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Research Article

Antimicrobial Activity of Various Extracts of *Centaurea balsamita* Lam. And *Centaurea coronopifolia* Lam.

Nuray YILDIRIM[1*], Mehtap AKIN[2], Hatice TANER SARAÇOĞLU[2]

Article Info

**Corresponding author*:

e-mail: nurayyildirim@comu.edu.tr

Institution: [1] Çanakkale Onsekiz Mart Üniversitesi Sağlık Hizmetleri Meslek Yüksekokulu

[2] Selçuk Üniversitesi, Fen Fakültesi, Biyoloji Bölümü

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ABSTRACT

Members of Centaurea have been widely used to treat various diseases in Turkish folk medicine. The antimicrobial activities of ethanol, acetone, ethyl acetate and chloroform extracts from *Centaurea balsamita* Lam. and *Centaurea coronopifolia* Lam. species were evaluated against some bacteria (*Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Streptococcus salivarius*) by broth microdilutation method. All the extracts exhibited antimicrobial activity of different range against the tested bacteria. The chloroform extract of *C. balsamita* was the most effective against *B. cereus* strain (0,039 µg/mL). Also, all the extracts tested against the microorganisms had strong antimicrobial activity against *S. aureus* strains (20 µg/mL), except the ethanol extracts which had lower activity.

Centaurea balsamita Lam. ve *Centaurea coronopifolia* Lam. Türlerine Ait Farklı Ekstraktların Antimikrobiyal Aktivitesi

ÖZET

Centaurea türleri Türk geleneksel tıbbında çeşitli hastalıkların tedavisinde yaygın olarak kullanılmaktadır. Bu araştırma kapsamında, *Centaurea balsamita* Lam. and *Centaurea coronopifolia* Lam. türlerinin etanol, aseton, etil asetat ve kloroform ile hazırlanan ekstraktlarının bazı bakterilere karşı (*Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, , *Salmonella typhimurium*, *Streptococcus salivarius*) antimikrobiyal aktiviteleri broth mikrodilüsyon yöntemi ile araştırılmıştır. Bitki ekstraktları test edilen mikrooganizmalara karşı farklı aralıklarda antimikrobiyal etki göstermiştir. Tüm ekstraktlar içinde *C. balsamita*'nın kloroform ekstresi *B. cereus*'a karşı en yüksek antimikrobiyal aktiviteyi göstermiştir (0,039 µg/mL). Ayrıca, etanol ekstraktı dışında tüm ekstraktlar *S. aureus* suşlarına karşı yüksek antimikrobiyal aktivite göstermiştir (20 µg/mL).

1. INTRODUCTION

Plant-based medicine has been used from the ancient times, which remains today [1]. Use of plant-based medicine is increasing overtime, accordingly studies on the therapeutic effects of these plants gain more importance [2, 3]. Previous studies have shown

that many medicinal plant extracts contain robust antimicrobial agents against a wide variety of pathological microorganisms [4, 5].

Centaurea is a medicinal herb from the Asteraceae family, which is particularly important as they are used to obtain bioactive compounds [6, 7]. The genus Centaurea has 500-600 species worldwide; widespread especially throughout the Mediterranean and western Asia. The genus is represented by 179 species, 111 of which are endemic, in flora of Turkey [8, 9]. Different species of Centaurea are named as peygamber çiçeği, timur dikeni, and zerdali dikeni in Anatolia [10]. Many Centaurea species have been used conventionally to treat diverse diseases such as abscess, common cold, hemorrhoids, peptic ulcers, malaria and herpes infections [11–13]. Many antimicrobial activity studies have been conducted on the genus and they have yielded promising results [14, 15]. Considering the results obtained from the studies in the literature, we can conclude that further research on Centaurea species is required. These studies are important in terms of contributing to the discovery of new drugs [16].

The widespread and indiscriminate usage of antibiotics has caused to arise of antibiotic resistance and rendered many existing drugs ineffective. Antibiotic resistance has become a prominent problem to public health and is considered by the World Health Organisation (WHO) as one of the most pressing problems medical science facing. This outcome has led the researchers to explore for new antimicrobial agents. As the antimicrobial effects of many herbs are still not discovered, researches on the discovery of natural antimicrobial agents are increasing [17–19].

In the light of the information given above, this study was conducted to investigate the antimicrobial activity of chloroform, ethyl acetate, acetone and ethanol extracts of C. balsamita Lam. and C. coronopifolia Lam. against different microorganisms by using broth microdilution assay. These species were choosed because Centaurea species are videly used for divers medicinal purposes, including as antimicrobials and their antimicrobial properties against selected bacteria have not yet been determined.

2. MATERYAL VE METOD

2.1. Plant Materials

Samples of *Centaurea balsamita* Lam. were gathered from Konya, Turkey and *Centaurea coronopifolia* Lam. were collected from Çankırı, Turkey in May and June. The plant samples were identified by Dr. Osman Tugay and Dr. Tuna Uysal from the Division of Botany, Department of Biology, Faculty of Science, Selçuk University. Voucher specimens were deposited with the collector numbers of *C. balsamita* NY-1201 and *C. coronopifolia* OT-2712-TU in the KNYA Herbarium at the Department of Biology, Selçuk University.

2.2. Preparation of The Plant Extracts

Tha aerial parts of the air-dried plant samples were finely ground in the aseptic conditions with the help of a laboratory mill. 20 g of ground samples were extracted with 200 mL of the solvent (chloroform, ethyl acetate, acetone and ethanol) for 8 h with a Soxhlet equipment. The extracts were filtered through a Whatman filter paper (No:1) and the solvent was evaporated under 40 °C by using a Rotary evaporator (Heidolph 4000). The residues were dissolved in DMSO and stored in a refrigerator at 4 °C until further analysis.

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2.3. Microorganisms

Antimicrobial activity of the samples was studied against eight bacteria strains. *Bacillus cereus* ATCC 14579, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 29988, *Escherichia coli* O157 ATCC 35190, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 6538, *Salmonella typhimurium* ATCC 14028, *Streptococcus salivarius* RSHE 606.

2.4. Antimicrobial Activity

The antimicrobial activity of the plant extracts was investigated by broth microdilution method according to principles and procedures of the Clinical Laboratory Standards Institute (CLSI) [20]. Briefly, 100 µL of Mueller Hinton Broth was added to the 96-well microplate. Two-fold dilutions of the plant extracts were dispensed into the wells. Inoculum suspensions of the bacteria strains were set to 0,5 McFarland turbidity. 100 µL of the inoculum was added to all wells, except the negative control. After the addition of the inoculum, each well contained $5x10^5$ CFU/mL bacterial concentration at the final. Then, the plates were left for incubation for 16-20 h at 37 °C. The lowest concentration of the extract that inhibited bacterial growth was evaluated as minimum inhibitory concentration (MIC).

3. RESULTS AND DISCUSSION

In determining the antimicrobial properties of a plant extract, the available screening methods can be grouped as diffusion and dilution methods. Diffusion methods are considered qualitative assays, while dilution methods are quantitative since they determine the minimal inhibitory concentration [5, 21]. Medicinal plant extracts may not diffuse properly due to the non-polar substances in their content. Therefore, diffusion methods are not suitable, as they may give false results [22]. Accordingly, the antimicrobial activity of the plant extracts was investigated using the broth microdilution method according to the CLSI procedures [20]. Lowest concentration of the plant extract that inhibited the visible growth of bacteria was evaluated as MIC value.

The antimicrobial activity of the extracts was screened in the concentration range of 160-0,3125 µg/mL. Gentamicin and the extracts which were effective were tested at lower concentrations in the range of 160-0,0003 µg/mL. Average data from the three replicated experiments of the *C. balsamita* extracts are presented in Table 1 and *C. coronopifolia* extracts in Table 2.

Table 1. Antimicrobial activity of *Centaurea balsamita* extracts (µg/mL)

	Chloroform	Ethyl acetate	Acetone	Ethanol	Gentamicin
B. cereus ATCC 14579	0,039	40	80	80	0,313
B. subtilis ATCC 6633	160	160	160	160	0,002
E. coli ATCC 29988	160	160	160	160	0,625
E. coli O157 ATCC 35190	160	160	160	160	0,313
S. aureus ATCC 25923	20	20	20	160	0,005
S. aureus ATCC 6538	20	20	20	160	0,039
S. typhimurium ATCC 14028	80	160	160	160	0,625
S. salivarius RSHE 606	80	160	160	160	1,25

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Table 2. Antimicrobial activity of *Centaurea coronopifolia* extracts (µg/mL)

	Chloroform	Ethyl acetate	Acetone	Ethanol	Gentamicin
B. cereus ATCC 14579	40	80	40	40	0,313
B. subtilis ATCC 6633	160	160	160	160	0,002
E. coli ATCC 29988	160	160	160	160	0,625
E.a coli O157 ATCC 35190	160	160	160	160	0,313
S.s aureus ATCC 25923	20	20	20	>160	0,005
S. aureus ATCC 6538	20	20	20	>160	0,039
Sa.a typhimurium ATCC 14028	160	160	160	160	0,625
S. salivarius RSHE 606	160	160	160	160	1,25

Although different extracts of the two *Centaurea* species used in our study mostly showed similar antimicrobial activity, some extracts were significantly effective against the test microorganisms. Chloroform extract of *C. balsamita* was found to be the most effective extract against the tested bacteria between the concentrations of 160-0,039 µg/mL. The extract showed its strongest activity against *B. cereus* (0,039 µg/mL) which was more effective than positive control gentamicin (0,313 µg/mL).

Chloroform, ethyl acetate and acetone extracts of both plants had strong antimicrobial activity against *S. aureus* strains (20 µg/mL), while ethanol extract was not effective in the concentration range tested. Tekeli et al. [5] investigated the antibacterial activities of *Centaurea* species and *C. solstitialis* subsp. *solstitialis* had the highest activity against *S. aureus* (0,5 mg/mL). Güven et al. [11], found significant antimicrobial activity of ethyl acetate extract of *C. amonicola* against *S. aureus* (62,5 µg/mL).

All extracts of *C. balsamita* and *C. coronopifolia* demonstrated moderate activity against *B. subtilis, E. Coli, S. typhimurium* and *S. salivarius* (160 µg/mL), except chloroform extract of *C. balsamita* which was more effective against *S. typhimurium* and *S. salivarius* (80 µg/mL). Cansaran et al. [23] studied the antimicrobial activities of *C. cankiriense* extracts and they found that *E. coli* was the most resistant strain to the plant extracts. The strongest effect was against *B. Cereus* with 7,8 µg/m MIC value.

This study provides evidence that *C. balsamita* and *C. coronopifolia* have antimicrobial effects on the tested bacteria. Many authors consider the extracts that have lower MIC value than 0,1 mg/mL have a potential as new antibiotics [24]. Both plants showed strong antimicrobial affect against *B. cereus* and *S. aureus* strains; especially chloroform extract of *C. balsamita* had the strongest antimicrobial effect against *B. cereus*, which was more effective than the positive control gentamicin. *B. cereus* is best known to cause food born diseases, in addition to that it is increasingly reported as an opportunistic pathogen in certain high-risk groups [25, 26]. Results show that the chloroform extract of *C. balsamita* has a potential to be used as an antibiotic agent against *B. cereus. S. aureus* is a leading opportunistic human pathogen and causes a wide variety of clinical diseases, which can be life-threatening [27]. Infections caused by *S. aureus* can be acquired in both hospital and community. Treatment of these infections is also challenging because of the strains that develop multidrug resistance, such as Methicillin-Resistant *Staphylococcus aureus* (MRSA) [28]. Considering the antibiotic drug resistance, *S. aureus* is perhaps the greatest concern because it causes diverse life-threatening illnesses and it can adopt to different environmental conditions [29]. In this aspect, this study shows promising results.

Previous studies report that sesquiterpene lactones, flavonoids and phenolic compounds have been isolated from the *Centaurea* species [30]. These investigations demonstrated that their high content of sesquiterpen lactones is mainly responsible for their antimicrobial activities [7]. The best extraction of sesquiterpen lactones can be achieved with chloroform extraction [31] which may be the reason for higher activity of the extract [19, 32].

3. CONCLUSION

In summary, plant-derived drugs have been used over the years to prevent and cure serious diseases [33]. Researching of medicinal plants as antimicrobial agents is gaining more importance due to increasing resistance to present antimicrobial drugs. Our study showed that both *C. balsamita* and C. coronopifolia have significant antimicrobial activity, especially against *B. cereus* and *S. aureus*. Results indicate that they have a potential to be used as antimicrobial agents. However, more research should be carried out to determine the compounds responsible for the antimicrobial activity, mechanisms of action, or pharmacokinetics of the extracts.

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The Declaration of Conflict of Interest

No conflict of interest or common interest has been declared by the authors. “No conflict of interest or common interest has been declared by the authors.”

Author’s Contribution

The first author contributed 65%, the second author 25%, the third author 10%.

The Declaration of Ethics Committee Approval

This study does not require ethics committee permission or any special permission.

The Declaration of Research And Publication Ethics

The authors of the paper declare that they comply with the scientific, ethical and quotation rules of ETOXEC in all processes of the paper and that they do not make any falsification on the data collected. In addition, they declare that Environmental Toxicology and Ecology and its editorial board have no responsibility for any ethical violations that may be encountered, and that this study has not been evaluated in any academic publication environment other than Environmental Toxicology and Ecology

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