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ORIGINAL PAPER

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BIOACTIVE COMPOUNDS, ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF TWO *Cirsium* SPECIES

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ABSTRACT

In the GC-MS analysis, 81 bioactive phytochemical compounds were identified in hexane extracts of *Cirsium creticum* and *Cirsium italicum*. Terpenoids constituted the main fractions of *C. italicum* (70.95%), while hydrocarbons were most abundant compounds of *C. creticum* (41.11%). The antibacterial activity and antifungal activity of extracts from two *Cirsium* species was tested using microdilution methods. According to the results of antibacterial activity, the highest inhibition effect of *C. creticum* was found on *B. subtilis, E. coli* and *P. aeruginosa.* The highest inhibition effect of *C. italicum* was found on *B. subtilis.* In the results of antifungal activity, the highest inhibition effect of *C. creticum* was found on *P. chrysogenum* and the highest inhibition effect of *C. italicum* was found on *C. krusei*. The present work is the first report on hexane extracts compounds of two *Cirsium* species as well as antibacterial and antifungal activities.

Key words: antibacterial activity, antifungal activity, *C. creticum, C. italicum,* active compounds

INTRODUCTION

The Asteraceae family is one of the largest families of flowering plants, consisting of approximately 1600 genera and over 23,000 species. *Cirsium creticum* (Lam.) d'Urv. subsp. and *Cirsium italicum* (Savi) DC. belongs to the Asteraceae family. A ethnobotanical research carried out in Catalca-Turkey reported that fruits of *C. creticum* (esekcalisi) can be used as remedy against mushroom poisoning in traditional treatments [Genc and Ozhatay 2006]. Some parts of *Cirsium* species, especially the roots or whole plants have been used in traditional medicine for treatment some diseases such as haemorrhaging, inflammation of the liver and kidney, and a variety of abdominal and intestinal disorders [Kim 1997]. It was revealed that the extracts of different *Cirsium* species had possess the antioxidant, antibacterial, anti-cancer, hepatoprotective, vasorelaxant and anti-diabetic activities according to recent reported studies [Borawska et al. 2010, Nazaruk et al. 2008, Yoo et al. 2008, Kim et al. 2008, Perez et al. 2001]. Additionally, *C. creticum* can be eaten as raw vegetable or cooked in a meal after peeling its stems [Kizilarslan and Ozhatay 2012]. It was reported that the extract from *C. italicum* prepared by boiling seeds (kisa kangal) can be effective for the treatment of haemorrhoid [Yesilada et al. 1999].

Antibiotics are considered as important weapons in fighting bacterial infections and humans have

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greatly benefited from them to treatment some disease since their introduction to human life. However, their health benefits are under discussion over the past few decades because of many commonly used antibiotics have become less and less effective against certain illnesses, many of them produce toxic reactions and some of them gain drug-resistant against bacteria. For this reason, it has become important to find new drugs with less resistance against microorganisms. In recent years, different drugs have been obtained from plants and natural sources gain importances in the prevention and treatment of human diseases. Natural products from some of higher plants can be a new source of antimicrobial agents with possibly biological mechanisms of their action [Runyoro et al. 2006]. Therefore the effects of several plant extracts on bacteria have been investigated by a number of researchers in the world [Reddy et al. 2001].

The aim of the present research was to identify of compounds and to determine antibacterial, antifungal activities of *C. creticum* (Lam.) d'Urv. subsp. *creticum* (Asteraceae) and *C. italicum* (Savi) DC. (Asteraceae) in hexane extracts. In the literature, there is no study on the composition of compounds in hexane extracts of *C. creticum* and *C. italicum* with their antibacterial, antifungal activities.

MATERIALS AND METHODS

Plant material and extractions. *C. creticum* (NGBB 7230) and *C. italicum* (NGBB 6807) which are wild plant species in Trakya region (Turkey), were collected in June 2016 and June 2017. The plants identified by University of Namik Kemal, Faculty of Science, Department of Biology. The whole plant was grounded and powder-homogenized after drying at room temperature. For obtain extracts, hexane was used as solvent after maceration at room temperature of powdered whole plants for each *Cirsium* species. The extractions were done two times and solvents were evaporated under vacuum using a rotary evaporator (Büchi Labortechnik, Flawil, Switzerland, Model: R-210 Rotavapor, B-491 Heating Bath, V-700 Vacuum Pump).

Conditions of GC-MS analysis

Chromatographic analysis of hexane extracts of two *Cirsium* species were done with Hewlett-Packard HP 6890 series GC/MS. GC/MS apparatus was combined with a mass selective detector and the capillary column used was an HP-5MS (5% phenyl methyl siloxane, 30 m × 250 µm × 0.25 µm). Carrier gas was helium and its flow rate was 1.0 ml/min with 5µl injection volume. Samples were analysed with the column held initially 180° for 1 min after injection, then increased to 250° with an 8°C/min heating ramp with a 1 min hold time. The temperature was then increased to 300°C with 2°C/min heating ramp for 10 minutes. The injection was performed in split mode (split ratio: 10 : 1). Interface and injector temperatures were 250°C and 280°C, respectively. Run time was 49 min. MS scan range was m/z 20−440 using electron impact (EI) ionization (70 eV) and an ion source temperature of 250°C.

On comparison of the mass-spectra of the constituents with Wiley 9 and NIST library. The relative percentage of separated compounds were determined from Total Ion Chromatography by the computerized integrator. Retention indices were determined externally with a series of n-alkanes (C_6–C_{22}), under the same chromatographic [Sabudak and Goren 2011].

Determination of antibacterial and antifungal activities. Microdilution methods were used to investigations the antibacterial and antifungal effects of extracts obtained from the plants. Antibacterial activity was carried out on two Gram-positive bacterial reference strains [*Staphylococcus aureus* (ATCC 43300), *Bacillus subtilis* (NRRL NRS-744)] and four Gram-negative bacterial reference strains [*Escherichia coli* (ATCC 35218), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus mirabilis* (ATCC 12453), *Salmonella typhimurium* (ATCC 14028)] were grown in nutrient agar at 37° for 18 h. Antifungal activity test was carried out against six fungal reference strains; *Candida albicans* (ATCC 90028), *Candida glabrata* (ATCC 90030), *Candida parapsilosis* (ATCC 22019), *Candida krusei* (ATCC 6258), *Penicillium chrysogenum* (ATCC 48271) and *Aspergillus fumigatus* (ATCC 204305) were grown in Sabouraud dextrose agar (SDA) at 27°C. For both antimicrobial and antifungal testing, sterile microtiter plates with 96 "U" type wells were used. As bacterial growth medium Mueller-Hinton broth (Gibco®) was used.

For fungus growth RPMI 1640-L glutamin (Gibco®) was used as the medium and pH was adjusted to 7 by adding 34.53 gr/l MOPS as buffer. Prepared medium was sterilized by filtration method and stored at + 4° until use. Bacterial inoculums prepared in Mueller-Hinton broth by using a Densitometer (Densimat; BioMèrieux). The McFarland bacterial suspension standard at a density of 0.5, approximately 10^8 CFU/ml. Fungus inoculums were prepared in RPMI 1640-L glutamin. By this process, stock yeast suspensions containing $1-5 \times 10^6$ cells were obtained in millilitres. Stock yeast suspension was diluted first with 1/50 and then 1/20, resulting in a final concentration of $1-5 \times 10^3$ cells/ml to be used in the test. The stock concentrations of extracts obtained from the plants was 1000 mg/ml for *C. creticum* and 1411 mg/ml for *C. italicum*. Serial dilutions to 12 wells are made. As antimicrobic control Penicillin G and Gantamycin was used as standard according to CLSI M-100 [2017] for determination of antimicrobial activity. For antifungal control, Fluconazole as was used as standard for determination of antifungal activity according to CLSI M27-A3 [2008].

RESULTS AND DISCUSSION

GC-MS analysis of *C. creticum* and *C. italicum*. The GC-MS chromatogram analysis of C. creticum indicated the presence of forty peaks and compounds in hexane extract. Similarly, forty bioactive compounds were identified for *C. italicum* in hexane extract. These compounds were characterized their retention indices (RI), their retention time (RT) and concentration (peak area %). These identified compounds of two *Cirsium* species are presented in Table 1 and Table 2. According to chemical class distribution, hydrocarbons (41.11%) and terpenoids (33.26%) most abundant compounds of the hexane extracts of *C. creticum* (Tab. 3). Additionaly, *C. creticum* hexane extracts were abundant in esters (11.94%) and fatty acids (9.25%) (Tab. 3). The most abundant hydrocarbons in hexane extract of *C. creticum* were identified as 1-docesen (7.57%), 1-nonadecene (7.2%), cyclotetracosane (6.07%), 1-hexadecene (5.81%) and tetratetracontane (4.0%). Smaoui et al. [2012], reported 1-nonadecene from *Streptomyces* sp. TN 256 strain exhibited antibacterial activity and strong antifungal activity against *Candida albicans* [El-Sakhawy et al. 1998] and 1-hexadecene and cyclotetracosane from Gymura segetum's leaf extracts showed potent antimicrobial activities [Lay-Jing et al. 2012].

In the total terpenoids constituents of *C. creticum*, globulol was dominant which accounted for 9.29% as seen Table 1. It was reported that globulol has been exhibit antifungal activity against a variety of fungal species and against bacteria [Tan et al. 2008]. The other identified terpenoids from *C. creticum* were lup-20(29)-en-3-yl-acetate (9.27%), norolean-12-ene (4.1%), olean-12-en (3.47%), thunbergol (2.9%) and lupeol (1.99%). The anticancer, antiprotozoal, anti-inflammatory, antimicrobial and chemopreventive properties of lupeol and lup-20(29)-en-3-yl-acetate were reported in a recent research [Gallo and Sarachine 2009]. It has been reported that thunbergol which isolated from the brown alga *Sargassum thunbergii* has been found significant potently radical scavenging activities [Youngwan et al. 2006].

Palmitic acid (hexadecanoic acid) (7.54%) and linoleic acid methyl ester (8.83%) are the main fatty acid esters identified in the *C. creticum* hexane extract. Reifen et al. [2015], suggested that α-linolenic acid alone has potential as an anti-inflammatory agent. Palmitic acid is a saturated fatty acid, Aparna et al. [2012] reported that their structural and kinetic studies on Palmitic acid, Palmitic acid may an anti-inflammatory compound as an inhibitor of phosphlipase A_2.

A total of fourty constituents were determined, accounting for 99.67% of the *C. italicum* hexane extracts (Tab. 2). Terpenoids (70.95%) were most abundant compounds of *C. italicum* total constitutens (Tab. 3). This indicates that the hexane extract of *C. italicum* is a rich source of terpenoids compounds. The chromatogram analyses of hexane extract of *C. italicum* indicated that the main nine major peaks were the most prevailing compounds in extract. These compounds were identified as lupeol (17.99%), moretenol (6.2%), (3β-21-α-diacetoxy-18,22,22-trimethyl-17,27,29,30-tetranor-c-homoolean-14--ene) (9.54%), betulin (5.28%), Z-13-docosenamide (Erucamide) (8.41%), 22(29)-hopene (diploptene) (6.97%), methyl commate A (5.34%), lup-20(29)-en--3-yl-acetate (13.09%) and norolean-12-ene (5.81%) are shown in Table 2. Except Erucamide and norolean-

Table 1. The composition of hexane extract obtained from *C. creticum*

No	RI	Compounds	Percentage (%)	No	RI	Compounds	Percentage (%)
1	873	1-Hexadecene	5.81	22	1355	5-(1-methylpropyl)-Nonane	0.67
2	920	1-Nonadecene	7.2	23	1367	10-Nonadecanone	0.6
3	931	6,10,14-trimethyl-2-Pentadecanone	0.65	24	1380	9-Tricosene	0.68
4	949	Palmitoleic acid, methyl ester	0.24	25	1405	1,2-Cyclohexanedicarboxylic acid, didecyl ester	0.34
5	963	Palmitic acid	7.54	26	1467	Tetratetracontane	4.00
6	971	1-Docosene	7.57	27	1541	Hexanoic acid, 2-propenyl ester (Allyl caproate)	0.22
7	1005	1-Hexacosanol (ceryl alcohol)	0.61	28	1550	Heneicosane	0.23
8	1009	Phytol	0.75	29	1736	2-propenyl nonanoate	0.58
9	1020	Methyl-Linoleate	8.83	30	1803	Stigmasterol	0.45
10	1027	Stearic acid	0.85	31	1867	γ-Sitosterol	0.98
11	1027	Tetrahydro geraniol	0.43	32	1901	Olean-12-ene	3.47
12	1038	Cyclotetracosane	6.07	33	1950	Lupeol	1.99
13	1111	Oleic acid	0.86	34	2037	Norolean-12-ene	4.10
14	1127	Ethyl-Cyclodocosane	3.50	35	2073	Veridiflorol	0.52
15	1181	Octacosane	0.69	36	2084	Lup-20(29)-en-3-yl- acetate	9.27
16	1213	Di-*n*-octyl phthalate	0.47	37	2168	Neophytadiene	0.54
17	1241	1-Tricosene	1.68	38	2211	Thunbergol	2.90
18	1312	Tetracosane	2.44	39	2245	Globulol	9.29
19	1317	5-butyl-Nonane	0.57	40	2267	2-tert-butyl-4,6-bis(3,5-ditert--butyl-4-hydroxybenzyl) phenol	1.17
20	1333	Nonadecane	0.94				
21	1338	Vinyl decanoate	0.32				

RI: retention indices

Table 2. The composition of hexane extract obtained from *C. italicum*

No	RI	Compounds	Percentage (%)	No	RI	Compounds	Percentage (%)
1	907	1-Bromo2-methyl-decane	0.25	22	1561	Vitamin E	0.4
2	921	Hexadecanoic acid, methyl ester (Methylpalmitate)	0.35	23	1628	Ergost-5-en-3β-ol	0.51
3	950	5-methyl-2-isopropyl-3-Cyclo-hexen-1-one	1.29	24	1659	Stigmasterol	2.22
4	954	(E)-Verbenol	0.08	25	1714	β-sitosterol	3.5
5	969	9,12,15-Octadecatrienoic acid, methyl ester	0.78	26	1737	24- propylidene- Cholest-5--en-3β-ol	1.03
6	976	5,7-dimethyl octa hydrocoumarin	1.92	27	1742	Methyl CommateA	5.34
7	987	Ethyl linoleate	0.25	28	1766	Clionasterol	0.57
8	993	Tetradecanamide	0.42	29	1784	Betulin	5.28
9	1059	(Z)-13-Docosenamide (Erucylamide)	8.41	30	1800	3,5-Cholestadien-7-one	0.16
10	1120	Pentacosane	0.20	31	1807	Benzylpalmitate	0.27
11	1125	2-monopalmitine	0.29	32	1838	Humalene-1,6-dien-3-ol	0.19
12	1231	Hexacosane	0.85	33	1847	Norolean-12-ene	5.81
13	1240	Diolein	0.7	34	1873	Moretenol	6.2
14	1317	Squalene	0.2	35	1885	Lupeol	17.99
15	1359	1-Eicosanol	0.2	36	1892	3β-21-α-diacetoxy-18,22,22--trimethyl-17,27,29,30-tetranor--c-homoolean-14-ene	9.54
16	1366	Tetracontane	1.62	37	1906	Cycloart-23-ene-3β-25-diol	0.43
17	1490	Solanesol	0.15	38	1969	Neophytadiene	0.38
18	1517	17-Pentatriacontene	0.14	39	1982	(22(29))-Hopene	6.97
19	1528	Pentatriacontane	0.4	40	1995	Lup-20(29)-en-3-yl- acetate	13.09
20	1534	Cholesterol	0.22				
21	1559	α-Tocopheryl- β-D-Mannoside	1.4				

RI**:** retention indices

Table 3. The chemical class distribution of two *Cirsium* species in hexane extracts

Compounds	*C. creticum*	*C. italicum*
Terpenoids	33.26	70.95
Hydrocarbons	41.11	3.22
Esters	11.94	2.29
Fatty acids	9.25	–
Cumarin / Phenolic comp. / Flavones	1.17	3.72
Carbonyl compounds	1.25	1.29
Alcohols	0.61	0.47
Sterol	1.43	8.65
Other functional groups (amide, alkyl halogen)	–	8.83 amide 0.25 alkyl hal.
Total (%)	100.02	99.67

Table 4. Antibacterial activity of hexane extracts from two *Cirsium* species expressed by the MIC values

Bacterial reference strain	*C. creticum* (mg/ml)	*C. italicum* (mg/ml)	Penicillin G[1]/ Gentamycin (mg/ml)
Staphylococcus aureus	125.0	–	0.125[1]
Bacillus subtilis	31.25	22.56	0.125
Escherichia coli	31.25	90.56	0.062
Pseudomonas aeruginosa	31.25	–	0.125
Proteus mirabilis	62.5	–	0.125
Salmonella typhimurium	125.0	–	0.125

[1] Penicillin was used only for *Staphylococcus aureus*

Table 5. Antifungal activity of hexane extracts from two *Cirsium* species expressed by the MIC values

Fungal reference strain	*C. creticum* (mg/ml)	*C. italicum* (mg/ml)	Fluconazole (mg/ml)
Candida albicans	125.0	352.25	0.002
Candida glabrata	125.0	352.25	0.032
Candida parapsilosis	62.5	176.38	0.002
Candida krusei	62.5	88.19	0.125
Penicillium chrysogenum	1.95	176.38	0.256
Aspergillus fumigatus	0.98	176.38	0.256

12-ene, the other compounds identified as terponoids compounds from these compounds. Lup-20(29)-en-3--yl-acetate (13.09%) and lupeol (17.99%) were among the most abundant terpenoids of *C. italicum* according to GC-MS analyses. Lupeol and lup-20(29)-en-3--yl-acetate are a triterpene were reported to possess pharmacological activities including anticancer, antiprotozoal, anti-inflammatory, antimicrobial and chemopreventive properties [Gallo and Sarachine 2009]. Srivastava and Singh reported that methyl commate A is a pentacyclictriterpene glycoside and has antifungal activity [Srivastava and Singh 2015].

Different sterol compounds were found in considerable amounts (8.65%) in *C. italicum* sterols. Sterols are important constituents various vehicles including natural sources, and as part of a healthy diet and life style, are important dietary components [Berger and Jones 2004]. Among the sterols, β-sterol (3.5%) and stigmasterol (2.22%) were predominant in *C. italicum.* A mixture of stigmasterol and sitositerol was shown to possess antiinflammatory activity after topical application [Gomez et al. 1999].

Additionally, erucylamide (8.41%) was found high content in *C. italicum* hexane extract. Erucamide is a fatty acid amide known to enhance neovascularization in regenerating skeletal muscle [Wakamatsu et al. 1990] and modulate water balance in the visceral organs and in the cerebrospinal fluid [Hamberger and Stenhagen 2003]. In contrast to *C. creticum*, *C. italicum* have not fatty acids in hexane extract.

Antibacterial and antifungal potential of *C. creticum* and *C. italicum*. The antibacterial and antifungal activity of *C. creticum* hexane extract as summarized at Table 4 and 5. As shown Table 4, *C. creticum* exhibited antibacterial activity against all bacterial strains. The antibacterial activity of *C. creticum* on *B. subtilis*, E. coli and *P. aeruginosa* was found similar with a MIC level of 31.25 mg/ml and inhibition effects were determined higher than *C. creticum* hexane extract the other bacterial strains. *S. aureus* and *S. typhimurium* seems more resistant than all other bacterial strains according our results. On the other hand, *C. italicum* exhibited antibacterial activity only two bacterial strains which were *E. coli* and *B. subtilis*. The highest inhibition effect of *C. italicum* was found on *Bacillus subtilis* (NRRL NRS-744) with a MIC level of 22.56 mg/ml (Tab. 4). Nevertheless, the antibacterial activity of *C. italicum* hexane extract higher than *C. creticum* against *Bacillus subtilis* at seems from Table 4.

For *C. creticum* and C. *italicum* extracts, the results established that the obtained extracts was unable to inhibit the grown of tested fungal strains. The highest inhibition effect of *C. creticum* was found against A. *fumigatus* with a MIC level of 0.98 mg/ml. *C. creticum* also showed higher inhibitory effect against *P. chrysogenum* than other fungal strains. On the other hand, the highest inhibitory effect of *C. italicum* was found on *C. krusei* with a MIC level of 88.19 mg/ml (Tab. 5). When compared two species with respect to antifungal activity, it seems that *C. creticum* extract more effective than *C. italicum* against selected fungal strains.

CONCLUSIONS

The aim of the present paper was to analyze compounds of hexane extracts of *C. creticum* and *C. italicum* and to determine their antibacterial and antifungal activities. This is the first investigation on compounds of hexane extracts of *C. creticum* and *C. italicum* and their antifungal and antibacterial activities.

The chemical class distributions of the two *Cirsium* species are reported Table 3. According to chemical class distributions, the compounds were separated into nine classes, terpenoids, hydrocarbons, esters, fatty acids, phenolic compounds-flavonoids, carbonyl compounds, alcohols, sterols, and other functional groups. On the whole, terpenoids (70.95 %) constituted the main fractions of *C. italicum* (Savi) DC. while hydrocarbons (41.11 %) constituted the main fractions of *C. creticum* (Lam.) d'Urv. subsp. in hexane extracts.

The antimicrobial activity results exhibited that *C. creticum* extracts has much more effective against all tested microorganisms except *B. subtilis*. While *C. creticum* showed inhibitory effect against all bacterial strains, the highest antibacterial activity was found on *B. subtilis* for *C. italicum*. On the other hand, the highest antifungal activity determined against *P. chrysogenum* for *C. creticum* and against *C. krusei* for *C. italicum.*

In conclusion, the hexane extracts of *C. creticum*

and *C. italicum* are rich terpenoids, hydrocarbons, esters and fatty acids. In this study the composition and its variation were explored for *C. creticum* and *C. italicum* in hexane extracts. As a conclusion, this study revealed that the differences between two wild *Cirsium* species in respect to volatiles and antimicrobial activity. It should be noted that these two *Cirsium* species from obtained location are examined for the first time for constituents in the hexane extracts and antimicrobial activities.

GC-MS analysis of hexane extracts of *C. creticum* and *C. italicum* revelated the existence of various pharmaceutically important chemical compounds with different chemical structure. The presence of various bioactive compounds confirm the application of this plants for various ailments by traditional practitioners. Further studies are needed on these extracts in order to isolate, identify, characterize and illustrate the structure of these compounds to elucidate their exact mechanism of action against various disorders.

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