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Chemical Composition and Antimicrobial Activity of Endemic *Onopordum caricum*

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Abstract: The antimicrobial activity of the hexane, chloroform, ethyl acetate and ethanol extracts of the aerial parts of *O. caricum* Hub.-Mor. (*Asteraceae*) was evaluated against microorganisms including multi-resistant bacteria using a paper disc diffusion method. The chemical composition of the chloroform extract of the plant was determined by Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS). The ethanol and chloroform extracts exhibited significant antibacterial activity The compounds of the chloroform extract were betuline (Lup-20(29)-ene-3β,28-diol), (3α)-12-oleanen-3-yl-acetate, 3β-lup-20(29)-en-3-ol (lupeol), β-amyrin (3β-olean-12-en-3-ol) and 6,10,14-trimethyl-2-pentadecanone (hexahydrofarnesyl acetone). It has become clear that chloroform and ethanol extracts of *O. caricum* has a potential to inhibit the growth of multi-resistant *S. maltophilia*, *S. aureus* and some staphylococci. Hence, the extracts of *O. caricum* may be useful as alternative antimicrobial agents for multi-antibiotic resistant bacteria.

Key words: Antimicrobial activity · Chemical composition · *Onopordum caricum*

INTRODUCTION

Over the years, antibiotic use led to the emergence of infectious bacteria that are resistant to one or more antibiotics. As a result, there are strains of bacteria today for which only one effective drug treatment is available and in some cases, there are no treatments available [1]. For this reason, there is an increasing interest in medicinal plants as an alternative to synthetic drugs, particularly against microbial agents because of the growth of antibiotic resistance [2].

Some species of *Onopordum* are used in traditional medicine. For example, the fruits of *O. tauricum* are saled in herb and spice seller as "devedikeni tohumu" and used to treat liver diseases. The flowering branches of *O. acanthium* are used as diuretic and antipyretic and the roots are used as diuretic, antipyretic, stomachache and appetizing [3]. Several species of the *Onopordum* exhibit antimicrobial [4] and antioxidant activity [5].

O. caricum is a local endemic to Sandras mountain (Mugla-Turkey)[6]. There are no previous studies on the antimicrobial activity and chemical composition of *O. caricum*. Also, there is no information about its traditional uses.

The aim of this study was to identify the chemical composition and evaluate the antimicrobial activity of several extracts of *O. caricum* against different microorganisms including multi-resistant bacteria.

MATERIALS AND METHODS

Plant Material: *O. caricum* naturally growing plants belonging to *Asteraceae* were collected at the flowering stage from Mugla, Turkey. A voucher specimen (Herbarium No: O.V. 5556) has been deposited in the Herbarium of Faculty of Arts and Science, University of Mugla, Turkey. The plant was identified immediately after collection and air-dried at room temperature for later analysis.

Preparation of *O. caricum* Crude Extracts: The air dried and powdered aerial parts of *O. caricum* were extracted successively with hexane, chloroform, ethyl acetate and ethanol in a soxhlet apparatus until the last portion of the extract became colorless. Solvents of all the extracts were removed under low vacuum by using rotary evaporation. Crude extracts were maintained at +4°C until used.

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Test Microorganisms: Three Gram-negative standard test bacteria: *Enterobacter aerogenes* RSKK 720, *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922; 4 Gram-positive standard test bacteria: *Micrococcus luteus* NRRL B-4375, *Bacillus subtilis* ATCC 6633, *Streptococcus mutans* CNCTC 8/77 and *S. aureus* ATCC 25923; 2 yeasts: *Candida albicans* ATCC 10239 and *Candida tropicalis* RSKK 665 and multi-resistant strains of *S. maltophilia* and various species of *Staphylococcus* were used. The strains of MU coded (the multi-resistant bacteria) were obtained from Mugla University Culture Collection.

Antimicrobial Assay: The antimicrobial activity of *O. caricum* crude extracts was tested using the disc diffusion method [7-9] with bacterial cell suspension concentration equilibrated to a 0.5 McFarland standard. A 100 µL of each bacterial suspension was spread on a Mueller-Hinton agar plate. Sterile paper discs (6 mm diameter) were impregnated with 20 µL of each extract dissolved in the solvent used for extraction at 25 mg/mL. The discs were allowed to dry and then placed on the inoculated agar. The plates were incubated at appropriate temperature and time for microorganisms. Discs of hexane, chloroform, ethyl alcohol and ethyl acetate were used as controls. After incubation time, zone of inhibition was measured. The experiment was performed in triplicate.

Column Chromatography (CC): Firstly, CC was performed to highlight the structure of the active extract. For CC, silica-gel 60 (70-230 mesh) as adsorbent in a column with 2x80 cm measurements was used and mobile phases were respectively 95:5, 90:10 and 85:15 hexane: acetone systems [10]. The active fraction was evaporated to dryness. About 5 mg of the residue was mixed with 50 ml of dry pyridine and 75 ml bis(trimethylsilyl)trifluoracetamide, heated at 80 °C for 20 min and analyzed by GC-MS.

Gas Chromatography (GC) and GC-MS Analysis: GC and GC/MS analyses were performed under the experimental conditions as reported earlier [10]. Identification of the components was based on GC retention indices and computer matching of MS with those of standards (NIST and Wiley and a personal library of 320 spectra), as well as by comparison with the fragmentation patterns of MS reported in the literature [11] and, whenever possible, by coinjection with authentic compounds.

RESULTS AND DISCUSSION

The antimicrobial activity of the extracts of *O. caricum* were tested *in vitro* by using the paper disc diffusion method against the microorganisms. If the extracts had any effect on the Gram-negative and Gram-positive test bacteria, its antibacterial activity was evaluated on the multi-resistant strains of *S. maltophilia* and various species of *Staphylococcus*. The results of the antimicrobial activity of the extracts are shown in Table 1.

Since ethyl acetate extract of *O. caricum* did not have any effect on Gram-negative test bacteria, its antibacterial activity was not determined on antibiotic resistant strains of *S. maltophilia*. The hexane extract didn't show inhibition effect on Gram-negative bacteria, except *P. aeruginosa*. The ethanol extract had antimicrobial effect on many of Gram-negative bacteria and the inhibition zones ranged between 9-23 mm. Its maximum activity was on *S. maltophilia* MU 64 and MU 99. The extract inhibited all the multi-resistant strains of *S. maltophilia*, except *S. maltophilia* MU 136.

The chloroform extract inhibited all of the multi-resistant Staphylococci, except *Staphylococcus* sp. MU28 and the inhibition zones ranged between 9-20 mm. The ethanol and ethyl acetate extracts inhibited the growth of most of the multi-resistant Staphylococci, except *S. capitis* MU27 and *Staphylococcus* sp. MU28.

Staphylococci are among the most commonly encountered pathogens in clinical practice. *S. aureus* is a major cause of nosocomial infections, food poisoning, osteomyelitis, pyoarthritis, endocarditis, toxic shock syndrome and a broad spectrum of other disorders [12, 13].

In this study, all strains of multi-resistant *S. maltophilia* tested were inhibited by the ethanol extract, except *S. maltophilia* MU 136 and also most of these strains were inhibited by the chloroform extract.

The ethanol and chloroform extracts had shown strong activities against *S. maltophilia*, *S. aureus* and *Staphylococcus* sp. which were resistant to various antibiotics. Multidrug resistance is common and increasing among Gram-negative nonfermenters and a number of strains have now been identified to exhibit resistance to essentially all commonly used antibiotics, including antipseudomonal penicillins and cephalosporins, aminoglycosides, tetracyclines, fluoroquinolones, trimethoprim-sulfamethoxazole and carbapenems [14]. *S. maltophilia* is one of the important

Table 1: Antimicrobial activity of *O. caricum* extracts

	Inhibition zone (mm)			
Strains	Hexane extract	Chloroform extract	Ethanol extract	Ethyl acetate extract
E. aerogenes RSKK 720	-	-	13	-
P. aeruginosa ATCC 27853	13	15	9	-
E. coli ATCC 25922	-	12	11	-
S. maltophila MU 23	-	15	12	NT
S. maltophila MU 25	-	-	16	NT
S. maltophila MU 52	-	8	18	NT
S. maltophila MU 53	-	15	15	NT
S. maltophila MU 63	-	-	17	NT
S. maltophila MU 64	-	13	22	NT
S. maltophila MU 69	-	9	19	NT
S. maltophila MU 94	-	12	20	NT
S. maltophila MU 99	-	16	23	NT
S. maltophila MU 136	-	-	-	NT
S. maltophila MU 137	-	-	18	NT
M. luteus NRRL B-23	11	19	15	-
B. subtilis ATCC 6633	9	19	-	8
S. aureus ATCC 25923	-	12	13	10
S. mutans CNCTC 8/77	-	-	-	-
S. capitis MU 27	-	14	-	-
Staphyl. sp. MU 28	-	-	-	-
S. epidermidis MU 30	-	11	19	9
S. xylosus MU 34	-	13	15	11
S. xylosus MU 35	-	15	14	10
S. xylosus MU 37	-	19	15	11
S. aureus MU 38	-	20	15	11
S. aureus MU 40	-	13	16	10
S. xylosus MU 42	-	11	15	9
S. lentus MU 43	-	13	15	10
S. aureus MU 46	-	15	16	9
C. albicans ATCC 10239	-	-	-	-
C. tropicalis RSKK 665	10	-	-	13

(-): No activity, NT: No studied

Table 2: Chemical composition of chloroform extract of *O. caricum*

	Compounds	%	Method
1	Hexahydrofarnesyl acetone (6,10,14-trimethyl-2-pentadecanone)	8.78	GC, GC-MS
2	Betuline (Lup-20(29)-ene-3β,28-diol)	18.02	GC-MS
3	(3α)-12-Oleanen-3-yl-acetate	20.32	GC-MS
4	Lupeol (3β-Lup-20(29)-en-3-ol)	23.85	GC-MS
5	β-Amyrin (3β-olean-12-en-3-ol)	17.56	GC-MS
	Total	88.53	

members of this group. *S. maltophilia* has received much attention in the last decade because of its role as a pathogenic microorganism in an increasing number of clinical syndromes [15], such as bacteremia, infections of the respiratory and urinary tracts, skin and soft tissue infections, biliary tract infection, meningitis, serious wound infections, conjunctivitis and endocarditis [16]. The treatment of infections caused by this microorganism is difficult because *S. maltophilia* is frequently resistant to most of the widely used antibiotics [17]. There are possible alternative therapies for these strains by using *O. caricum*. Such therapies are very important because these of organisms are difficult to treatment and often require alternative therapy.

The hexane and ethyl acetate extracts inhibited the growth of *C. tropicalis* and the inhibition zones were 10 mm and 13 mm, respectively. Other extract did not show anticandidal activity.

There are no previous studies on the antimicrobial activity of *O. caricum*. Although there are some studies on the antimicrobial activity of other *Onopordum* species. For example, the antimicrobial activities of the water and methanol extracts of *Onopordum cynarocephalum* [18]; the antimycobacterial properties of *Onopordum anatolicum* was determined [19].

The identified compounds and their percentages are listed according to their elution order on SE-52. More than 88% of the chloroform extract of bio-active fraction was identified. Total of 5 compounds were detected in the chloroform extract, which was rendered bio-active by using GC and GC/MS methods. As can be seen from Table 2, the main constituents are triterpenes. Five detected compounds were identified by using reference substances and NIST 2005, Wiley Library and special library established in our laboratory.

The components of the chloroform extract were separated into sesquiterpene and triterpene classes, including hexahydrofarnesyl acetone (8.78%), betuline (18.02%), lupeol (23.85%), β-amyrin (17.56%) and (3α)-12-oleanen-3-yl-acetate (20.32%).

There are no previous studies on the chemical composition of *O. caricum*. Although there are some studies of the metabolites from *Onopordum illyricum* [20] and *Onopordum acanthium* [21].

CONCLUSION

This study, is the first document on the *in vitro* antimicrobial features and chemical composition of *O. caricum*. In this study, it has become clear that chloroform and ethanol extracts of *O. caricum* has a great potential to inhibit the growth of multi-resistant *S. maltophilia*, *S. aureus* and some staphylococci. Hence, the extracts of *O. caricum* may be useful as alternative antimicrobial agents for multi-antibiotic resistant bacteria.

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