifera*), pomegranate (*Punica granatum* L.) and common fig (*Ficus carica* L.) produced in Kermanshah, west of Iran.

Methods: The antibacterial activity of fruit extracts were examined by broth micro-dilution and agar disk diffusion methods. Their antioxidant activity were measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferric reducing antioxidant power, β-carotene/linoleic acid bleaching and thiobarbituric acid (TBA) methods. The contents of total phenolic (TP) and total flavonoid (TF) of extracts were determined using Folin Ciocalteu and colorimetric methods, respectively.

Results: The highest and lowest antibacterial effects were found for *P. granatum* and *F. carica* extracts, respectively. The most antibacterial effect was observed against *Bacillus subtilis*, followed by *B. cereus* and *Staphylococcus aureus*, respectively. The similar sensitivity was observed for *Listeria monocytogenes*, *Salmonella typhimurium* and *Escherichia coli* O157:H7. *P. granatum* extract had a significant higher DPPH radical scavenging (0.16 ± 0.07 mg/ml), ability to prevent the bleaching of β-carotene (0.09 ± 0.01 mg/ml), ferric reducing power (0.33 ± 0.03 mg/ml) and TBA (0.27 ± 0.01 Meq malondialdehyde/g) values. The highest TP and TF were found in *P. granatum*, followed by *V. vinifera*, *M. pumila*, and *F. carica*, respectively, which were in consistent with the results of antioxidant and antibacterial properties.

Conclusions: The strong *in vitro* antioxidant activities of investigated fruit extracts support their traditional application in the treatment and/or prevention of different diseases.

Introduction

Since ancient times, the medicinal plants, fresh fruits, spices and vegetables have been recommended for prevention and/or treatment of inflammation, different types of cancer and food poisoning.[1-3] Moreover, there are numerous investigations indicating a significant positive correlation between the intake of fresh fruits and vegetables with human health and performance.[4-6] The remarkable interest in application of natural compounds such as medicinal plants and fruits has burgeoned due to their bioactive constituents and subsequently biochemical properties.[7] Indeed, the edible and medicinal plants, herbs, spices and fruits contain high relatively concentrations of terpenes, alkaloids, flavonoids, coumarins and other secondary metabolites, which have strong antibacterial, antifungal, antiviral, and antioxidant properties effects.[6,8-10]

On the other hand, due to growing concerns regarding application of synthetic additives in different foods further investigations for discovery new alternative natural products with plant, animal and microorganism origins is of an unquestionable interest.[11] A new natural compound with antimicrobial and antioxidant activities can decrease oxidation and microbial deterioration of fresh food products, potential risk of food-borne illnesses and

***Corresponding Author:** Yasser Shahbazi, E-mail: y.shahbazi@razi.ac.ir

economic losses.[6] In this context, the essential oils and extracts, which were obtained from different plant organs including bud, seed, root, leave, stem, wood, bark and flower, can be considered as alternative natural compounds with the possibility to control of spoilage microorganisms and food-borne pathogens.[12-14] Numerous studies have indicated that essential oils and/or extracts of natural compounds such as cinnamon, basil, peppermint, mustard, clove, olive oil and rosemary exhibit strong antibacterial activity against important food-borne pathogens including *Salmonella enteritidis*, *Escherichia coli* O157:H7, *Bacillus cereus*, *Bacillus subtilis*, *Listeria monocytogenes* and *Staphylococcus aureus*.[6,15-18] Essential oils and extracts are able to replace the most commonly used synthetic preservatives e.g. butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) in fat and fat-containing foods, which cause liver and DNA damages.[7,15]

Although different essential oils and extracts have been long discovered for their biological properties including antibacterial, antifungal, antiviral and antioxidant activities,[1,6,19,20] the recent enhancement of interest in 'green' consumerism has given rise to a renewal scientific awareness of them.[18] A considerable volume of scientific studies have suggested that the consumption of apple (*Malus pumila*), grape (*Vitis vinifera*), pomegranate (*Punica granatum* L.) and common fig (*Ficus carica* L.) is correlated with lower risks of oxidative and infectious diseases.[19-22] However, the antibacterial and antioxidant activities as well as mechanisms of their bioactivity still remain unknown. Therefore, the aims of the present study were to evaluate the antioxidant and antibacterial activities present in some fruits including apple, grape, pomegranate and common fig produced in Kermanshah, west of Iran.

Materials and Methods

Preparation of methanolic extracts of fruits

Newly ripe *M. pumila*, *V. vinifera*, *P. granatum* and *F. carica* were purchased from a local market in Kermanshah, west of Iran. Authentications of the fruits were conducted by Dr. Seyed Mohammad Masoumi (Faculty of Agriculture, Razi University, Kermanshah, Iran). Representative vouchers specimens (*M. pumila*: 3253; *V. vinifera:* 884; *P. granatum*: 1399 and *F. carica*: 6300) were deposited in the herbarium of the Research Center of Natural Resources of Tehran, Iran.

The preparation of methanolic extracts of fruits were conducted according to the previously published method by Basiri *et al.*,[23] with some minor modifications. For this purpose, each fruit was washed using tap water, dried in shadow at room temperature and powdered using mechanical blender. Then, 1 g of fine-powdered fruits was dissolved in 20 ml methanol and kept overnight at room temperature. The extract was filtered through Whatman filter paper no.3, subjected to a rotary evaporator at a maximum temperature of 40 ºC and stored in an amber vial in the refrigerator until antibacterial and antioxidant analyses.

Preparation of bacterial strains

A panel of microorganisms included *S. aureus* (ATCC 6538), *B. subtilis* (ATCC 6633), *B. cereus* (ATCC 11774), *L. monocytogenes* (ATCC 19118), as Gram-positive bacteria as well as *S. typhimurium* (ATCC 14028) and *E. coli* O157:H7 (ATCC 10536) as Gram-negative bacteria were used for antibacterial examinations of the methanolic extracts of aforementioned fruits. The strains were purchased from the culture collection of the Iranian Research Organization for Science and Technology (IROST), Tehran, Iran and maintained on slants of Brain Heart Infusion agar (BHI; Merck, Germany). The preparation of bacterial inoculants (10^6 CFU/ml) was conducted according to our previously published method.[8]

Antibacterial analysis of methanolic extracts of fruits

Determination of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC)

The *in vitro* antibacterial activity of the methanolic extracts of *M. pumila*, *V. vinifera*, *P. granatum* and *F. carica* were evaluated based on broth micro-dilution test proposed by Shahbazi and Shavisi.[7] For this purpose, methanolic extracts were dissolved in BHI broth containing 5% (v/v) dimethyl sulfoxide (DMSO) to reach a final concentrations of 10 mg/ml. Then, selected dilutions were made in a concentration ranging between 0.05-10 mg/ml. The 96-well sterile micro-dilution plates with U-bottom wells were prepared by pouring 180 µl of BHI broth containing different concentrations of extracts and 20 µl of the tested microorganisms. The last row well containing 180 µl BHI broth without the natural compounds and 20 µl of inoculum was used as parallel positive control. The plates were covered with sterile plate sealers. Contents of each well were mixed on a plate shaker for 2 min before incubation at 37 °C for 24 h. The MIC was defined as the lowest concentration of extract showing no white pellet on the well bottom of the test microorganisms. To determine MBC value, 20 µl of each well without any invisible growth was sub-cultured on BHI agar and incubated at 37 °C for 24 h.

Agar disk diffusion assay

For agar disk diffusion assay, 0.1 ml of each bacterial suspension (10^8 CFU/ml) was uniformly spread on BHI agar medium using a sterile cotton swab. Then, the sterile paper discs (6 mm in diameter) were incorporated with 10 µl of diluted

fruit extracts was placed on the surface of each BHI agar. The plates were incubated for 24 h under appropriate cultivation temperature (37°C). The area of the inhibition zone was calculated as πr^2. [13,24]

Antioxidant analysis of methanolic extracts of fruits

1, 1-Diphenyl-2-picrylhydrazyl (DPPH) assay

The 1, 1-diphenyl-2-picrylhydrazyl hydrate (DPPH) assay was used for determination of antioxidant properties of fruit extracts. Briefly, stock solutions (100 mg/ml) of each extract and the synthetic standard antioxidant BHT were prepared in methanol. Selected diluted concentrations were mixed to 1 ml of DPPH methanol solution. The mixtures were shaken and allowed to stand for 30 min at room temperature in the dark. Test was conducted for investigated extracts and BHT (Merck, Darmstadt, Germany) as synthetic antioxidant. The ultraviolet (UV) absorbances of these solutions were measured at 517 nm using visible light spectrophotometer. The percent of DPPH radical scavenging activity was calculated as follows:[25]

$$DPPH\ scavenging\ effect(I;\%) = [(A_{blank} - A_{sample})/A_{blank}] * 100 \quad \text{Eq.(1)}$$

Where A_{blank} is the absorbance of the blank sample and A_{sample} is the absorbance of the extract fruit. The sample concentration providing 50% inhibition (IC_{50}) was calculated from the curve of radical scavenge activity (I %) against sample concentration.

Ferric reducing power

Ferric reducing power of the fruit extracts were evaluated based on the previously method reported by Singh *et al.*[26] The reducing power of the extracts were determined at 690 nm in the Microplate Reader.

β-Carotene/linoleic acid bleaching assay

The method of Martucci *et al.* was used to determine the bleaching of β-carotene in linoleic acid emulsion system at 490 nm using a UV-vis spectrophotometer.[27]

Thiobarbituric acid reactive substances (TBA) assay

The thiobarbituric acid reactive substances (TBA) values, a secondary product of lipid peroxidation, of extracts were evaluated according to the method of Singh *et al.* [26] The TBA value (Meq malondialdehyde/g) of each extract was calculated as following formula:[26]

$$TBA\ value = \frac{[50 \times (A-B)]}{M} \quad \text{Eq.(2)}$$

Where A is the absorbance of test sample, B is the absorbance of reagent blank and M is the mass of the sample (mg).

The total amount of flavonoid and phenolic compounds

The content of total phenolic (TP) in fruit methanolic extracts was determined by Folin Ciocalteu method.[28] The amount of total phenolic compounds were expressed as gallic acid equivalents (GAE) in milligrams per gram dry fruit extract. The total flavonoid (TF) content was determined using colorimetric method.[29] The results were expressed in mg rutin in g dry matter by comparison with standard rutin treated in the same conditions.

Statistical analysis

All experiments were repeated three times and the results were expressed as mean ± SD. The statistical analysis was performed using SPSS 16.0 software program (SPSS, Chicago, IL, USA). Statistical significance levels used was $P < 0.05$.

Results and Discussion

In few last decades, there has been especial interest in the use of abundant naturally occurring antimicrobials and antioxidants such as herbs, spices, plants and fruits for medicinal applications and as food additives.[30,31] In the present study, methanolic extracts of *M. pumila*, *V. vinifera*, *P. granatum* and *F. carica* were evaluated to compare their total phenolic and flavonoid contents, as well as antibacterial and antioxidant properties.

Tables 1 and 2 summarize antibacterial effects of *M. pumila*, *V. vinifera*, *P. granatum* and *F. carica* extracts against some food-borne pathogenic bacteria.

Table 1. Antibacterial effect *M. pumila*, *V. vinifera*, *P. granatum* and *F. carica* extracts indicated as Minimum Inhibitory/Bactericidal Concentrations-MIC/MBC (mg/ml).

	M. pumila		*V. vinifera*		*P. granatum*		*F. carica*	
Bacteria	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Staphylococcus aureus	>10	>10	8	10	8	8	>10	>10
Bacillus subtilis	8	8	6	6	4	4	10	10
Bacillus cereus	8	10	6	8	4	6	10	10
Listeria monocytogenes	>10	>10	>10	>10	>10	>10	>10	>10
Salmonella typhimurium	>10	>10	>10	>10	>10	>10	>10	>10
***Escherichia coli* O157:H7**	>10	>10	>10	>10	>10	>10	>10	>10

Table 2. Antibacterial effect of *M. pumila*, *V. vinifera*, *P. granatum* and *F. carica* extracts by agar disk diffusion assay.

	Inhibition zone (mm)			
Bacteria	***M. pumila***	***V. vinifera***	***P. granatum***	***F.. carica***
Staphylococcus aureus	ND	7.06	12.56	ND
Bacillus subtilis	3.14	12.56	28.26	3.14
Bacillus cereus	3.14	12.56	28.26	3.14
Listeria monocytogenes	ND	3.14	3.14	ND
Salmonella typhimurium	ND	3.14	3.14	ND
***Escherichia coli* O157:H7**	ND	3.14	3.14	ND

ND: Not detected

As it can be seen, most precise results on the antibacterial properties of fruit extracts were obtained through the disc diffusion method. The antimicrobial activities of essential oils and extracts can be classified into three levels:[6] weak activity (inhibition zone lower than 12 mm), moderate activity (inhibition zone between 12 and 20 mm) and strong activity (inhibition zone higher than 20 mm). Accordingly, the highest antibacterial effect was found for methanolic extracts of *P. granatum* followed by *V. vinifera*, *M. pumila* and *F. carica*, respectively. Indeed, *P. granatum* and *V. vinifera* extracts were active against all the tested microorganisms, whose zones of inhibition were in the range of 3.14-28.26 mm. It was also worth noting that *M. pumila* and *F. carica* extracts were active only against the two Gram-positive bacteria (*B. subtilis* and *B. cereus*) with zones of inhibition 3.14 mm and MIC between 8-10 mg/ml. The higher antimicrobial activities of *P. granatum* and *V. vinifera* extracts compared to *M. pumila* and *F. carica* extracts are most likely due to the presence of higher polyphenolic compounds such as catechins, epicatechin, epicatechin-3-o-gallate, monomeric flavanols and procyanidins, tannins and flavonoids.[23] As it can be seen in Table 3, the highest total phenolic and flavonoid compounds were found in methanolic extracts of *P. granatum* (TP: 265.72 mg gallic acid/g and TF: 60.46 mg rutin/g), followed by *V. vinifera* (TP: 146.4 mg gallic acid/g and TF: 37.06 mg rutin/g), *M. pumila* (TP: 93.16 mg gallic acid/g and TF: 14.43 mg rutin/g), and *F. carica* (TP: 45.89 mg gallic acid/g and TF: 9.88 mg rutin/g), respectively. The results of the phenolic and flavonoid compounds of fruit extracts were good in agreement with the results of antibacterial properties. Khan and Hanee reported that bioactive compounds of *P. granatum* extract including polyphenols, tannins, flavonoids and anthocyanins had antibacterial effects against *E. coli*, *P. aeruginosa* and *S. aureus*.[32]

Moreover, Al-Zoreky *et al.* investigated *in vitro* (agar diffusion) antimicrobial activity of methanolic extract of *P. granatum*. Based on their results, *P. granatum* was a potent inhibitor for *L. monocytogenes*, *S. aureus*, *E. coli* O157:H7 and *Yersinia enterocolitica* by inhibition zones of 3.68, 14.42, 4.12 and 5.32 mm, respectively, Their phytochemical analyses revealed the presence of high active inhibitors in peels, including phenolics and flavonoids.[33] Jayaprakasha *et al.*, and Kakaei and Shahbazi, indicated that proanthocyanidins contained in red grape seed extract have potent antibacterial activity and should be considered as a potential antibacterial agent in the food active packaging.[22,34] In another study, *M. pumila* was presented as a rich source of phenolic compounds (proanthocyanidin B1 and B2, catechin, epicatechin, cyanidin-3-O-galactoside and Quercetin-3-O-rhamnoside) and a good antibacterial agent against *B. subtilis* and *B. cereus*.[35] According to the literature review by Sirisha *et al.*, methanolic *F. carica* L extract contained low level of phenolic compounds such as oleanolic acid, rusolic acid, α-hydroxy ursolic acid, protocatechic acid and maclinic acid and therefore did not exhibit strong antibacterial effect against Gram-positive and Gram-negative bacteria. [36]

Table 3. Antioxidant activity, total phenolic and flavonoid compounds of *M. pumila*, *V. vinifera*, *P. granatum* and *F. carica* extracts (mean ± SD).

	M. pumila	***V. vinifera***	***P. granatum***	***F. carica***	**BHT**
DPPH radical-scavenging activity (IC_{50}; mg/ml)	0.36 ± 0.22^{b}	0.23 ± 0.43^{c}	0.16 ± 0.07^{d}	0.54 ± 0.23^{a}	0.018 ± 0.01^{e}
Ferric reducing power (EC_{50}; mg/ml)	0.67 ± 0.02^{b}	0.55 ± 0.01^{c}	0.33 ± 0.03^{d}	0.98 ± 0.01^{a}	0.0 ± 0.0^{e}
β-Carotene bleaching inhibition (EC_{50}; mg/ml)	0.19 ± 0.01^{b}	0.12 ± 0.01^{c}	0.09 ± 0.01^{d}	0.24 ± 0.00^{a}	0.012 ± 0.001^{e}
Thiobarbituric acid (Meq malondialdehyde/g)	0.46 ± 0.01^{b}	0.35 ± 0.02^{c}	0.27 ± 0.01^{d}	0.62 ± 0.02^{a}	0.087 ± 0.001^{e}
Total phenolic (mg gallic acid/g)	93.16 ± 9.12^{c}	146.4 ± 14.71^{b}	265.72 ± 6.21^{a}	45.89 ± 6.33^{d}	-
Total flavonoid (mg rutin /g)	14.43 ± 1.11^{c}	37.06 ± 2.14^{b}	60.46 ± 3.20^{a}	9.88 ± 0.21^{d}	-

$^{a-e}$ Columns representing different values are labeled with different letters ($P < 0.05$).

In general, the possible mechanism for antibacterial effect of polyphenolic compounds might be attributed to the presence of -OH group in the phenolic compounds which can easily bind to the active site of enzymes by changing the cell metabolism of microorganisms as well as attachment with minerals, vitamins and carbohydrates and making them unavailable for microorganisms.[23,37,38]

By comparing among bacterial strains, the most antibacterial effect was observed against *B. subtilis*, followed by *B. cereus* and *S. aureus*, respectively. Moreover, the similar sensitivity was observed for *L. monocytogenes*, *S. typhimurium* and *E. coli* O157:H7. The most important reason for the differences in microorganism's susceptibility to antimicrobial agents might be attributed to the outer cytoplasmic membrane surrounding the thin peptidoglycan structure of Gram-negative bacteria, which lead to restrict diffusion of hydrophobic compounds through its lipopolysaccharide covering.[15,37] Moreover, the periplasmatic space contains enzymes which are capable of breaking down foreign molecules introduced from the outside.[39] Lv *et al.*,[6] investigated the antimicrobial efficacy of selected plant essential oils and extracts against four food-related microorganisms including *E. coli*, *S. aureus* and *B. subtilis*. Accordingly, the Gram-negative bacteria was found to be the most resistant to the examined natural compounds, which was good in accordance with our findings. In another study, Gilles *et al.*,[13] examined the antimicrobial properties of three common Australian *Eucalyptus* species using agar disc diffusion method and concluded that Gram-positive bacteria were more sensitive than Gram-negative bacteria. *S. aureus* was the most sensitive strain, while *P. aeruginosa* was the most resistant.

In vitro antioxidant properties of *M. pumila*, *V. vinifera*, *P. granatum* and *F. carica* extracts were examined using DPPH radical scavenging, ferric reducing antioxidant power, β-carotene/linoleic acid bleaching and TBA methods and their results are exhibited in Table 3. As it can be observed, *P. granatum* extract had a significant higher DPPH radical scavenging (0.16 ± 0.07 mg/ml), ability to prevent the bleaching of β-carotene (0.09 ± 0.01 mg/ml), ferric reducing power (0.33 ± 0.03 mg/ml) and TBA (0.27 ± 0.01 Meq malondialdehyde/g) value. It was found that *V. vinifera*, *M. pumila* and *F. carica* extracts showed remarkable antioxidant activities, but *V. vinifera* extract exerted more DPPH radical scavenging (0.23 ± 0.43 mg/ml), ability to prevent the bleaching of β-carotene (0.12 ± 0.01 mg/ml), ferric reducing power (0.55 ± 0.01 mg/ml) and TBA (0.35 ± 0.02 Meq malondialdehyde/g) other *M. pumila* and *F. carica* extracts. Similar results were also found in previous studies where reported that all parts of *V. vinifera* and *P. granatum* had high antioxidant activities.[10,21-23,32] According to these results, the DPPH radical scavenging activities of *V. vinifera* and *P. granatum* extracts were ranged 0.18-0.27 and 0.14-0.25 mg/ml, respectively, which are good in agreement with our findings. It seems that the ranges of antioxidant activities of *M. pumila* and *F. carica* extracts were remarkably higher than those of reported in the literature.[40-42] for example, Ćetković *et al.*[41] investigated antioxidant activity of different varieties of apple collected from Serbia using their ability to DPPH radical scavenging activity and found that IC_{50} values were varied from 6.33 mg/ml to 26.11 mg/ml. Based on the results of Suárez *et al.*[40] DPPH radical scavenging and ferric reducing power values of apple extract obtained from Spain were 6.66 and 7.73 mg/ml, respectively. Yang *et al.*[42] reported that IC_{50} value of water *F. carica* extract on scavenging DPPH radicals was 0.72 mg/ml and indicated that it could be used as a free radical inhibitor and primary antioxidant. In general, differences in the antioxidant activities of investigated extracts among studies could be explained by variations in phenolic content of extracts and antioxidant procedures used in tests.[10,23] Similar to the antibacterial activities, the antioxidant properties of fruit extracts are related to phytochemical contents especially total phenolic compounds such as anthocyanins in skins and seeds of fruits.[10] As previously described, the highest total phenolic and flavonoid compounds were found in methanolic extracts of *P. granatum*, followed by *V. vinifera*, *M. pumila*, and *F. carica*, respectively, which are in consistent with the results of antioxidant properties. In confirmation with our findings, Ćavar *et al*. concluded that there was significant correlations between antioxidant assays and the phenolic compounds.[43] Indeed, the hydroxyl group of phenolic compounds inhibit the production of reactive oxygen species and scavenging of free radicals.[37] It has been shown that ursolic acid, oleanolic acid and other triterpenoids present in *F. carica* extract are efficient protectors against lipid peroxidation and hence these are potent antioxidants.[36]

Conclusion

The findings of present study revealed the *in vitro* antibacterial and antioxidant activities of methanolic extracts of *M. pumila*, *V. vinifera*, *P. granatum* and *F. carica*. The strong *in vitro* antioxidant activities of investigated fruit extracts support their traditional application in the treatment and/or prevention of different diseases. According to our results, *M. pumila*, *V. vinifera*, *P. granatum* and *F. carica* extracts may be recommended as a fruit based antibacterial and antioxidant food additives for increasing of shelf life of stored food commodities.

Conflict of interests

The author claims that there is no conflict of interest.

References

1. Badawy ME, Abdelgaleil SA. Composition and antimicrobial activity of essential oils isolated from egyptian plants against plant pathogenic bacteria and fungi. Ind Crops Prod. 2014;52:776-82. doi:10.1016/j.indcrop.2013.12.003
2. Saei Dehkordi SS, Tajik H, Moradi M, Khalighi Sigaroodi F. Chemical composition of essential oils in *Zataria multiflora* boiss. from different parts of Iran and their radical scavenging and antimicrobial activity. Food Chem Toxicol. 2010;48(6):1562-7. doi:10.1016/j.fct.2010.03.025
3. Lansky EP, Newman RA. *Punica granatum* (pomegranate) and its potential for prevention and treatment of inflammation and cancer. J Ethnopharmacol. 2007;109(2):177-206. doi:10.1016/j.jep.2006.09.006
4. Denev P, Kratchanova M, Ciz M, Lojek A, Vasicek O, Nedelcheva P, et al. Biological activities of selected polyphenol-rich fruits related to immunity and gastrointestinal health. Food Chem. 2014;157:37-44. doi:10.1016/j.foodchem.2014.02.022
5. Bobinaitė R, Viskelis P, Bobinas Č, Mieželienė A, Alenčikienė G, Venskutonis PR. Raspberry marc extracts increase antioxidative potential, ellagic acid, ellagitannin and anthocyanin concentrations in fruit purees. LWT-Food Sci Technol. 2016;66:460-7. doi:10.1016/j.lwt.2015.10.069
6. Lv F, Liang H, Yuan Q, Li C. In vitro antimicrobial effects and mechanism of action of selected plant essential oil combinations against four food-related microorganisms. Food Res Int. 2011;44(9):3057-64. doi:10.1016/j.foodres.2011.07.030
7. Shahbazi Y, Shavisi N. Interactions of *Ziziphora clinopodioides* and *Mentha spicata* essential oils with chitosan and ciprofloxacin against common food-related pathogens. LWT-Food Sci Technol. 2016;71:364-9. doi:10.1016/j.lwt.2016.04.011
8. Shahbazi Y, Shavisi N, Karami N, Kakaei S. Chemical composition and *in vitro* antibacterial activity of *Ferulago angulata* (schlecht.) boiss essential oil. Pharm Sci. 2015;21(1):6-11. doi:10.15171/ps.2015.10
9. Shahbazi Y, Shavisi N, Mohebi E. Effects of *Ziziphora clinopodioides* essential oil and nisin, both separately and in combination, to extend shelf life and control *Escherichia coli* O157:H7 and *Staphylococcus aureus* in raw beef patty during refrigerated storage. J Food Saf. 2016;36(2):227-36. doi:10.1111/jfs.12235
10. Xu C, Zhang Y, Cao L, Lu J. Phenolic compounds and antioxidant properties of different grape cultivars grown in china. Food Chem. 2010;119(4):1557-65. doi:10.1016/j.foodchem.2009.09.042
11. Volpe MG, Siano F, Paolucci M, Sacco A, Sorrentino A, Malinconico M, et al. Active edible coating effectiveness in shelf-life enhancement of trout (*Oncorhynchusmykiss*) fillets. LWT - Food Science and Technology. 2015;60(1):615-22. doi:10.1016/j.lwt.2014.08.048
12. Campana R, Casettari L, Fagioli L, Cespi M, Bonacucina G, Baffone W. Activity of essential oil-based microemulsions against *Staphylococcus aureus* biofilms developed on stainless steel surface in different culture media and growth conditions. Int J Food Microbiol. 2017;241:132-40. doi:10.1016/j.ijfoodmicro.2016.10.021
13. Gilles M, Zhao J, An M, Agboola S. Chemical composition and antimicrobial properties of essential oils of three Australian eucalyptus species. Food Chem. 2010;119(2):731-7. doi:10.1016/j.foodchem.2009.07.021
14. Shahbazi Y. Chemical composition and *in vitro* antibacterial activity of *Mentha spicata* essential oil against common food-borne pathogenic bacteria. J Pathogens. 2015;2015:1-5. doi:10.1155/2015/916305
15. Burt S. Essential oils: Their antibacterial properties and potential applications in foods-a review. Int J Food Microbiol. 2004;94(3):223-53. doi:10.1016/j.ijfoodmicro.2004.03.022
16. Zhang Y, Ma Q, Critzer F, Davidson PM, Zhong Q. Physical and antibacterial properties of alginate films containing cinnamon bark oil and soybean oil. LWT - Food Science and Technology. 2015;64(1):423-30. doi:10.1016/j.lwt.2015.05.008
17. Teixeira B, Marques A, Pires C, Ramos C, Batista I, Saraiva JA, et al. Characterization of fish protein films incorporated with essential oils of clove, garlic and origanum: Physical, antioxidant and antibacterial properties. LWT - Food Science and Technology. 2014;59(1):533-9. doi:10.1016/j.lwt.2014.04.024
18. Turgis M, Han J, Caillet S, Lacroix M. Antimicrobial activity of mustard essential oil against *Escherichia coli* O157: H7 and *Salmonella typhi*. Food Control. 2009;20(12):1073-9. doi:10.1016/j.foodcont.2009.02.001
19. Solomon A, Golubowicz S, Yablowicz Z, Grossman S, Bergman M, Gottlieb HE, et al. Antioxidant activities and anthocyanin content of fresh fruits of common fig (*Ficus carica* l.). J Agric Food Chem. 2006;54(20):7717-23. doi:10.1021/jf060497h
20. Hamauzu Y, Yasui H, Inno T, Kume C, Omanyuda M. Phenolic profile, antioxidant

property, and anti-influenza viral activity of chinese quince (*Pseudocydonia sinensis* schneid.), quince (*Cydonia oblonga* mill.), and apple (*Malus domestica* mill.) fruits. J Agric Food Chem. 2005;53(4):928-34. doi:10.1021/jf0494635
21. Mohebi E, Shahbazi Y. Application of chitosan and gelatin based active packaging films for peeled shrimp preservation: A novel functional wrapping design. LWT-Food Sci Technol. 2017;76:108-16. doi:10.1016/j.lwt.2016.10.062
22. Kakaei S, Shahbazi Y. Effect of chitosan-gelatin film incorporated with ethanolic red grape seed extract and *Ziziphora clinopodioides* essential oil on survival of *Listeria monocytogenes* and chemical, microbial and sensory properties of minced trout fillet. LWT-Food Sci Technol. 2016;72:432-8. doi:10.1016/j.lwt.2016.05.021
23. Basiri S, Shekarforoush SS, Aminlari M, Akbari S. The effect of pomegranate peel extract (PPE) on the polyphenol oxidase (PPO) and quality of pacific white shrimp (*Litopenaeus vannamei*) during refrigerated storage. LWT-Food Sci Technol. 2015;60(2):1025-33. doi:10.1016/j.lwt.2014.10.043
24. Shahbazi Y, Ahmadi F, Karami N. Screening, determination and confirmation of tetracycline residues in chicken tissues using Four-Plate Test, ELISA and HPLC-UV methods: Comparison between correlation results. Food Agric Immunol. 2015;26(6):821-34. doi:10.1080/09540105.2015.1036357
25. Wannes WA, Mhamdi B, Sriti J, Jemia MB, Ouchikh O, Hamdaoui G, et al. Antioxidant activities of the essential oils and methanol extracts from myrtle (*Myrtus communis* var. *Italica* L.) leaf, stem and flower. Food Chem Toxicol. 2010;48(5):1362-70. doi:10.1016/j.fct.2010.03.002
26. Singh G, Kapoor I, Singh P, De Heluani CS, De Lampasona MP, Catalan CA. Comparative study of chemical composition and antioxidant activity of fresh and dry rhizomes of turmeric (*Curcuma longa linn.*). Food Chem Toxicol. 2010;48(4):1026-31. doi:10.1016/j.fct.2010.01.015
27. Martucci JF, Gende LB, Neira L, Ruseckaite RA. Oregano and lavender essential oils as antioxidant and antimicrobial additives of biogenic gelatin films. Ind Crops Prod. 2015;71:205-13. doi:10.1016/j.indcrop.2015.03.079
28. Motamed SM, Naghibi F. Antioxidant activity of some edible plants of the turkmen sahra region in northern iran. Food Chem. 2010;119(4):1637-42. doi:10.1016/j.foodchem.2009.09.057
29. Siahpoosh A, Javedani F. Antioxidative capacity of Iranian citrus deliciosa peels. Free Radic Res. 2012;2(2):62-7. doi:10.5530/ax.2012.2.2.11
30. Klančnik A, Piskernik S, Jeršek B, Možina SS. Evaluation of diffusion and dilution methods to determine the antibacterial activity of plant extracts. J Microbiol Methods. 2010;81(2):121-6. doi:10.1016/j.mimet.2010.02.004
31. Shahbazi Y. Chemical composition and *in vitro* antibacterial effect of *Ziziphora clinopodioides* essential oil. Pharm Sci. 2015;21(2):51-6. doi:10.15171/ps.2015.17
32. Khan JA, Hanee S. Antibacterial properties of *Punica granatum* peels. Int J Appl Biol Pharm Technol. 2011;2(3):23-7.
33. Al-Zoreky N. Antimicrobial activity of pomegranate (*Punica granatum* l.) fruit peels. Int J Food Microbiol. 2009;134(3):244-8. doi:10.1016/j.ijfoodmicro.2009.07.002
34. Jayaprakasha GK, Selvi T, Sakariah KK. Antibacterial and antioxidant activities of grape (*Vitis vinifera*) seed extracts. Food Res Int. 2003;36(2):117-22. doi:10.1016/s0963-9969(02)00116-3
35. Du WX, Olsen CW, Avena Bustillos RJ, Friedman M, McHugh TH. Physical and antibacterial properties of edible films formulated with apple skin polyphenols. J Food Sci. 2011;76(2):M149-M55. doi:10.1111/j.1750-3841.2010.02012.x
36. Sirisha N, Sreenivasulu M, Sangeeta K, Chetty CM. Antioxidant properties of ficus species–a review. Int J PharmTech Res. 2010;2(4):2174-82.
37. Gyawali R, Ibrahim SA. Natural products as antimicrobial agents. Food Control. 2014;46:412-29. doi:10.1016/j.foodcont.2014.05.047
38. Naczk M, Shahidi F. Phenolics in food and nutraceuticals. Boca Raton, Florida: CRC Press; 2004.
39. Sivaraj A, Devagnanaroopan J, Palani B, Senthilkumar B. Antibacterial activity of *Eucalyptus oblique* aqueous leaf extract on selective *Vibrio* species of *Penaeus monodon* culture hatchery. World J Pharm Sci. 2017;5(1):36-9.
40. Suárez B, Álvarez ÁL, García YD, del Barrio G, Lobo AP, Parra F. Phenolic profiles, antioxidant activity and *in vitro* antiviral properties of apple pomace. Food Chem. 2010;120(1):339-42. doi:10.1016/j.foodchem.2009.09.073
41. Ćetković G, Čanadanović Brunet J, Djilas S, Savatović S, Mandić A, Tumbas V. Assessment of polyphenolic content and *in vitro* antiradical characteristics of apple pomace. Food Chem. 2008;109(2):340-7. doi:10.1016/j.foodchem.2007.12.046
42. Yang XM, Yu W, Ou ZP, Liu WM, Ji XL. Antioxidant and immunity activity of water extract and crude polysaccharide from *Ficus carica* L. fruit. Plant Foods Hum Nutr.

2009;64(2):167-73. doi:10.1007/s11130-009-0120-5
43. Ćavar S, Maksimović M, Šolić ME, Jerković Mujkić A, Bešta R. Chemical composition and antioxidant and antimicrobial activity of two *Satureja* essential oils. Food Chem. 2008;111(3):648-53. doi:10.1016/j.foodchem.2008.04.033