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GC-MS Analysis of Anti-*Candida* and Antioxidant Activities of Hydroalcoholic Leaf Extract of *Chaerophyllum macropodum*

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Abstract

Background: *Chaerophyllum* is a genus from the family Apiaceae. *Chaerophyllum macropodum* as a wild species is endemic to Iran and Turkey. The leaves of this plant are used in the food industry, as well as medicine. Since resistance has increased in some *Candida* species, side effects of chemical drugs and the use of medicinal plants are very important.
Objectives: We reviewed the antioxidant and anti-*Candida* effects of hydroalcoholic leaf extracts of *C. macropodum*.
Methods: For the evaluation of the antioxidant potential of *C. macropodum* extracts, DPPH radical scavenging test, reducing power assay, and β-carotene bleaching test were performed. Moreover, the total free phenolic and flavonoid contents were measured. A GC-MS instrument was used for analyzing the extract components. In addition, hydroalcoholic extraction was performed by agar well-diffusion method against 40 *Candida* species isolated from patients.
Results: According to the DPPH test, a moderate antiradical activity was attributed to *C. macropodum* leaf extract; maximum inhibition (6.38) was reported at 1000 μg/mL. Based on the findings, the total phenolic and flavonoid contents were 57.43 $\pm$ 0.25 and 138 $\pm$ 19.80 (mg/g), respectively. The minimum inhibitory concentrations for *C. albicans* and *C. glabrata* were 300 and 400 μg/μL at 48 hours, respectively.
Conclusions: In this study, a moderate antioxidant activity was attributed to *C. macropodum* leaf extracts. In addition, the results confirmed that leaf extracts had inhibitory activity against bacteria and *Candida* species. Therefore, they may be applied as antioxidant, antibacterial, and antifungal agents in food protection.

Keywords: *Chaerophyllum*, *Candida*, Antioxidant Activity, Hydroalcoholic Extract

1. Background

Chaerophyllum genus, from the Apiaceae family, includes nearly 110 species in temperate and sub-temperate regions of Asia, Africa, and Europe. *Chaerophyllum* (chervil) is a native herb with weak stems and leaves, similar to parsley or parsley-like leaves. *C. macropodum* is known as "Gazarsare" in Mahdishahr (Sangsar) and "Jafari Frangi Kohi" in Iran. Eight *Chaerophyllum* species have been found in Iran, including *C. macropodum*, which is a wild species endemic to Iran and Turkey. The leaves of this plant are used in the food industry, as well as medicine. Previous studies have confirmed the presence of phenolic acids and constituents with cytotoxic and antimicrobial activities in *Chaerophyllum* species (1-4).

Candida species, particularly *Candida albicans*, are common pathogens in patients with organ transplantation, AIDS, and neoplastic disease, immunocompromised patients, and users of immunosuppressive drugs and broad-spectrum antibiotics. Recently, the use of medicinal plants has been highlighted, as natural products with therapeutic properties are cost-effective, available, and beneficial. In addition, chemical drugs have more side effects. Treatment of some diseases using medicinal plants, especially herbs, is in its infancy. On the other hand, researchers try to detect the active constituents of extracts to treat different diseases.

2. Objectives

We aimed to examine the anti-*Candida* and antioxidant potential of hydroalcoholic leaf extracts of *C. macropodum* via GC analysis.

3. Methods

3.1. Materials

Sigma-Aldrich supplied Gallic acid, trichloracetic acid (TCA), vitamin C, Quercetin, DPPH, Folin-Ciocalteu reagent, and butylated hydroxytoluene (BHT). The spectrophotometric assays were performed with a spectrophotometer (Analytik Jena, Germany).

3.2. Collection of Plants

After collecting *C. macropodum* leaves from Roudbarak in Mahdishahr (Semnan, Iran), they were confirmed by professional botanists. A voucher sample was deposited at the Research Center for Medicinal Plants. The dried samples were powdered for further analysis.

3.3. Extract Preparation

For extraction, the powdered leaf (50 g) was mixed with 6:3:1 ethyl acetate: methanol: distilled water (500 mL) overnight. The temperature was adjusted to 40°C in a Soxhlet system for eight hours before the evaporation solvent was incubated at room temperature.

3.4. Total Phenolic Content (TPC)

The Folin-Ciocalteu method was used according to the literature (5, 6) with some modifications to evaluate TPC. Following two hours of incubation, absorbance was read at 765 nm. For the measurements, the standard Gallic acid (GA) curve was applied, presented as grams of GA equivalent (GAE) per 100 g of dry weight (7).

3.5. Total Flavonoid Content (TFC)

To determine TFC, the extract (1 mL) in methanol was mixed in dilutions of standard quercetin solution in a flask (10 mL) of distilled water (4 mL). During five minutes of incubation, 5% sodium nitrite (0.3 mL) was added. In addition, aluminum chloride (0.3 mL) was added before six minutes of incubation, followed by 1 M sodium hydroxide (2 mL). OD was determined at 510 nm against a blank reagent. TFC was expressed as mg of quercetin equivalent per gram of sample (8-10). To obtain the extract concentrations, the standard curve of quercetin was used. TFC was calculated as follows:

$TFC = R \times D.F \times V/w$

where R represents the standard curve result, D.F denotes the dilution factor, V indicates the stock solution volume, and W represents the plant weight.

3.6. Antioxidant Assay

For the antioxidant assay, after dissolving the sample in 95% methanol at 1 mg/mL, it was diluted for preparation of series concentrations. The reference chemicals were used for comparisons.

3.6.1. DPPH Radical Scavenging Assay (DRSA)

DRSA was conducted and the absorbance was read at 517 nm based on studies by Burits and Bucar (11), Cuendet et al. (12), and Kirby and Schmidt (13):

DPPH radical scavenging percentage: $I\% = (1 - As/Ac) \times 100$

where Ac indicates the control absorbance, while As denotes the sample solution absorbance.

3.6.2. Reducing Power Assay

For this purpose, the method proposed by Hinneburg et al. (14) was applied. To measure absorbance, a spectrophotometer was used at 700 nm after mixing the supernatant (2.5 mL) with $FeCl_3$ (0.1%, 0.5 mL) and distilled water (2.5 mL). An increase in absorbance was indicative of increased reducing activity.

3.6.3. β-Carotene Bleaching Test (BCBT)

To prepare the stock solution of linoleic acid and β-carotene, linoleic acid (25 μL), as well as Tween 40 (200 mg), was added after dissolving β-carotene (0.5 mg) in chloroform (1 mL), since β-carotene is not considered water-soluble. Following that, 100 mL of oxygen-saturated distilled water was added during 30 minutes via vigorous shaking at 100 ml/min. Finally, the mixture (2500 μL) was added to tubes and then, the extract (350 μL) was added at 2 g/L.

OD was measured at 490 nm after incubation. A similar procedure was applied using a positive BHT control, along with a blank. Comparisons were made in terms of antioxidant potential between the extract, BHT, and the blank. All assays were conducted three times, and the relative antioxidant activity (RAA) was estimated as follows (15):

$RAA = A_{sample}/A_{BHT}$

3.7. Preparation of Microbial Strains and Inoculum

3.7.1. Serial Dilutions

For a final concentration of 400 $\mu g/\mu L$, 2 g of *C. macropodum* extract was dissolved in 5 mL of DMSO (16). To prepare serial dilutions of 400, 350, 300, 250, 200, 150, and 100 $\mu g/\mu L$, sterile distilled water was used. The reference strain was *Candida albicans* (ATCC10231). Gram-positive and Gram-negative bacteria included *Staphylococcus aureus* and *Escherichia coli*, respectively. Finally, 40 *Candida*

isolates were obtained from women with vulvovaginal candidiasis, including 34 *C. albicans* and six *C. glabrata* isolates (17).

After culturing the bacterial strains in nutrient agar (NA), incubation was performed for 24 hours at 37°C. Following the inoculation of the *Candida* isolates in Sabouraud dextrose broth (SDB, Merck, Germany), incubation was performed for 24 hours at 35°C. Then, using sterile distilled water, the yeasts were washed three times and the suspension was used to prepare the 1×10^6 CFU/mL concentration (0.5 MF standard). The positive and negative controls included the fluconazole disc for fungi (10 μg/disc; Hi-Media, India) and gentamicin (10 μg/disc) for bacteria, and DMSO, respectively.

3.7.2. Broth Microdilution for MIC Measurements

After adding the yeast inoculum (100 μl, 10^6 cells/mL) on SDA (Merck, Germany) and bacterial inoculum (100 μl, 10^6 cells/mL) on Mueller-Hinton agar (MHA, Merck, Germany), a borer was used to punch six 7-mm wells in the SDA medium; then, 100 μL of the extract dilutions was added, along with DMSO 100% as the negative control (16). The zone of inhibition was measured after incubating the plates at 35°C for 24 hours to determine the anti-*Candida* activity. The experiments were repeated twice.

3.8. GC-MS Analysis

A GC-MS system (Agilent) with a VF-5 ms silica capillary column (DB-35 ms, 30 m $\times$ 250 μm $\times$ 0.15 μm) was used for the GC-MS analysis (18), using helium gas (99.99%) as the carrier at a constant flow of 1 mL/min; the temperature of the injector and transfer line was 200°C and 250°C, respectively. On the other hand, the oven temperature increased from 60°C to 280°C at 10°C/min. After maintaining the isothermal temperature for 60 seconds, it increased to 280°C at 10°C/min. In the next step, the sample (1 μL) was injected in the splitless mode (50 - 600 amu; split ratio, 1:10). The total running time was 57 minutes. The retention indices, as well as fragmentation patterns, were compared with the computer library data and literature to identify the extract components. Using the Willey library, the recognized constituents were matched.

3.9. Statistical Analysis

One-way ANOVA was applied at 95% confidence level in SPSS version 16. In addition, the correlation of antioxidant activity with TPC and TFC was determined via linear regression analysis in Excel 2003.

4. Results

4.1. Extraction Yield, TPC, and TFC

The extraction method, growth, and climatic conditions, as well as genetic factors, influence TPC, TFC, and antiradical activity. However, according to the literature (18), the use of a different solvent system, i.e., ethyl acetate: methanol: distilled water, is preferable for the extraction of antioxidant constituents. The percentage of hydroalcoholic extraction yield of *C. macropodum* leaf extract was 1.86 w/w%.

4.2. Antioxidant Potential

The high antioxidant potential was confirmed on DRSA, reducing power assay, and BCBT.

4.2.1. DRSA

In this primary assay, odd electrons of DPPH are quenched by the extract to decrease absorbance at 517 nm. The leaf extract of *C. macropodum* showed high antiradical activity in comparison with the standard BHT with the highest inhibition at 1000 μg/mL (14.47) (Table 1).

4.2.2. Reducing Power Activity

Compared to vitamin C, the reducing potential of the hydroalcoholic extract was not dose-dependent. The reducing power percentage is demonstrated in Table 1. The leaf extract of *C. macropodum* showed maximum reducing power ($P \leq 0.05$). Analysis of the association between the reducing power activity and phenolic content indicated an R^2 of 0.89, while its correlation with the flavonoid content was estimated at $R^2 = 0.88$. Based on these values, phenolic compounds may have greater involvement in the reducing activity, compared to flavonoid compounds.

4.2.3. BCBT

The intensity of yellow color will reduce following the reaction between β-carotene and radicals produced via linoleic acid oxidation. The RAA of *C. macropodum* extract was measured as follows:

$RAA = A_{sample}/A_{BHT}$

where A_{BHT} represents the BHT absorbance (standard) and A_{sample} denotes the absorbance of *C. macropodum*. The calculated RAAs for *C. macropodum* leaf extract and BHT are presented in Table 1.

4.3. Bioactive Content

4.3.1. TPC Measurements

For the spectrophotometric measurement of TPC (expressed as GAE), the Folin-Ciocalteu method was applied

Table 1. Effect of the Test Compounds in the DPPH Assay, β-Carotene Bleaching, and Reducing Power of the Hydroalcoholic *C. macropodum* Extract with Respect to Standard Vit.c and BHT

Test Compound	IC_{50}	Inhibition (%)	β-Carotene Bleaching (RAA%)	Reducing Power (%)
C. macropodum	27.63	6.38	37.61 ± 0.92	94.48 ± 3.75
BHT	14.55	14.47	100	

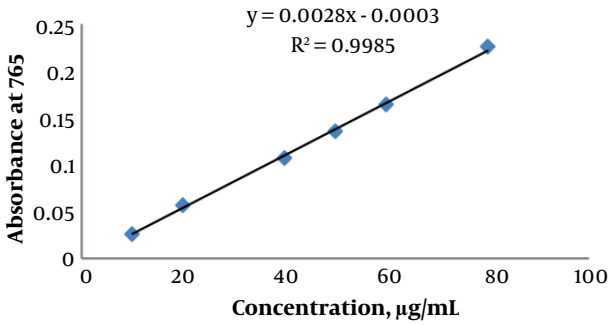

Figure 1. Calibration curve of standard Gallic acid for determination of total phenol content

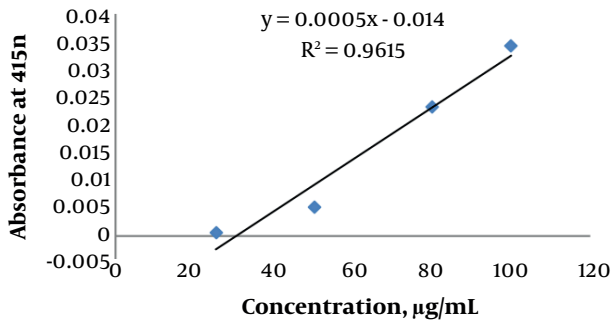

Figure 2. Calibration curve of standard Quercetin for determination of total flavonoid content in *C. macropodum*

and the standard curve equation was determined (Figure 1):

Absorbance = 0.038 × (μg GA) - 0.0132 (R^2 = 0.9859)

TPC was calculated by adding the absorbance value in this equation. As shown in Table 2, TPC of *C. macropodum* leaf extract was 57.43 ± 0.25.

Table 2. Antioxidant Constituents of *Chaerophyllum macropodum*[a]

Extract	Total Phenols (GAE) (mg/g)[b]	Total Flavonoids (QU) (mg/g)[c]
C. macropodum	57.43 ± 0.25	138 ± 19.80

[a] Values represent means ± standard deviations for triplicate experiments.
[b] Expressed as GAE.
[c] Expressed as Quercetin equivalent (QU).

4.3.2. TFC Measurements

For the spectrophotometric measurement of TFC, the Folin-Ciocalteu method was applied and the standard curve equation was measured (Figure 2):

Absorbance = 0.0005 × (μg quercetin) - 0.014 (R^2 = 0.9615)

TFC was calculated after entering the absorbance value in the equation. The mean TFC was 138 ± 19.80 mg/g (Table 2).

4.4. Anti-Candida Activity

The anti-*C. albicans* (n = 34) and anti-*C. glabrata* (n = 6) activities of hydroalcoholic extracts were confirmed in our analyses (Table 3).

4.5. GC-MS Analysis

The presence of phytocomponents was confirmed based on the GC-MS analysis. 15 compounds were detected in *C. macropodum* (Table 4).

5. Discussion

Recently, the use of medicinal plants has attracted the researchers' attention. Polyphenols are secondary metabolites, scavenging and quenching free radicals. Coruh et al. (4) showed that TPC of *C. macropodum* was 34 μg GA/mg. Ebrahimabadi et al. (19) reported that TPC was 29.3 and 30.2 μg GA/mg in the methanolic leaf and flower extracts of *C. macropodum*, respectively. According to a study by Alavi et al. (20), TPC of the dried extract of *C. macropodum* was 1.34 μg/mg. In our study, TPC was 57.43 μg GA/mg in the hydroalcoholic leaf extracts of *C. macropodum*, which is higher than the level reported in the mentioned studies.

On the other hand, 15 compounds were identified in the hydroalcoholic leaf extracts of *C. macropodum* in this research. The major chemical constituents included palmitic acid, linolenic acid, linoleic acid, nonacosane, trimethyloctane, ethylilnoleolate, neophytadiene, benzothiazole, pyridinecarboxaldehyde, and oleic acid. Based on the findings, omega-3, -6, and -9 and palmitic acid were respectively unsaturated and saturated fatty acids. Unsaturated fatty acids showed higher concentrations than saturated fatty acids. The GC-MS analysis showed that

Table 3. Antimicrobial Activity of the Hydroalcoholic Extract of *C. macropodum*

Variable	Inhibition Zone	MIC	Gentamicin	Fluconazole
Candida albicans	9	300 μg/μL	-	20
Candida glabrata	8	400 μg/μL	-	-
Escherichia coli	7	400 μg/μL	20	-
Staphylococcus aureus	10	300 μg/μL	21	-

Table 4. Chemical Analysis of the Constituents of the Hydroalcoholic Extract of *C. macropodum* Leaves Based on GC Peak Areas

No	Components	Peak Area, %	RT
1	Pentenylamine	0.058	5.308
2	Butanoic acid (active valeric acid)	0.070	8.704
3	Silanol (formic acid)	0.160	11.216
4	4-vinyl-2-methoxy-phenol	0.129	22.162
5	Thiourea	0.108	28.715
6	Neophytadiene	6.524	29.205
7	Hexadecanoicacid (palmitic acid)	27.527	35.866
8	Octadecatrienoic acid (linolenic acid)	20.296	36.175
9	Ethylilnoleolate	6.551	36.245
10	Octadecadienoic acid linoleic acid	12.299	39.378
11	Trimethyloctanal	6.588	42.662
12	Pyridinecarboxaldehyde	4.102	44.343
13	Benzothiazole	4.333	44.381
14	9-Octadecenoic acid (oleic acid)	3.522	45.765
15	Nonacosane	7.728	47.165
Total		99.99	

Abbreviation: RT, retention time.

fatty acids were dominant, which is in agreement with a study by Shafaghat (21). Different results regarding the amount of various fatty acids and chemical components may be related to various factors, including season, period of growth, geographical location, altitude, and methods.

Youssef and Noaman (22) showed which benzothiazole derivatives have antibacterial and antifungal activities. Keri et al. (23) conducted a comprehensive systematic review of benzothiazole-based constituents and examined antifungal, antibacterial, antitubercular, antileishmanial, antimalarial, and other medicinal agents. The researchers showed that neophytadiene, a diterpenoid hydrocarbon that belongs to the group of phytanes, has antioxidant and antimicrobial activities (24-26). In the methanolic extract of *Apamarga kshara*, neophytadiene was a superior TvCK inhibitor compared to other identified compounds (27).

Tao et al. (28) showed that octanal derivatives exhibited strong antifungal activity against *Penicillium* species. In addition, anti-*Candida* and antibacterial activities of some *Chaerophyllum* species showed moderate inhibitory effects (28-30). Ebrahimisadr et al. (31) reported that the extracts of *C. macropodum* have high antileishmania activities. Our results showed that hydroalcoholic leaf extract of *C. macropodum* (nearly 17.3% of the total chemical compound) has an inhibitory activity against bacteria and *Candida*, which is in agreement with the mentioned research and a study by Durmaz et al. (3). It may be due to the presence of compounds, such as benzothiazole, neophytadiene, and octanal, isolated from the hydroalcoholic extract. Therefore, *C. macropodum* is a candidate for the treatment of some diseases and is used in food industries, as well.

On the other hand, the TPC results showed about 57.43 μg GA/mg in hydroalcoholic *C. macropodum* leaf extract, which is higher than the amount reported in other studies (4, 19, 20). Differences in the chemical compounds, content, and therapeutic outcomes related to antifungal and antibacterial activities may be due to various factors, such as season, extraction method, growth period, geographic location, and altitude. There may be a new *Chaerophyllum* species in our region (Rodbarak, Semnan, Iran), based on the plant ecotype theories. Therefore, we will conduct further research on DNA barcoding.

References

1. Dall'Acqua S, Innocenti G. Antioxidant compounds from Chaerophyllum hirsutum extracts. *Fitoterapia*. 2004;**75**(6):592–5. doi: 10.1016/j.fitote.2004.05.007. [PubMed: 15351116].
2. Dall'Acqua S, Viola G, Piacente S, Cappelletti EM, Innocenti G. Cytotoxic constituents of roots of Chaerophyllum hirsutum. *J Nat Prod*. 2004;**67**(9):1588–90. doi: 10.1021/np040046w. [PubMed: 15387667].
3. Durmaz H, Sagun E, Tarakci Z, Ozgokce F. Antibacterial activities of Allium vineale, Chaerophyllum macropodum and Prangos ferulacea. *Afr J Biotechnol*. 2006;**5**(19).
4. Coruh N, Celep AGS, Özgökçe F. Antioxidant properties of Prangos ferulacea (L.) Lindl., Chaerophyllum macropodum Boiss. and Heracleum persicum Desf. from Apiaceae family used as food in Eastern Anatolia and their inhibitory effects on glutathione-S-transferase. *Food chemistry*. 2007;**100**(3):1237–42. doi: 10.1016/j.foodchem.2005.12.006.
5. Zheng W, Wang SY. Antioxidant activity and phenolic compounds in selected herbs. *J Agric Food Chem*. 2001;**49**(11):5165–70. [PubMed: 11714298].

6. Liu M, Li XQ, Weber C, Lee CY, Brown J, Liu RH. Antioxidant and antiproliferative activities of raspberries. *J Agric Food Chem*. 2002;**50**(10):2926–30. [PubMed: 11982421].
7. Wolfe K, Wu X, Liu RH. Antioxidant activity of apple peels. *J Agric Food Chem*. 2003;**51**(3):609–14. doi: 10.1021/jf020782a. [PubMed: 12537430].
8. Tesoriere L, Butera D, Gentile C, Livrea MA. Bioactive components of caper (Capparis spinosa L.) from Sicily and antioxidant effects in a red meat simulated gastric digestion. *J Agric Food Chem*. 2007;**55**(21):8465–71. doi: 10.1021/jf0714113. [PubMed: 17887802].
9. Tepe B, Sokmen M, Akpulat HA, Sokmen A. Screening of the antioxidant potentials of six Salvia species from Turkey. *Food Chemistry*. 2006;**95**(2):200–4. doi: 10.1016/j.foodchem.2004.12.031.
10. Tlili N, Nasri N, Khaldi A, Triki S, Munné-Bosch S. Phenolic compounds, tocopherols, carotenoids and vitamin C of commercial caper. *J Food Biochem*. 2011;**35**(2):472–83.
11. Burits M, Bucar F. Antioxidant activity of Nigella sativa essential oil. *Phytother Res*. 2000;**14**(5):323–8. [PubMed: 10925395].
12. Cuendet M, Hostettmann K, Potterat O, Dyatmiko W. Iridoid glucosides with free radical scavenging properties from Fagraea blumei. *Helvetica Chimica Acta*. 1997;**80**(4):1144–52. doi: 10.1002/hlca.19970800411.
13. Kirby AJ, Schmidt RJ. The antioxidant activity of Chinese herbs for eczema and of placebo herbs–I. *J Ethnopharmacol*. 1997;**56**(2):103–8. [PubMed: 9174970].
14. Hinneburg I, Dorman HJD, Hiltunen R. Antioxidant activities of extracts from selected culinary herbs and spices. *Food chem*. 2006;**97**(1):122–9. doi: 10.1016/j.foodchem.2005.03.028.
15. Tomaino A, Cimino F, Zimbalatti V, Venuti V, Sulfaro V, De Pasquale A, et al. Influence of heating on antioxidant activity and the chemical composition of some spice essential oils. *Food chem*. 2005;**89**(4):549–54. doi: 10.1016/j.foodchem.2004.03.011.
16. Sadeghi Nejad B, Rajabi M, Zarei Mamoudabadi A, Zarrin M. In vitro anti-Candida activity of the hydroalcoholic extracts of heracleum persicum fruit against phatogenic Candida species. *Jundishapur J Microbiol*. 2014;**7**(1). e8703. doi: 10.5812/jjm.8703. [PubMed: 25147655]. [PubMed Central: PMC4138661].
17. Bineshian F, Yadegari MH, Sharifi Z, Akbari Eidgahi M, Nasr R. Identification of Candida species using MP65 gene and evaluation of the Candida albicans MP65 gene expression in BALB/C mice. *Jundishapur J Microbiol*. 2015;**8**(5). e18984. doi: 10.5812/jjm.8(5)2015.18984. [PubMed: 26060567]. [PubMed Central: PMC4458353].
18. Mohammadi A, Malek F, Dehpour A, Nazari H. Chemical composition and antibacterial activity of the essential oils of some medicinal plants. *Afr J Agr Res*. 2013;**8**(24):3151–8. doi: 10.5897/AJAR12.1941.
19. Ebrahimabadi AH, Djafari-Bidgoli Z, Mazoochi A, Jookar Kashi F, Batooli H. Essential oils composition, antioxidant and antimicrobial activity of the leaves and flowers of Chaerophyllum macropodum Boiss. *Food Control*. 2010;**21**(8):1173–8. doi: 10.1016/j.foodcont.2010.01.014.
20. Alavi SM, Farasat Kish R, Sadeghpour Tabaei A, Babaei T. [Comparison of the analgesic effect of sufentanil and morphine in patient controlled analgesia method after coronary artery by pass surgery]. *Iran J Surg*. 2010;**17**(4). Persian.
21. Shafaghat A. Biological activity and chemical compounds of the hexane extracts from Chaerophyllum macropodum in two different habitats. *J Med Plant Res*. 2013;**7**(20):1406–10. doi: 10.5897/JMPR12.067.
22. Youssef AM, Noaman E. Synthesis and evaluation of some novel benzothiazole derivatives as potential anticancer and antimicrobial agents. *Arzneimittelforschung*. 2007;**57**(8):547–53. doi: 10.1055/s-0031-1296647. [PubMed: 17915643].
23. Keri RS, Patil MR, Patil SA, Budagumpi S. A comprehensive review in current developments of benzothiazole-based molecules in medicinal chemistry. *Eur J Med Chem*. 2015;**89**:207–51. doi: 10.1016/j.ejmech.2014.10.059. [PubMed: 25462241].
24. Cheng MC, Lin LY, Yu TH, Peng RY. Hypolipidemic and antioxidant activity of mountain celery (Cryptotaenia japonica Hassk) seed essential oils. *J Agric Food Chem*. 2008;**56**(11):3997–4003. doi: 10.1021/jf703593v. [PubMed: 18473476].
25. Cheng MC, Chang WH, Chen CW, Li WW, Tseng CY, Song TY. Antioxidant properties of essential oil extracted from pinus morrisonicola hay needles by supercritical fluid and identification of possible active compounds by GC/MS. *Molecules*. 2015;**20**(10):19051–65. doi: 10.3390/molecules201019051. [PubMed: 26492232].
26. Palic R, Stojanovic G, Alagic S, Nikolic M, Lepojevic Z. Chemical composition and antimicrobial activity of the essential oil and CO2 extracts of the oriental tobacco, Prilep. *Flavour Frag J*. 2002;**17**(5):323–6. doi: 10.1002/ffj.1084.
27. Shaikh S, Aaqil H, Rizvi SMD, Shakil S, Abuzenadah AM, Gupta P, et al. Comparative inhibition study of compounds identified in the methanolic extract of apamarga kshara against trichomonas vaginalis carbamate kinase (TvCK): An enzoinformatics approach. *Interdisc Sci Comput Life Sci*. 2016;**8**(4):357–65. doi: 10.1007/s12539-015-0120-0.
28. Tao N, Jia L, Zhou H, He X. Effect of octanal on the mycelial growth of Penicillium italicum and P. digitatum. *World J Microbiol Biotechnol*. 2014;**30**(4):1169–75. doi: 10.1007/s11274-013-1539-2. [PubMed: 24162951].
29. Demirci B, Koşar M, Demirci F, Dinc M, Başer KHC. Antimicrobial and antioxidant activities of the essential oil of Chaerophyllum libanoticum Boiss. et Kotschy. *Food chem*. 2007;**105**(4):1512–7. doi: 10.1016/j.foodchem.2007.05.036.
30. Kürkçüoğlu M, Başer KHC, Işcan G, Malyer H, Kaynak G. Composition and anticandidal activity of the essential oil of Chaerophyllum byzantinum Boiss. *Flavour Frag J*. 2006;**21**(1):115–7. doi: 10.1002/ffj.1539.
31. Ebrahimisadr P, Majidiani H, Bineshian F, Jameie F, Ghasemi E, Ghaffarifar F. Evaluation of the cytotoxicity effect of chaerophyllum extract on leishmania major and J774 cell line in vitro. *Jundishapur J Nat Pharm Prod*. 2017;**12**(3 (Supp)). e38948. doi: 10.5812/jjnpp.38948.