Evaluation of phytochemical analysis and antimicrobial activities Allium essential oil against the growth of some microbial pathogens

Article *in* Microbial Pathogenesis · November 2017

DOI: 10.1016/j.micpath.2017.11.055

CITATIONS
7

READS
59

2 authors:

Behrooz Alizadeh Behbahani
Agricultural Sciences and Natural Resources University of Khuzestan
50 PUBLICATIONS **301** CITATIONS
SEE PROFILE

Abbas Ali Imani Fooladi
249 PUBLICATIONS **2,043** CITATIONS
SEE PROFILE

Microbial Pathogenesis 114 (2018) 299–303

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

Evaluation of phytochemical analysis and antimicrobial activities Allium essential oil against the growth of some microbial pathogens

Behrooz Alizadeh Behbahani, Abbas Ali Imani Fooladi*

Applied Microbiology Research Center, Systems Biology and Poisoning Institute, Baqiyatallah University of Medical Sciences, Sheikh Bahaei Street, Molla Sadra Street, Vanak Sq., Tehran 984359-44711, Iran

ARTICLE INFO

Keywords:
Microbial pathogens
Essential oil
Antimicrobial effect
Phytochemical analysis

ABSTRACT

In this experimental study, the Allium essential oil (AHEO) was extracted through the hydrodistillation method. AHEO components were identified through gas chromatography/mass spectrometry (GC/MS), and its antioxidant properties and total phenolic content were examined through the methods of 2,2-diphenyl-1-picrylhydrazyl (DPPH), ß-carotene/linoleic acid inhibition and Folin-Ciocalteu, respectively. The antimicrobial effect of AHEO was evaluated on *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Candida albicans* through the methods of well diffusion, disk diffusion, minimum inhibitory concentration, and minimum bactericidal/fungicidal concentration. Phytochemical constituents (alkaloids, tannins, saponins, flavone and glycosides) were evaluated. 5-chloroorcylaldehyde with a percentage of 45.6% was the major compound of AHEO. Increasing concentration of AHEO had a significant effect ($p \leq 0.05$) on inhibition zone diameter. The MICs of the AHEO varied from 0.25 mg/ml to 2 mg/ml. The MBCs/MFCs of the AHEO varied from 0.25 mg/ml to 4 mg/ml. The results of phytochemical screening of AHEO showed the existence of alkaloids, tannins, saponins, flavone and glycosides. There was also little difference between disk diffusion and well diffusion methods, and the data was well distributed throughout the X and Y components.

1. Introduction

It was estimated that 30% of people in developed countries suffer from foodborne diseases [1]. Therefore, it is highly appreciated to find out ways overcoming these issues. One solution is to find food grade anti-pathogenic compounds such as Allium.

Allium genus (*Alliaceae* family) in which the *Allium hushidari*, *Allium sativum* (garlic) and other onions are in same biological genus [2,3]. It is one of the valuable members of Allium with antifungal, antibacterial, antiprotozoal, antiviral, and antihelmintic properties [2,4,5] It was reported that Allium genus extracts are good potentials to reduce the growth of the organisms by lowering oxygen uptake as well as inhibiting the synthesis of proteins, lipids, and nucleic acids and damaging the membranes. Allicin (diallyl thiosulfinate) was identified as a main component, which is responsible for remarkable antibacterial activities of Allium family [3,6]. Research has shown that the long-term application of chemical preservatives leads to the outbreak of numerous complications including cancers. Therefore, utilization of such compounds has now decreased. Alternatively, application of medicinal plants has recently increased due to their less side effects.

Multivariate analysis (MVA) technique can be applied to accomplish trade studies across multiple dimensions while considering the impacts of all variables on the interest responses [7].

The purpose of this research was to identify the chemical substances and the antioxidant potential and antimicrobial of AHEO together with its scavenging activity of free radicals and oxidation inhibition power. Thus, MVA including partial least squares–discriminant analysis (PLS–DA) and principal component analysis (PCA) were performed for first time to evaluate the effect of AHEO on the microbial pathogens.

2. Materials and methods

2.1. Chemicals, reagents, and microbial media

Sabouraud dextrose agar (SDA), sabouraud dextrose broth (SDB), Mueller hinton agar (MHA) and mueller hinton broth (MHB) were purchased from Merck Chemicals (Darmstadt, Germany). 2,2-diphenyl-1-picrylhydrazyl (DPPH) and bacteriological peptone were supplied from Sigma-Aldrich (USA) and BDH Chemicals Ltd (England), respectively. Tryptone soya broth (TSB) and sodium chloride were prepared

* Corresponding author.
E-mail addresses: imanifouladi.a@bmsu.ac.ir, imanifouladi.a@gmail.com (A.A. Imani Fooladi).

https://doi.org/10.1016/j.micpath.2017.11.055
Received 20 November 2017; Received in revised form 27 November 2017; Accepted 27 November 2017
Available online 28 November 2017
0882-4010/

from East Anglia Chemicals, UK. plant was collected from Kurdistan, Iran.

2.2. Essential oil preparation and determination of the extraction yield

50 g plant powdered was transferred to the glass clevenger apparatus containing 750 ml of distilled water and hydro distillation technique, with the distillation rate of 1 ml/min, was performed. Extraction of AHEO was performed for 3 h and the essential oil was collected in vials and stored at 4 °C [8].

2.3. Identification of the essential oil chemical composition

Determination of the essential oil components present in AHEO was carried out using gas chromatography coupled with mass spectrometer. Injection volume was 0.2 µl. The carrier gas (helium) rate and an ionization energy were 1.1 ml/min and 70 eV, respectively [9]. The compounds were recognized by comparing their mass spectra and retention indexes (RI) with those of the standard samples and those given in previous studies. Quantification of each component relative amounts was carried out according to the area percentage method without considering the calibration factor.

2.4. Estimation of total phenolic content (TPC) and phytochemical constituents

The method of Folin-Ciocalteu was carried out for the determination of TPC. The result was expressed as mg of Gallic Acid Equivalents (GAE) per gram of the essential oil. Phytochemical constituents (alkaloids, tannins, saponins, flavone and glycosides) were evaluated based on qualitative methods [10].

2.5. Antioxidant activity

2.5.1. DPPH free radical-scavenging assay

For this purpose, 3.9 ml of the stock solution of DPPH (0.004 g of DPPH in 100 ml of methanol) was mixed with 0.1 ml of each extract and was kept at a dark place for 30 min. Then, its absorbance was read at 517 nm using spectrophotometer. The radical scavenging percent of DPPH was following equation (1):

$$\%\ \text{Scavenging activity} = [(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})]/(\text{Abs}_{\text{control}})] \times 100 \quad (1)$$

Where, $\text{Abs}_{\text{control}}$ and $\text{Abs}_{\text{sample}}$ refers to the absorbance of DPPH + ethanol and DPPH + sample, respectively [10]. The results were reported as IC_{50} (amount of antioxidant required to reduce the DPPH concentration to 50% of its initial value).

2.5.2. ß-Carotene-linoleic acid (B-CL) assay

Potential antioxidant activity was assessed with the oxidation prevention by linoleic acid and inhibition of the development of conjugated hydro-peroxides and volatile compounds. The B-CL assay was performed according to Akhbari et al. (2017) [11]. The average inhibition percent was calculated as follow:

$$\%\text{Inhibition} = [(AA_{120} - AC_{120})/ (AC_0 - AC_{120})] \times 100 \quad (2)$$

Where, AA_{120}, AC_{120}, and AC_0 are the absorbance for the antioxidant activity of sample after 120 min, control sample after 120 min, and control sample at the beginning of experiment (t = 0), respectively. All tests were performed in triplicate.

2.6. Preparation of the microbial strains

Microbial strains of *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Bacillus cereus* (ATCC 14579), *Bacillus subtilis* (ATCC 23857), *Staphylococcus aureus* (ATCC 25923), *Streptococcus pyogenes* (ATCC 19615), and *Candida albicans* (ATCC 5027) were used in this study. All American type culture collections were supplied from the department of food science and technology at Ferdowsi University of Mashhad (FUM), Mashhad, Iran. Bacterial suspension was diluted by Ringer solution until the solution turbidity equalized with 0.5 McFarland standard solution. The suspension must have contained 1.5×10^8 CFU/ml.

2.7. Antimicrobial susceptibility test

2.7.1. Disk diffusion agar (DDA)

Different concentrations of AHEO at 0.25, 0.5, 1, 2, 4, and 8 mg/ml were prepared in a suitable solvent. AHEO sterilized via a 0.45 µm syringe microfilter. Then the blank discs were immersed in the extract solutions for 15 min. Finally, these discs were immobilized on the medium surface. After that, the petri-dishes were incubated at 37 °C for 24 h and 27 °C for 72 h for bacteria and fungi, respectively. The antimicrobial effect was determined in term of IZ diameter (mm) [12].

2.7.2. Well diffusion agar (WDA)

The plates containing 0.25, 0.5, 1, 2, 4, and 8 mg/ml of AHEO were used, which were contaminated with specific microorganisms with sterilized pastor pipette specified for well creation. Next, the media including the bacteria and fungi were incubated at 37 and 27 °C for 24 and 72 h, respectively. After that, the extent of the inhibition zone was measured as mm and the mean values were reported [13].

2.7.3. Determination of minimum inhibitory concentration (MIC) through micro dilution broth

National Committee for Clinical Laboratory Standards (NCCLS) was applied for identification and valuation of MIC. Different concentrations of AHEO was prepared as 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32 and 64 mg/ml. Identification was performed using of three phenyl-tetrazolium. MIC was reported as the first concentration in which microbial growth did not occurred and no red color was synthesized [14].

2.7.4. Minimum bactericidal/fungicidal concentrations (MBC and MFC)

In order to asses MBC and MFC, all wells with opacity-free conditions were cultured separately on the media of MHA and SDA. Then the bacteria and fungi containing media were incubated for 24 and 72 h, respectively. The essential oil concentration, in which bacteria or fungi did not grow, was reported as MBC and MFC [13].

2.8. Statistical analysis

Obtained data were analyzed using SPSS (Version18.0, SPSS Inc., Chicago, USA) and Microsoft Windows Excel 2016. The data were initially analyzed by one-way ANOVA and Duncan's mean comparison test at the 5% significance level. All experiments were triplicated.

2.9. Multivariate data analysis (PLS–DA and PCA)

Multivariate data analysis including principal component analysis and partial least squares–discriminant analysis (PLS–DA) were performed on mean centering and unit variance scaling of variables by Simca (Umetrics Inc., Ume˚a University, Sweden). The model's quality was determined by the goodness-of-fit parameter (R2X or R2Y) and predictive ability parameter (Q2), as well as permutation testing (for PLS–DA models). Data points were randomly assigned a 100 times-class, and the corresponding R2Y and Q2 calculated and compared with the cross-validated using cross-validated analysis of variance (CV-ANOVA) and original. The data of PLS–DA models with variable influences of projection > 1, which the corresponding jackknife-based 95% CIs were not close to or including 0 were considered discriminative [15,16].

Table 1
Antimicrobial effect of AHEO concentrations for some microbial pathogens (DDA).

Microorganism	DDA (mm)					
	0.25	0.5	1	2	4	8
S. pyogenes	9.10 ± 0.54	11.50 ± 0.50	13.60 ± 0.52	15.20 ± 0.45	17.60 ± 0.50	19.20 ± 0.50
B. subtilis	8.70 ± 0.50	10.50 ± 0.57	12.00 ± 0.28	13.90 ± 0.45	15.40 ± 0.52	17.80 ± 0.45
S. aureus	7.00 ± 0.52	7.90 ± 0.50	9.90 ± 0.50	12.20 ± 0.50	13.80 ± 0.45	15.20 ± 0.52
B. cereus	6.50 ± 0.57	7.70 ± 0.57	9.60 ± 0.52	11.00 ± 0.50	13.00 ± 0.45	14.00 ± 0.45
E. coli	–	7.20 ± 0.28	8.80 ± 0.50	10.10 ± 0.50	12.00 ± 0.50	13.00 ± 0.28
P. aeruginosa	–	7.00 ± 0.50	7.50 ± 0.54	9.00 ± 0.50	10.90 ± 0.45	11.80 ± 0.50
C. albicans	8.90 ± 0.50	11.20 ± 0.50	12.90 ± 0.50	14.70 ± 0.45	16.30 ± 0.28	17.90 ± 0.50

3. Results and discussion

3.1. Chemical compounds

Water distillation technique was used for extraction of AHEO (had yellowish color) with the extraction efficiency of 0.06 (v/w). The results of gas chromatography showed that AHEO consists of 14 compounds, which allocated 96.5% of total composition of AHEO. 5-chloroorcylaldehyde was the major compound of AHEO (45.6%). Other AHEO's compounds included methylthiomethyl disulfide (28.2%), pentythiophene (5.4%), tricosane (5.2%), dimethyl trisulfide (4.5%), carvacrol (1.2%), and thymol (0.5%). The results showed that sulfide compounds allocate an important part of AHEO composition. The results were somehow in line with other reports.

Fasihzadeh et al. (2016) [17] identified AHEO compounds and reported that the AHEO extraction efficiency was 0.06 (v/w) and the major compounds were 1-butene, 1-(methylthio)-(Z) (18.21%), methyl methylthiomethyl disulfide (8.41%), dimethyl tetra sulfide (6.47%), and piperitenone oxide (4.55%). Mahboubi et al. (2014) [18] reported that the extraction efficiency of AHEO was 0.04 (v/w), and AHEO consisted of 11 compounds as follow; 5-chloroorcylaldehyde (55.1%), methyl methylthiomethyl disulfide (24.6%), Tricosan (6.3%), pentylthiophene (3.8%), and dimethyl trisulfide (3.1%). Ismail et al. (2013) [19] showed that *Allium hirtifolium* mainly comprised of 9-hexadecenoic acid, 11,14-eicosadienoic acid, and n-hexadecanoic acid. The comparison between the results of this study and other researchers showed that the compounds identified in the AHEO differ in type and amount of the constituents. Constituents of the AHEO can vary due to the weather conditions, growth stage and place, and the time plants were taken [14].

3.2. Antioxidant activity, total phenolic content (TPC) and phytochemical constituents

Total phenol, calculated with Folin-Ciocalteu procedure, was 168.9 ± 0.45 μg/ml in terms of gallic acid (GAE). DPPH and ß-carotene/linoleic acid inhibition tests for assessing antioxidant activity of AHEO showed IC_{50} of 1.2 ± 0.35 mg/g and 28.6%, respectively. The results of phytochemical screening of AHEO showed the existence of alkaloids, tannins, saponins, flavone and glycosides.

The antioxidant activity of the AHEO was directly related to its phenolic and flavonoid compounds, which were in line with others (Kanatt et al. (2007) [20], Peterson et al. (2001) [21], Bamdad et al. (2006) [22] and Alizadeh Behbahani et al. [14]. Ghasemi Pirbalouti et al. (2015) [23], reported total phenol content of AHEO 44.28 mg GAE/g. They indicated that there is a direct relationship between total phenol content of AHEO and its antioxidant activity. Ghahremani-majd et al. (2012) [24], assessed total phenol and antioxidant activity of *Allium hirtifolium* by DPPH, ferric reducing antioxidant potential (FRAP assay), and ABTS. They reported that the antioxidant activity of Allium hirtifolium was mainly due to its phenolic compounds. Mahboubi et al. (2014) [18] calculated the antioxidant activity of AHEO through DPPH and ß-carotene/linoleic acid inhibition and reported that AHEO antioxidant activity were equal to 56.1 and 32.3%, respectively.

The correlation between antioxidant activity and the compounds in essential oils and plant extracts is not readily possible. This can be attributed to the difference in the type and number of effective compounds present in their composition. In general, the number of hydroxyl groups in antioxidants' structure is not mainly responsible for their functionalities. Position of hydroxyl groups, presence of other functional groups, such as double bonds and the combination of hydroxyl and ketone groups, will play an important role in the antioxidant activity. The reason for the difference in the results of the antioxidant activity and phenolic compounds of AHEO in several studies, can be related to the climatic conditions, the method of drying and the difference between the methods of measuring phenolic compounds and antioxidant activities [10]. Singleton et al. (1999) [25] reported that the number of different phenolic compounds could cause various responses using the Folin–Ciocalteu procedure. It was reported that the presence of some inorganic substances such as hydroxyammonium chloride, iron sulfate, and hydrazine could react with the Folin–Ciocalteu reagent and increase the apparent phenolic concentration [26,27].

3.3. Antimicrobial activity

The results of antimicrobial effects of AHEO on 7 microbial strains were assessed using diffusion agar method and well diffusion agar at 6

Table 2
Antimicrobial effect of AHEO concentrations for some microbial pathogens (WDA).

Microorganism	WDA (mm)					
	0.25	0.5	1	2	4	8
S. pyogenes	9.90 ± 0.45	11.80 ± 0.54	14.20 ± 0.50	16.00 ± 0.50	18.00 ± 0.50	19.60 ± 0.50
B. subtilis	9.00 ± 0.54	11.10 ± 0.50	12.80 ± 0.52	15.10 ± 0.54	16.20 ± 0.28	18.10 ± 0.50
S. aureus	7.50 ± 0.50	8.50 ± 0.54	10.50 ± 0.54	12.70 ± 0.50	14.90 ± 0.54	16.30 ± 0.50
B. cereus	7.50 ± 0.50	8.70 ± 0.28	10.30 ± 0.50	12.00 ± 0.52	13.50 ± 0.45	14.60 ± 0.52
E. coli	7.00 ± 0.50	7.90 ± 0.50	9.00 ± 0.54	10.00 ± 0.54	12.60 ± 0.52	14.10 ± 0.50
P. aeruginosa	7.00 ± 0.52	7.50 ± 0.52	8.00 ± 0.50	9.40 ± 0.28	11.00 ± 0.45	12.40 ± 0.50
C. albicans	9.90 ± 0.52	11.50 ± 0.54	13.80 ± 0.52	15.70 ± 0.54	17.20 ± 0.50	18.30 ± 0.50

Table 3
MIC and MBC/MFC of AHEO for some microbial pathogens.

Microorganisms	MIC	MBC/MFC
S. pyogenes	0.25	0.25
B. subtilis	0.25	0.5
S. aureus	0.5	1
B. cereus	1	2
E. coli	1	2
P. aeruginosa	2	4
C. albicans	0.25	0.5

concentrations (Tables 1 and 2). The results showed that the Gram-positive *Streptococcus pyogenes* was the most sensitive strain to AHEO. The highest resistance to AHEO was observed for Gram-negative *Pseudomonas aeruginosa*. The antimicrobial activity of the DDA was reduced by diluting AHEO and at 0.25 mg/ml concentration, the inhibition zone was not observed for *Pseudomonas aeruginosa* and *Escherichia coli*. In DDA procedure, the greatest inhibition zone diameter of AHEO (19.20 ± 0.50) was observed at a concentration of 8 mg/ml, which was related to *Streptococcus pyogenes*. The WDA technique, which can provide direct contact with microorganisms, showed greater inhibition zone than the DDA method, which AHEO must penetrate from disk to the surface of the culture medium. Factors such as disk diameter, thickness, temperature, and soaking time of the disk in the AHEO can be effective on the results obtained from the DDA method [28,29].

In general, the sensitivity of the studied microorganisms from the most resistant to the most sensitive strains was as follows; *Pseudomonas aeruginosa* > *Escherichia coli* > *Bacillus cereus* > *Staphylococcus aureus* > *Bacillus subtilis* > *Candida albicans* > *Streptococcus pyogenes*. There are different literature indicating bacteri/fungi cidal, antiprotozoal, and antiviral effects of Allium genus, but there are few works about AHEO [18,30–32]. The results were in good match with others such as Ismail et al. (2013) [19]. They investigated antimicrobial activity of *Allium hirtifolium* by DDA and microdilution broth on 10 pathogen microorganisms. Their results showed that gram-positive bacteria are more susceptible than gram-negative bacteria.

The results of the MIC and MBC/MFC of AHEO were presented in Table 3. The results showed that the MIC of AHEO for the studied strains was in the range of 0.25–2 mg/ml. Strains of *Streptococcus pyogenes*, *Bacillus subtilis,* and *Candida albicans* were more susceptible. *Pseudomonas aeruginosa* was the most resistant microbial strain. The results of the MBC/MFC varied between 0.25 and 4 mg/ml.

Mahboubi et al. (2014) [18], reported that the MIC and minimal lethal concentration (MLC) ranges of 0.06–2 and 0.25–2 µl/ml, respectively. They reported higher antimicrobial activity of AHEO against bacteria, yeast, and fungi. According to the compounds identified in AHEO, the main role in the antimicrobial activity of AHEO is related to sulfide compounds, thymol, and carvacrol. It was reported that sulfur compounds in *Allium* spp are major compounds exhibiting antimicrobial properties. Sulfides containing three or more sulfur atoms in their structures are effective fungicidal agents [18,33]. Methyl, thiomethyl disulfide, and dimethyl trisulfide were the main sulfides present in *Allium hirtifolium* extracts. Phenolic compounds such as carvacrol and thymol were also founded in AHEO, which had wide range of antimicrobial activities [34–36]. Thymol and carvacrol play their antimicrobial role by inhibiting the ergosterol biosynthesis and destruction of the membrane integrity [36]. There are various mechanisms for antimicrobial effects of plant extracts [37–39]. Phenolic compounds can act with two different mechanisms through the cell membrane and cell wall of the microorganisms [40]. Phenolic compounds can make their destructive role on the bacterial cell membrane by interacting with the membrane proteins through their hydroxyl groups [23,41and42].

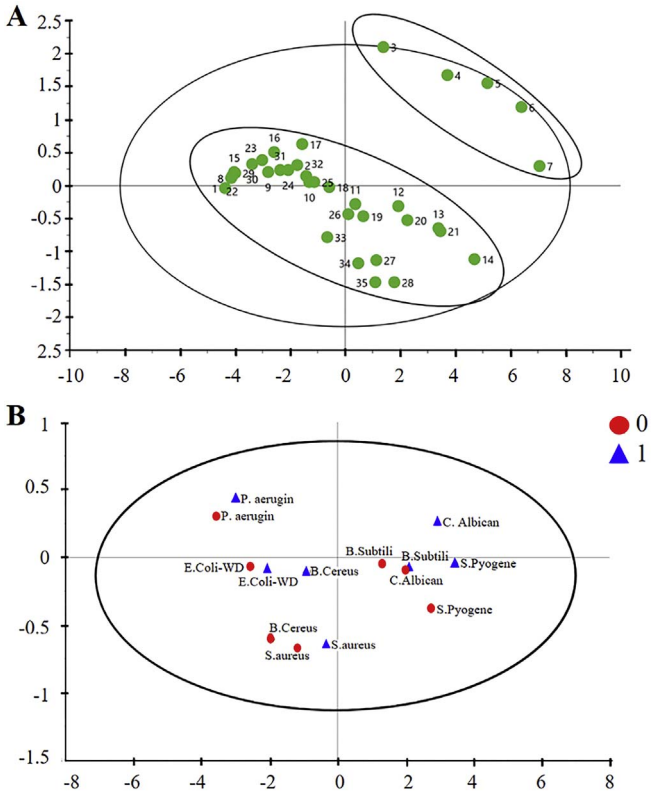

Fig. 1. Principal component analysis (PCA) *in vitro*.

3.4. Statistical multivariate methods: partial least squares–discriminant analysis (PLS–DA) and principal component analysis (PCA)

Fig. 1 shows the results of the AHEO effect on 7 bacterial strains by PCA. Results showed no outlier data. There was also little difference between DDA and WDA methods, and the data was well distributed throughout the X and Y components. The largest difference was observed for *Candida albicans* microbial strains. Although the different concentrations of the essential oil caused the difference between the inhibition zone, but none of the multivariate statistical models such as: partial least squares regression and optimized potential for liquid simulations were fitted on the samples, which could be due to a non-significant difference between the types of microorganisms.

4. Conclusion

The results showed that AHEO had antimicrobial effect on pathogenic strains under laboratory conditions. According to the compounds identified in AHEO, the main role in the antimicrobial activity of AHEO was related to sulfide, Thymol, and carvacrol compounds. The results of phytochemical screening of AHEO showed the existence of alkaloids, tannins, saponins, flavone and glycosides. According to the PCA results, there was no outlier data and all data were well distributed along the X and Y component.

Acknowledgments

The authors wish to express their profound gratitude sincerely to Applied Microbiology Research Center, Systems Biology and Poisoning institute, Baqiyatallah University of Medical Sciences.

References

[1] L. Cao, J.Y. Si, Y. Liu, H. Sun, W. Jin, Z. Li, et al., Essential oil composition, antimicrobial and antioxidant properties of Mosla chinensis Maxim, Food Chem. 115

(2009) 801–805.
[2] M. Taran, M. Rezaeian, M. Izaddoost, In vitro antitrichomonas activity of *Allium hirtifloium* (Persian Shallot) in comparison with metronidazole, Iran. J. Public Health 35 (2006) 92–94.
[3] H.G. Azadi, S.M. Ghaffari, G.H. Riazi, S. Ahmadian, F. Vahedi, Antiproliferative activity of chloroformic extract of Persian Shallot, *Allium hirtifolium*, on tumor cell lines, Cytotechnology 56 (2008) 179–185.
[4] G.S. Moore, R.D. Atkins, The fungicidal and fungistatic effects of an aqueous garlic extract on medically important yeast-like fungi, Mycologia (1977) 341–348.
[5] T. Ariga, T. Seki, Antithrombotic and anticancer effects of garlic-derived sulfur compounds: a review, Biofactors 26 (2006) 93–103.
[6] C. Borek, Antioxidant health effects of aged garlic extract, J. Nutr. 131 (2001) 1010S–5S.
[7] B. Worley, R. Powers, Multivariate analysis in metabolomics, Curr. Metabolomics 1 (2013) 92–107.
[8] Z. Sayyari, R. Farahmandfar, Stabilization of sunflower oil with pussy willow (Salix aegyptiaca) extract and essential oil, Food Sci. Nutr. 5 (2017) 266–272.
[9] M. Božović, S. Garzoli, M. Sabatino, F. Pepi, A. Baldisserotto, E. Andreotti, et al., Essential oil extraction, chemical analysis and anti-Candida activity of *Calamintha nepeta* (L.) Savi subsp. glandulosa (Req.) Ball—new approaches, Molecules 22 (2017) 203.
[10] B. Alizadeh Behbahani, F. Shahidi, F.T. Yazdi, S.A. Mortazavi, M. Mohebbi, Antioxidant activity and antimicrobial effect of tarragon (Artemisia dracunculus) extract and chemical composition of its essential oil, J. Food Meas. Charact. 11 (2017) 847–863.
[11] M. Akhbari, S. Tavakoli, Z. Ghanbari, M. Dadgarnia, A. Mazoochi, Evaluation of biological activity and analysis of volatile fraction from *Pterocarya fraxinifolia* in vegetative stage from Iran, J. Sci. Islamic Repub. Iran 28 (2017) 119–126.
[12] M.D. Antunes, G. da Silva Dannenberg, Â.M. Fiorentini, V.Z. Pinto, L.-T. Lim, E. da Rosa Zavareze, et al., Antimicrobial electrospun ultrafine fibers from zein containing eucalyptus essential oil/cyclodextrin inclusion complex, Int. J. Biol. Macromol. 104 (2017) 874–882.
[13] A. Bouyahya, N. Dakka, A. Talbaoui, A. Et-Touys, H. El-Boury, J. Abrini, et al., Correlation between phenological changes, chemical composition and biological activities of the essential oil from Moroccan endemic Oregano (Origanum compactum Benth), Ind. Crops Prod. 108 (2017) 729–737.
[14] B. Alizadeh Behbahani, F. Shahidi, F.T. Yazdi, S.A. Mortazavi, M. Mohebbi, Use of Plantago major seed mucilage as a novel edible coating incorporated with Anethum graveolens essential oil on shelf life extension of beef in refrigerated storage, Int. J. Biol. Macromol. 94 (2017) 515–526.
[15] A.A. Moazzami, J.-X. Zhang, A. Kamal-Eldin, P. Åman, G. Hallmans, J.-E. Johansson, et al., Nuclear magnetic resonance–based metabolomics enable detection of the effects of a whole grain rye and rye bran diet on the metabolic profile of plasma in prostate cancer patients, J. Nutr. 141 (2011) 2126–2132.
[16] T.-L. Kammies, M. Manley, P.A. Gouws, P.J. Williams, Differentiation of foodborne bacteria using NIR hyperspectral imaging and multivariate data analysis, Appl. Microbiol. Biotechnol. 100 (2016) 9305–9320.
[17] S. Fasihzadeh, Z. Lorigooini, N. Jivad, Chemical constituents of *Allium stipitatum* regel (Persian shallot) essential oil, Der Pharm. Lett. 8 (2016) 175–180.
[18] M. Mahboubi, N. Kazempour, Chemical composition, antioxidant and antimicrobial activity of *Allium hirtifolium* essential oil, J. Microbiol. Biotechnol. Food Sci. 3 (2014) 402–405.
[19] S. Ismail, F.A. Jalilian, A.H. Talebpour, M. Zargar, K. Shameli, Z. Sekawi, et al., Chemical composition and antibacterial and cytotoxic activities of *Allium hirtifolium* boiss, BioMed Res. Int. 1–8 (2013).
[20] S.R. Kanatt, R. Chander, A. Sharma, Antioxidant potential of mint (*Mentha spicata* L.) in radiation-processed lamb meat, Food Chem. 100 (2007) 451–458.
[21] D.M. Peterson, C.L. Emmons, A.H. Hibbs, Phenolic antioxidants and antioxidant activity in pearling fractions of oat groats, J. Cereal Sci. 33 (2001) 97–103.
[22] F. Bamdad, M. Kadivar, J. Keramat, Evaluation of phenolic content and antioxidant activity of Iranian caraway in comparison with clove and BHT using model systems and vegetable oil, Int. J. Food Sci. Technol. 41 (2006) 20–27.
[23] A. Ghasemi Pirbalouti, Y. Ahmadzadeh, F. Malekpoor, Variation in antioxidant, and antibacterial activities and total phenolic content of the bulbs of mooseer (Allium hirtifolium Boiss), Acta Agric. Slov. 105 (2015) 15–22.
[24] H. Ghahremani-majd, F. Dashti, D. Dastan, H. Mumivand, J. Hadian, M. Esna-Ashari, Antioxidant and antimicrobial activities of Iranian mooseer (Allium hirtifolium Boiss) populations, Hortic. Environ. Biotechnol. 53 (2012) 116–122.
[25] V.L. Singleton, Analysis of total phenols and other oxidation substrates and their antioxidants by means of Folin-Ciocalteu reagent, Meth Enzymol. 299 (1999) 152–178.
[26] R.L. Prior, X. Wu, K. Schaich, Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements, J. Agric. Food Chem. 53 (2005) 4290–4302.
[27] R. Mustafa, A.A. Hamid, S. Mohamed, F.A. Bakar, Total phenolic compounds, flavonoids, and radical scavenging activity of 21 selected tropical plants, J. Food Sci. 75 (2010) 28–35.
[28] F. Tabatabaei Yazdi, B. Alizadeh Behbahani, S.A. Mortazavi, Investigating the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the *Lavandula stoechas* L. and *Rosmarinus officinalis* L. extracts on pathogen bacterias “in vitro”, J. Paramed. Sci. 5 (2014) 91–101.
[29] F. Tabatabaei Yazdi, B. Alizadeh Behbahani, A. Vasiee, S.A. Mortazavi, F. Tabatabaie Yazdy, An investigation on the effect of alcoholic and aqueous extracts of Dorema aucheri (Bilhar) on some pathogenic bacteria in vitro, J. Paramed. Sci. 6 (2015) 58–64.
[30] R. Fateh, M.J. Nasiri Kashani, M. Motevallian, M. Falahati, A. Yazdanparast, In vitro antifungal activity of *Allium hirtifolium* in comparison with miconazole, Med. J. Islamic Repub. Iran (MJIRI) 24 (2010) 17–22.
[31] H. Wang, T. Ng, Ascalin, a new anti-fungal peptide with human immunodeficiency virus type 1 reverse transcriptase-inhibiting activity from shallot bulbs, Peptides 23 (2002) 1025–1029.
[32] Y. Yamada, K. Azuma, Evaluation of the in vitro antifungal activity of allicin, Antimicrob. Agents Chemother. 11 (1977) 743–749.
[33] P. Rose, M. Whiteman, P.K. Moore, Y.Z. Zhu, Bioactive S-alk (en) yl cysteine sulfoxide metabolites in the genus Allium: the chemistry of potential therapeutic agents, Nat. Product. Rep. 22 (2005) 351–368.
[34] N. Didry, L. Dubreuil, M. Pinkas, Activity of thymol, carvacrol, cinnamaldehyde and eugenol on oral bacteria, Pharm. Acta Helv. 69 (1994) 25–28.
[35] M. Mahboubi, F.G. Bidgoli, Antistaphylococcal activity of Zataria multiflora essential oil and its synergy with vancomycin, Phytomedicine 17 (2010) 548–550.
[36] A. Ahmad, A. Khan, F. Akhtar, S. Yousuf, I. Xess, L. Khan, et al., Fungicidal activity of thymol and carvacrol by disrupting ergosterol biosynthesis and membrane integrity against Candida, Eur. J. Clin. Microbiol. Infect. Dis. 30 (2011) 41–50.
[37] I. Ahmad, A.Z. Beg, Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens, J. Ethnopharmacol. 74 (2001) 113–123.
[38] H. Rodríguez, J.A. Curiel, J.M. Landete, B. de las Rivas, F.L. de Felipe, C. Gómez-Cordovés, et al., Food phenolics and lactic acid bacteria, Int. J. Food Microbiol. 132 (2009) 79–90.
[39] H. Thormar, Lipids and Essential Oils as Antimicrobial Agents, John Wiley & Sons, 2010.
[40] T. Taguri, T. Tanaka, I. Kouno, Antibacterial spectrum of plant polyphenols and extracts depending upon hydroxyphenyl structure, Biol. Pharm. Bull. 29 (2006) 2226–2235.
[41] A. Ghasemi Pirbalouti, M. Fatahi-Vanani, L. Craker, H. Shirmardi, Chemical composition and bioactivity of essential oils of Hypericum helianthemoides. Hypericum perforatum and Hypericum scabrum, Pharm. Biol. 52 (2014) 175–181.
[42] B. Alizadeh Behbahani, F.T. Yazdi, F. Shahidi, S.A. Mortazavi, M. Mohebbi, Principle component analysis (PCA) for investigation of relationship between population dynamics of microbial pathogenesis, chemical and sensory characteristics in beef slices containing Tarragon essential oil, Microb. Pathog. 105 (2017) 37–50.