Comparative in vitro Study of the biological activity and chemical composition extracts of Helicteres isora L. obtained by water and subcritical water extraction

Zohreh Didar

Department of Food Science and Technology, Neyshabur Branch, Islamic Azad University, Neyshabur, Iran

Correspondence to: E-mail: z_didar57@yahoo.com

Received 25 August 2019; Revised 13 September 2019; Editorial decision 3 November 2019.

Abstract

Objectives: Subcritical water extraction technique is considered as an environmentally extraction technique. The aim of this study was to compare the different characteristics of water extract and subcritical water extract of Helicteres isora L.

Materials and Methods: Water extraction was performed under the following conditions: 25°C, 24 h, and solid-to-water ratio 1:30. Subcritical water extract was carried out under specific conditions (pressure = 10 bar, temperature = 160°C, solid-to-water ratio = 1:30, time = 30 min). Chemical composition analysis was performed using GC–Mass chromatography. Anti-biofilm activity in the terms of anti-attach and removal of biofilm were assessed using the ELISA reader method and reading absorbance at 570 nm. Anti-microbial activity against Bacillus cereus, Staphylococcus aureus, Staphylococcus saprophyticus, and Bacillus subtilis was investigated by measurement of inhibitory zone diameter. Anti-enzymatic and antioxidant properties were also assessed.

Results: The results of GC–Mass analysis showed some components extracted in subcritical method which were absent in water extract such as octadecanoic acid, hexadecanoic acid, and berberin. Antioxidant activity of the two tested extracts revealed that subcritical water extract had more antioxidant capacity than water extract (P ≤ 0.05). The two tested extracts exhibited anti-enzymatic activity against polyphenol oxidase enzyme with better performance of subcritical water extract. Anti-biofilm activity of the two extracts implies that, in the case of preventing biofilm formation, both extracts had similar efficiency but in the removal of biofilm, subcritical water extract showed better performance. Both extracts had anti-microbial activity against B. cereus, S. aureus, S. saprophyticus, and B. subtilis with better performance of subcritical water extract. Anti-enzymatic assay also showed similar results.

Conclusions: Subcritical water extract of H. isora showed more antioxidant activity as well as anti-biofilm, anti-bacterial, and anti-enzymatic activity rather than ordinary water extract.

Key words: Helicteres isora L; subcritical water; anti-biofilm; antioxidant; anti-bacterial.
Introduction

Plant materials possess various bio-molecule components with beneficial biological activities including antioxidant, anti-microbial, antifungal, antiviral, anti-inflammatory, and anticancer activities (Cvetanović et al., 2018). Novel scientific trends application of natural products (such as plants extract, pure compounds, or standardized extracts) in the different fields of science such as therapy, prevention of numerous diseases, and food technology. Different researches affirmed beneficial of diet composed of plant in human health and prevention of cancers, cardiovascular diseases and diabetes, and osteoporosis and neurodegenerative diseases (Graf et al., 2005). Accordingly, there is interest for investigation of characteristics of plant material sources and their beneficial impacts. Food industry is one of the fields focused on application of natural products in food, especially for enhancement of the food functionality properties and replacement synthetic components with natural compounds. Extraction approach is one of the main factors affecting the efficiency of resulted plant extract. For this purpose, various techniques are available in which most of them are in the basis of extraction via organic solvents. Organic solvents have a negative impact on human health as well as environment. Some environmentally benign technologies are introduced by some researchers which imply on water usage as solvent. A disadvantage of these methods includes low solubility of many organic components in water and high energy need for solvent removal (Cvjetko Bubalo et al., 2015). Subcritical is a technique in which water acts as an excellent solvent and dissolves all the components and could be used for the extraction of non-polar and moderately polar compounds under the controlled test conditions.

Helicteres isora L. is a plant that belongs to the Sterculiaceae family. Various beneficial properties of H. isora were reported that include anticancer, antioxidant, antiperoxidative potency, and antibacterial potency (Kumar & Kumar Singh, 2014).

Subcritical water extraction has been applied to extract value-added by-products (e.g. bioactive phenolic compounds) from plants such as xanthone from mangosteen pericarps (Machmudaha et al., 2018), flavanones from defatted orange peel (Lachos-Pereza et al., 2018), antioxidants from mountain germander (Nastić et al., 2018), and Nannochloropsis salina oil (Eikani et al., 2019). Cvetanovic et al. (2017) pointed out subcritical water at specific conditions that resulted in suitable performance in extraction of apigenin (Cvetanovic et al., 2017). Gabaston et al. (2018) concluded that applying subcritical water extraction method resulted in more extraction of stilbenes from grapevine by-products (Gabaston et al., 2018).

The aim of this research was to compare water extract of H. isora, in terms of chemical composition, antioxidant property, anti-enzymatic, anti-microbial, and anti-biofilm activities using water and subcritical water extraction methods.

Materials and Methods

Plant material

Helicteres isora was purchased from local market and its variety was confirmed in Systematic Biology of Islamic Azad University, Neyshabur branch.

Preparation of subcritical water extract

The extraction of H. isora was performed under recommended conditions by Mohammadi et al. (2014): 160°C, 30 min, pressure = 10 bar, sample-to-solvent ratio = 1:30. The obtained extracts were filtered and stored in a refrigerator for further analysis.

Water extraction of H. isora

For water extraction, the method outlined by Kumar et al. (2013) was adapted. One hundred grams of H. isora were soaked in 3-L distilled water. The extraction method was cold percolation (25°C, 24 h) (Kumar et al., 2013).

Anti-microbial activity

Microbial strains of S. aureus (PTCC 1112), S. saprophyticus (PTCC 1440), B. subtilis (PTCC1720), and B. cereus (PTCC 1015) were purchased from Iranian Research Organization for Science and Technology (IROST) in a lyophilized form. Thereafter, lyophilized vials of bacteria were broken under sterile condition and transferred to a suitable culture medium (according to the recommendation of IROST) and incubated at 37°C for 24 h (Yolmeh et al., 2015). Microbial cells were harvested by centrifugation at 4000 g (ALC4232model). The MacFarland method was then applied for adjustment microbial population equal to 10^8 CFU ml^-1 (Moradian Eivari et al., 2015).

Anti-bacterial activity was evaluated using the disk diffusion method. For this reason, bacterial population equal to the population of 10^6 CFU ml^-1 was transferred to culture media. Then, 20 μl of extracts of H. isora poured on the disks and incubated at 37°C for 24 h. Inhibition zone was measured by the Guanglu 25-0 Digital Caliper and considered as the susceptibility of the bacteria (Mohammadi et al., 2015).

Anti-attach activity of H. isora extracts against biofilms of bacterial strains

Preparation of bacterial strains was carried out according to the following steps. To determine the biofilm formation, 200 μl of broth media containing bacterial strains (in population equal to 10^6 CFU ml^-1) were transferred to polystyrene microplates. Two hundred microliters of H. isora extracts were added to each well of a microplate and then, incubated at 37°C for 24 h. The wells containing sterile broth alone were used as control. After incubation, the broth culture medium was drained and each well was washed three times with 200 μl of phosphate-buffered saline (pH = 7.7) and reversed to dry, then washed with ethanol 95%, and stained with 100 μl of 1% Crystal Violet for 5 min. The remaining colour was washed three times with sterile distilled water. The microplate was dried for 30 min, and then the optical density at 570 nm was measured by ELISA Reader (AWARNNESS model). The biofilm formation was classified as follows: OD > 1, high levels of biofilm formation; 0.1 ≤ OD > 1, average biofilm formation; and OD ≤ 0.1, no biofilm formation (Noumi et al., 2017).

Biofilm removal activity of extracts of H. isora

To investigate the effect of extracts of H. isora on the removal of formed bacterial biofilms, a specific population (10^6 CFU ml^-1) of each bacterial strain was cultured in microplate and incubated at 37°C, 24 h. Then, extracts of H. isora were added to each well and incubated at 37°C for 150 min. Then the contents of each well were drained and the other steps of washing and staining of the microplate were similar to those described above (Todorov et al., 2018).

Anti-enzymatic activity of water extracts of H. isora

The anti-enzymatic activity of extracts against polyphenol oxidase of potato was determined as follows: first, potato samples were subjected to water and subcritical water extracts for 72 h and dried at room temperature (Fasih et al., 2016). Thereafter, to determine the activity
of polyphenol oxidase enzyme, 2.3 ml of phosphate buffer (pH = 7) and 0.6 ml of pyrocatechol 100 mmol ml⁻¹ were placed in a water bath at 25°C. The reaction was started by adding 0.1 ml of enzyme extract. Absorption was measured by a spectrophotometer (Jenway 6305, UK) at 420 nm in a 10-min period. In the control sample, distilled water was used instead of the enzyme extract (Lante et al., 2015).

Chemical composition

GC–MS analysis was performed by using Agilent 7890 A, injector 7683B, capillary column HP with the length of 30 m, ID 0.25 µm, and film thickness of 0.25 µm (Barupal et al., 2019).

DPPH radical scavenging assay

For the assessment of antioxidant activity of H. isora extracts, DPPH radical scavenging assay was applied. Ethanol solution of DPPH 0.05 mM (300 µl) was blended to 40-ml extract with 1000 µg/ml concentrations. After 5 min, absorbance was read at 517 nm. The radical scavenging activity of the plant extract was expressed as percentage of inhibition against control (Braca et al., 2002).

Statistical analysis

All analyses were performed in triplicate and are expressed as means ± standard deviation (SD). Mean values were considered as significantly different at P < 0.05 confidence level, after the ANOVA analysis. Comparison of means performed by Duncan.

Results and Discussion

Chemical composition of water extracts of H. isora

The obtained results of chemical composition analysis by GC–Mass chromatography revealed that various chemical components including aldehydes, alcohols, alkaloids, fatty acids, and esters are present in extracts of H. isora (Figure 1). Some differences in chemical composition of the two examined extracts were observed and some chemicals were present only in subcritical extracted extract (including hexadecanoic acid, Octadecanoic acid, and berberine). The main reason of these observations might be attributed to the specific characteristics of the subcritical method in extracting compounds. Subcritical water can be an alternative approach for the extraction of components with low polarity. With the aid of this method, several components with low polarity could be selectively extracted at various temperatures (100–374°C) and pressure. Under subcritical water conditions, increasing temperature resulted in weakening of hydrogen bonding of water and enhancement of dielectric constant of molecules of water (Teo et al., 2010). As the temperature of water reaches 225°C, the dielectric constant of water reaches close to the dielectric constant of methanol and ethanol at ambient temperature (Miller et al., 1998). Other reports pointed out more efficiency of subcritical water extraction method in extracting different chemical components. Nastić et al. (2018) reported that extraction of bioactive components such as gallic acid, catechin, chlorogenic acid, vanillic acid, caffeic acid, and epicatechin through subcritical water extraction from traditional Serbian medicinal plants had better performance (Nastić et al., 2018).

Antioxidant activity of water extract of H. isora

According to the results, antioxidant capacity of ordinary water extract and subcritical water extract of H. isora was 21.2% and 35.5%, respectively. This result approved the antioxidant capacity of two tested extract as well as higher antioxidant capacity of subcritical water extract, which could be attributed to the presence of chemical compounds with antioxidant characteristics such as hexadecanoic acid, octadecanoic acid, and berberine in subcritical water extract (Table 1). According to statistical analysis, antioxidant activity of the two extracts of H. isora significantly differed (P ≤ 0.05).

This result is in accordance with the results obtained from GC–Mass analysis of the two tested extracts. The subcritical method shows more extraction of chemical compounds with antioxidant potency resulted in more antioxidant activity of the extract. Some researchers also reported similar results. Higher antioxidant activity against 1,1-Diphenyl-2-picrylhydrazyl radicals was observed in subcritical water extracts of tested plant materials (G. macrorrhizum, T. chamaedrys) (Nastić et al., 2018).
Anti-attach activity of water extracts of *H. isora*

Biofilm formation causes remarkable issues in food industry in the view point of safety. This is more considerable in relation to the formation of biofilm from pathogenic bacteria.

Anti-biofilm activity included two different parts: prevention of biofilm formation (anti-attach property) and biofilm removal. In the present study, the effect of two tested extracts of *H. isora* was assessed in terms of anti-attach property and biofilm removal. The results of anti-attach activity of *H. isora* extracts are depicted in Table 2. Accordingly, all tested bacteria were capable of forming biofilm and their biofilm formation ability was moderate (OD > 0.1). In the case of both forms of *H. isora* extracts, biofilm formation was prevented (OD < 0.1). So, it could be concluded that both extracts have similar efficiency in preventing bacterial biofilm formation (Table 2).

### Table 1. GC-Mass analysis of subcritical water extract of *Helicteres isora L.*

| Components detected in the subcritical water extract | Components detected in the water extract | Biological activity |
|------------------------------------------------------|------------------------------------------|---------------------|
| Formic acid, 1-methyl ethyl ester                    | Formic acid, 1-methyl ethyl ester        | Preservative, anti-bacterial agent, treatment for warts |
| 1-Butanol, 2-methyl hexadecanoic acid                | 1-Butanol, 2-methyl hexadecanoic acid    | Anti-inflammatory property |
| 1-Octen-3-ol                                        | 1-Octen-3-ol                             | Anti-microbial activity |
| Heptadec-8-carboxylic acid                          | Heptadec-8-carboxylic acid               | Anti-inflammatory, stomachic, anticancer, analgesic, antibiotic, anticholera, antisyndemic, antibacterial |
| Berberine                                            |                                          |                     |

### Table 2. Anti-attach activity of water extracts of *Helicteres isora L.* against different bacterial strains biofilms

| Extract type                  | Optical density at 570 nm |
|-------------------------------|---------------------------|
|                               | *Bacillus cereus* | *Staphylococcus aureus* | *Staphylococcus saprophyticus* | *Bacillus subtilis* |
| Control                       | 0.167 ± 0.011A           | 0.254 ± 0.03A           | 0.146 ± 0.017BC               | 0.138 ± 0.019BD     |
| Ordinary water extract        | 0.014 ± 0.011A           | 0.013 ± 0.01A           | 0.007 ± 0.011BC               | 0.011 ± 0.011B      |
| Subcritical water extract     | 0.012 ± 0.011A           | 0.011 ± 0.01A           | 0.005 ± 0.011B                | 0.010 ± 0.011A      |

Means with different uppercase letters in the same row show significance (P ≤ 0.05). Means with different lowercase letters in the same column show significance (P ≤ 0.05).

### Table 3. Biofilm removal activity water extracts of *H. isora* against biofilm of bacterial strains

| Extract type                  | Optical density at 570 nm |
|-------------------------------|---------------------------|
|                               | *Bacillus cereus* | *Staphylococcus aureus* | *Staphylococcus saprophyticus* | *Bacillus subtilis* |
| Control                       | 0.401 ± 0.02A           | 0.254 ± 0.03A           | 0.146 ± 0.017BC               | 0.138 ± 0.019BD     |
| Water extract                 | 0.167 ± 0.011A           | 0.084 ± 0.011A           | 0.066 ± 0.011BC               | 0.052 ± 0.011B      |
| Subcritical water extract     | 0.09 ± 0.011A            | 0.075 ± 0.01B            | 0.054 ± 0.011BC               | 0.041 ± 0.12A       |

Means with different uppercase letters in the same row show significance (P ≤ 0.05). Means with different lowercase letters in the same column show significance (P ≤ 0.05).

The anti-attach property of *H. isora* extracts might be attributed to the existence of chemical components with bioactive characteristics. Some researchers reported the efficiency of plant extracts against bacterial biofilms. Mohammadi et al. (2019) confirmed anti-biofilm activity of cold extract of *Carum copticum* against biofilm of *B. cereus*, *S. aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Acinetobacter baumannii*, and *Klebsiella pneumonia*. According to their report, susceptibility of bacterial biofilm structure was varied against plant extract (Mohammadi et al., 2019).

Biofilm removal activity of water extracts of *H. isora* against biofilm of bacterial strains

The formation of biofilms, especially biofilms of pathogenic bacteria, causes important safety issues; so, various approaches can be used for removal of formed bacterial biofilms, including application of plant extracts and plant essential oils. Table 3 depicts the results biofilm removal activity of the two tested extracts. Accordingly, both extracts of *H. isora* were effective in removal of biofilms of *B. cereus*, *B. subtilis*, *S. aureus*, and *S. saprophyticus*. Only in the case of water extract of *H. isora*, optical density more than 0.1 implies retention the biofilm of *B. cereus* in a moderate magnitude (OD = 0.167 ± 0.011).

Anti-bacterial activity of water extracts of *H. isora*

Anti-microbial activity of *H. isora* extracts was assessed by measurement inhibition zone and the results are shown in Table 4. As it is apparent, both extracts have anti-microbial activity against *B. cereus*, *S. aureus*, *S. saprophyticus*, and *B. subtilis*. The highest inhibition zone belongs to *B. subtilis* and this bacteria showed more susceptibility against both types of *H. isora* extracts. The results revealed that the subcritical extract had more impact on all tested bacteria. This could be ascending to more bioactive components in subcritical water extract of *H. isora* than ordinary water extract, according to GC–Mass analysis.

Inhibition growth of microbes due to the plant extracts is reported by other researches. Mohammadi et al. (2019) showed that efficiency of different extracts of *C. coticum* against planktonic
Table 4. Inhibitory zone of water extracts of H. isora against different bacterial strains

| Extract type | Bacillus cereus | Staphylococcus aureus | Staphylococcus saprophyticus | Bacillus subtilis |
|--------------|----------------|----------------------|-----------------------------|-----------------|
| Water extract | 22 ± 0.2a | 27 ± 0.11b | 15 ± 0.4b | 29 ± 0.23a |
| Subcritical water extract | 25 ± 0.31b | 28.5 ± 0.24b | 16.5 ± 0.3b | 30.5 ± 0.26a |

Means with different uppercase letters in the same row show significance (P ≤ 0.05). Means with different lowercase letters in the same column show significance (P ≤ 0.05).

bacterial growth as the highest and the lowest inhibition zone belonged to S. aureus (25 ± 0.8 mm) and A. baumannii (7 ± 0.5 mm), respectively (Mohammadi et al., 2019).

Anti-enzymatic activity of water extracts of H. isora
Determination percentage of polyphenol oxidase activity inhibition by two water extracts of H. isora showed that subcritical water extract causes inhibition of 29% and the magnitude of enzyme activity inhibition for water extract was 11.2%. Statistical analysis revealed significant differences between the percentage of enzyme activity inhibition between the two extracts (P ≤ 0.05).

Different plant extracts could be effective in different enzyme inactivation. Wessels et al. (2014) reported the effect of different plant extracts such as oregano, rosemary, green tea, and 33 other plants on inhibition activity of polyphenol oxidase and concluded different levels of anti-enzymatic activity of the studied plant material. Accordingly, the main anti-enzymatic activity of these plants was ascending to the chemical components, especially those had phenolic structure (Wessels et al., 2014).

Conclusion
Water extracts of H. isora L. include ordinary water extract and subcritical water extract exhibited anti-microbial, anti-biofilm, and anti-enzymatic activity. In this concern, subcritical water extract had higher biological activity than ordinary water extract that might be due to more extraction of active components in this method than ordinary extraction.

Funding
No funding received for the study.

Acknowledgements
This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of Interest
None declared.

References
Aparna, V., Dileep, K. V., Mandal, P. K., Karthe, P., Sadasivan, C., Haridas, M. (2012). Anti-inflammatory property of n-hexadecanoic acid: structural evidence and kinetic assessment. Chemical Biology and Drug Design, 80: 434–439.

Barupal, T., Meena, M., Sharma, K. (2019). Inhibitory effects of leaf extract of Laurus nobilis on Curvularia lunata and characterization of novel inhibitory compounds by GC-MS analysis. Biotechnology Reports (Amsterdam, Netherlands), 23: e00335.

Braca, A., Sortino, C., Politi, M., Morelli, I., Mendez, J. (2002). Anti-oxidant activity of flavonoids from Licania licaniataeflora. Journal of Ethnopharmacology, 79: 379–381.

Cvetanovic, A., Svarc-Gajic, J., Gasic, U., Tesic, Z., Zengin, G., Zekovic, Z., Durovic, S. (2017). Isolation of apigenin from subcritical water extracts: optimization of the process. Journal of Supercritical Fluids, 120: 32–42.

Cvetanovic, A., et al. (2018). Comparative in vitro studies of the biological potential and chemical composition of stems, leaves and berries Aronia melanocarpa’s extracts obtained by subcritical water extraction. Food and Chemical Toxicology, 121: 458–466.

Cvetko Bubalo, M., Vidocev, S., Radovic Redovnikovic, I., Joki, S. (2015). Green solvents for green technologies. Journal of Chemical Technology and Biotechnology, 90: 1631–1639.

Deepak, P., Gopal, G. V. (2014). Phytochemical profile of Berberis tinctoria Lesc. bark using GC-MS analysis. European Journal of Experimental Biology, 4: 419–425.

Eikani, M. H., Khandan, N., Feyzi, E., Ebrahim, I. M. (2019). A shrinking core model for Nannochloropsis salina oil extraction using subcritical water. Renewable Energy, 131: 660–666.

Fasih, M., Ghorbani Noohooji, M., Rahimi, A. R. (2016). The effect of three medicinal plants essential oils on the activity of peroxidase and polyphenol oxidase enzymes in broccoli [Brassica oleracea L.var. Italica]. Journal of Medicinal Plants, 1: 60–77. [In persian].

Gabaston, J., Leborgne, C., Valls, J., Renouf, E., Richard, T., Waffo-Teguo, P., Merillon, J-M. (2018). Subcritical water extraction of stilbenes from grapevine by-products: a new green chemistry approach. Industrial Crops and Products, 126: 272–279.

Graf, R. A., Milbury, P. E., Blumberg, J. B. (2005). Flavonols, flavones, and human health: epidemiological evidence. Journal of Medicinal Food, 8: 281–290.

Kumar, N., Singh, A. K. (2014). Plant profile, phytochemistry and pharmacology of Axtarani [Helicteres isora Linn.]: a review. Asian Pacific Journal of Tropical Biomedicine, 4: S22–S26.

Kumar, V., Sharma, M., Lemos, M., Shriram, V. (2013). Efficacy of Helicteres isora L. against free radicals, lipid peroxidation, protein oxidation and DNA damage. Journal of Pharmacy Research, 6: 620–625.

Lachos-Pereza, D., Baseggio, A. M., Mayanga-Torresa, P. C., Marostica Juniorb, M. R., Rostagnoc, M A., Martinezb, J., Forster-Carneiroa, T. (2018). Subcritical water extraction of stilbenes from grapevine by-products: a new green chemistry approach. Industrial Crops and Products, 126: 272–279.

Lante, A., Tinello, F. (2015). Citrus hydrosols as useful by-products for tyrosinase inhibition. Innovative Food Science and Emerging Technologies, 27: 154–159.

Machmudaha, S., Sarah Dutta Lestaria, S. D., Wahyudionob, W., Kandab, H., Winardia, S., Gotob, M. (2018). Subcritical water extraction enhancement by adding deep eutectic solvent for extracting xanthone from mangosteen pericarps. The Journal of Supercritical Fluids, 133: 615–624.

Miller, D. J., Hawthorne, S. B., Gizir, A. M., Clifford, A. A. (1998). Solubility of polycyclic aromatic hydrocarbons in subcritical water from 298 K to 498 K. Journal of Chemical and Engineering Data, 43: 1043–1047.

Mohamadi, M., Maskoooki, A. M., Mortazavai, S. A., Kocheci, A., Nahardani, M., Pourfallah, Z. (2014). Extraction of phenolic compound from barberi by subcritical water and investigation of antioxidation properties of extracted juices. Journal of Food Science and Technology, 46: 49–59.

Mohamadi, M., Masoumipour, F., Hassanshahian, M., Jafarinasab, T. (2019). Study the antibacterial and antiobiofilm activity of Carum cuminum against antibiotic-resistant bacteria in planktonic and biofilm forms. Microbial Pathogenesis, 129: 99–105.

Mohamadi, N., Mirhosseini, M., Shriram, M., Dehgham Hamdan, A., Yazdani, N. (2015). Synthesizing ZnO nanoparticles by high-energy milling and investigating their antimicrobial effect. Journal of Shahid Sadoughi University Medical Science, 23: 2070–82. [In persian].
Moradian Eivari, A. K., Salehi, M., Malek Jafarian, M. (2015). Antimicrobial activity of Rosmarinus Officinalis on vancomycin-resistant Staphylococcus aureus isolated from Imam Reza Hospital patients of Mashhad. Journal of Neyshabur University Medical Science, 3: 39–44.

Nastić, N., Švarc-Gajić, J., Delerue-Matos, C., Barroso, M. F., Soares, G., Moreira, M. M et al. (2018). Subcritical water extraction as an environmentally-friendly technique to recover bioactive compounds from traditional Serbian medicinal plants. Industrial Crops and Products, 111: 579–589.

Noumi, E., et al. (2017). Phytochemical composition, anti-biofilm and anti-quorum sensing potential of fruit, stem and leaves of Salvadora persica L. methanolic extracts. Microbial Pathogenesis, 109: 169–176.

Teo, C. C., Tan, S. N., Yong, J. W., Hew, C. S., Ong, E. S. (2010). Pressurized hot water extraction (PHWE). Journal of Chromatography. A, 1217: 2484–2494.

Todorov, S. D., de Paula, O. A. L., Camargo, A. C., Lopes, D. A., Nero, L. A. (2018). Combined effect of bacteriocin produced by Lactobacillus plantarum ST8SH and vancomycin, propolis or EDTA for controlling biofilm development by Listeria monocytogenes. Revista Argentina De Microbiologia, 50: 48–55.

Tyagi, T., Agarwal, M. (2017). GC-MS analysis of invasive aquatic weed, Pista StratiotesL. and Eichhornia Crassipes (Mart.) Solms. International Journal of Current Pharmaceutical Research, 9: 111–117.

Wessels, B., Damm, S., Kunz, B., Schulze-Kaysers, N. (2014). Effect of selected plant extracts on the inhibition of enzymatic browning in fresh-cut apple. Journal of Applied Botany and Food Quality, 87: 16 – 23.

Xiong, C., Li, Q., Li, S., Chen, C., Chen, Z., Huang, W. (2017). In vitro antimicrobial activities and mechanism of 1-Octen-3-ol against food-related bacteria and pathogenic fungi. Journal of Oleo Science, 66: 1041–1049.

Yolmeh, M., Habbib-Naja, M. B., Najafzadeh, M. (2015). Study the effects of ultraviolet radiation on the growth of Escherichia coli and Bacillus cereus isolated from raw milk and raw rice. Iranian Food Science and Technology Research Journal, 4: 319–24 [In persian].