

## ANTIBACTERIAL ACTIVITIES OF EXTRACTS FROM TWELVE *CENTAUREA* SPECIES FROM TURKEY

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**Abstract** - Members of the genus *Centaurea* (Asteraceae) have been used in traditional plant-based medicine. The methanol extracts of twelve *Centaurea* species, of which five are endemic to Turkey flora, were screened for antibacterial activity against four bacteria (*Escherichia coli*, *Bacillus cereus*, *Salmonella enteritidis*, *Staphylococcus aureus*). The antibacterial activity was evaluated by the microdilution method and the minimum inhibition concentrations (MIC) of the extracts were determined. *C. cariensis* subsp. *microlepis* exhibited an antimicrobial effect on all tested microorganisms. The extracts from eight *Centaurea* species (*C. balsamita*, *C. calolepis*, *C. cariensis* subsp. *maculiceps*, *C. cariensis* subsp. *microlepis*, *C. kotschyi* var. *kotschyi*, *C. solstitialis* subsp. *solstitialis*, *C. urvillei* subsp. *urvillei* and *C. virgata*) possessed antibacterial activity against several of the tested microorganisms.

**Key words:** Medicinal plants, endemic, methanolic extract, antibacterial activity

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### INTRODUCTION

Plant-derived drugs remain an important resource, especially in developing countries, to combat serious diseases. Approximately 60-80% of the world's population still relies on traditional medicines for the treatment of common illness (WHO, 2002; Zhang, 2004). Plants contain several compounds that have potent biological activity (Mokbel and Hashinaga, 2006). For example, phenolic compounds and essential oil play a vital role as powerful natural biological agent (Cutter, 2000; Hao et al., 1998; Lis-Balchin and Deans, 1997; Puupponen-Pimia et al., 2001). Antibiotic use has led to the emergence of infectious bacteria that are resistant to one or more antibiotics (Falagas and Bliziotis, 2007). The situation has brought about the failure of the treatments of many

microbial diseases. A number of previous investigations have indicated that many medicinal plant extracts constitute a class of potent natural antimicrobial agent (Rayne and Mazza, 2007; Sufferidini et al., 2004). Therefore, many researchers have focused on the investigation of aromatic and medicinal plants as a source of new antimicrobial substances (Recio and Rios, 1989; Silver and Bostian, 1993).

Turkey's flora is comprised of over 3000 aromatic plant species (Baser, 2002), but there is no detailed information about most of these species. The Asteraceae plant family has ±2500 species and 1500 genera in the world (Wagstaff and Breitwieser, 2002). Some Asteraceae species have been used in many fields, including nutrition and medicinal industries (Roig, 1965). The genus *Centaurea* comprises more

than 500-600 species that are widespread all over the world, in particular around the Mediterranean and western Asia area (Mabberley, 1997); 179 species of the genus *Centaurea* are found in Turkey, among which 109 species are endemic (Davis et al., 1988; Guner et al., 2000). Some members of the genus are known by various local names such as “peygamber çiceği”, “zerdali diken”, “çoban kaldiran”, “Timur diken” and “boga diken” in Anatolia (Baytop, 1999; Sezik et al., 2001; Wagenitz, 1975). Many *Centaurea* species have long been used in traditional medicine to cure various ailments, such as hemorrhoids, abscess and the common cold (Baytop, 1999; Kargıoglu et al., 2008; Kargıoglu et al., 2010; Sezik et al., 2001).

Due to the above-mentioned pharmacological importance of *Centaurea* species; it is worthwhile to determine the antibacterial properties of *Centaurea*. Recently, some *Centaurea* species were examined for biological properties such as antioxidant capacity, antimicrobial activity and essential oil composition. The aim of this study was to determine the antibacterial activity of twelve *Centaurea* species growing in Turkey's flora.

## MATERIALS AND METHODS

### *Plant materials*

Samples of *Centaurea* species (*C. balsamita* Lam., *C. calolepis* Boiss., *C. carduiiformis* DC. subsp. *carduiiformis*, *C. cariensis* Boiss. subsp. *maculiceps*, *C. cariensis* Boiss. subsp. *microlepis*, *C. iberica*, *C. kotschy* (Boiss. et Heldr.) Hayek var. *kotschy* (Boiss. et Heldr.), *C. pterocaula*, *C. solstitialis* L. subsp. *solstitialis* L., *C. triumfettii* All., *C. urvillei* DC. subsp. *urvillei* DC. and *C. virgata* Lam.) were collected in May and June from Konya, Turkey. The plants were identified by Dr. Tuna Uysal from the Section of Botany, Department of Biology, Faculty of Science, Selcuk University. Five species (*C. calolepis* Boiss., *C. carduiiformis* DC. subsp. *carduiiformis*, *C. cariensis* Boiss. subsp. *maculiceps*, *C. cariensis* Boiss. subsp. *microlepis* and *C. kotschy* (Boiss. et Heldr.) Hayek var. *kotschy* (Boiss. et Heldr.)) are endemic to Turkey flora. The voucher specimens were deposited in the

KNYA herbarium at Department of Biology, Selcuk University.

### *Preparation of methanolic extracts*

Air-dried plant material was finely powdered using a laboratory mill. 15 g of each sample was exhaustively extracted with 300 ml methanol at room temperature under stirring and the extracts were filtered through a Whatman blue filter. After evaporation of the solvent at 40°C in rotary evaporator, the residues were stored at 4°C until further analysis.

### *Microorganisms*

The antibacterial activities of the *Centaurea* species were assessed against four bacteria species: *Escherichia coli* ATCC 25922, *Bacillus cereus* ATCC 14579, *Salmonella enteritidis* ATCC 13076 and *Staphylococcus aureus* ATCC 25923. Gentamicin was used as a positive control.

### *Microdilution method*

Minimum inhibition concentration (MIC) values were determined using 96-well microtiter plates by dissolving the sample in DMSO. Serial dilutions were made to obtain concentrations ranging from 8 to 0.0625 mg/ml. Suspensions of standard microorganisms were inoculated onto the microplates. The growth of the microorganisms was observed by using a microplate photometer (Thermo Scientific Multiskan). The MIC values were defined as the lowest concentrations of the plant extracts to inhibit the growth of microorganisms.

## RESULTS AND DISCUSSION

The available screening methods for the detection of antibacterial potency of plant extracts can be classified as diffusion and dilution methods. The diffusion methods are qualitative methods, while dilution methods are quantitative assays (with minimal inhibition concentration (MIC) (Vanden Berghe and Vlietinck, 1991)). The antibacterial activities of the methanolic extracts of twelve *Centaurea*

**Table 1.** Minimum inhibitory concentrations (MIC) of twelve *Centaurea* extracts (mg/ml)

| Species   | Microorganisms        |                |                  |                  |
|---|-----------------------|----------------|------------------|------------------|
|   | <i>S. enteritidis</i> | <i>E. coli</i> | <i>S. aureus</i> | <i>B. cereus</i> |
| <i>C. balsamita</i>                                 | -                     | 2              | 1                | -                |
| <i>C. calolepis</i>                                 | 2                     | -              | -                | -                |
| <i>C. carduiiformis</i> subsp. <i>carduiiformis</i> | -                     | -              | -                | -                |
| <i>C. cariensis</i> subsp. <i>maculiceps</i>        | -                     | 2              | 2                | 4                |
| <i>C. cariensis</i> subsp. <i>microlepis</i>        | 2                     | 4              | 2                | 4                |
| <i>C. iberica</i>                                   | -                     | -              | -                | -                |
| <i>C. kotschy</i> var. <i>kotschy</i>               | -                     | 2              | -                | -                |
| <i>C. pterocaula</i>                                | -                     | -              | -                | -                |
| <i>C. solstitialis</i> subsp. <i>solstitialis</i>   | -                     | 0.5            | 0.5              | -                |
| <i>C. triumfetti</i>                                | -                     | -              | -                | -                |
| <i>C. urvillei</i> subsp. <i>urvillei</i>           | -                     | 1              | 2                | -                |
| <i>C. virgata</i>                                   | 2                     | 1              | -                | -                |
| Gentamisin  | 0.004                 | 0.004          | 0.001            | 0.004            |

*rea* species were evaluated by using the MIC values in a micro-dilution method. Table 1 displays the *in vitro* antibacterial activities of the *Centaurea* species. The results show that eight plants exhibited antibacterial activity, while four species (*C. carduiiformis* subsp. *carduiiformis*, *C. iberica*, *C. pterocaula* and *C. triumfetti*) did not have any effect on all the tested bacteria. The extract of *C. cariensis* subsp. *microlepis* had an antibacterial effect on all tested microorganisms. *C. cariensis* subsp. *maculiceps* extract inhibited the bacteria with the exception of one bacterium (*S. enteritidis*). *S. enteritidis* was inhibited by three of the studied *Centaurea* species, namely, *C. calolepis*, *C. cariensis* subsp. *microlepis* and *C. virgata*, while seven *Centaurea* species had an antibacterial effect on *E. coli*.

*S. aureus* is the cause of many diseases, such as food poisoning, osteomyelitis, polyarthritis, endocarditis (Hajjeh et al., 1999; Rubin et al., 1999; Willet, 1992). Five studied *Centaurea* species exhibited a strong antibacterial effect on *S. aureus* and *C. solstitialis* subsp. *solstitialis* had the highest activity on the bacteria with a 0.5 mg/ml concentration. Therefore, *C. solstitialis* subsp. *solstitialis* may be used as an antibiotic for *S. aureus* infections. However, a lower concentration of gentamicin (0.001 mg/ml) had a strong antibacterial effect on the bacteria. *Bacillus* species, especially *B. cereus*, are responsible for foodborne diseases (Hillard et al., 2003). *B. cereus* was inhibited by two of the studied *Centaurea* species (*C. cariensis* subsp. *maculiceps* and *C. cariensis* subsp. *microlepis*) with 4 mg/ml concentration.

In a previous report, the antimicrobial activities of *C. calolepis*, *C. urvillei* subsp. *urvillei* were investigated by Karamenderes et al. (2006) who found that the species had an antimicrobial effect on two microorganisms (*Candida krusei* and *Cryptococcus neoformans*). In the present study, *C. calolepis* showed activity on *Salmonella enteritidis*, while *C. urvillei* subsp. *urvillei* was active on *Escherichia coli* and *Staphylococcus aureus*. Cansaran et al. (2010) examined the antimicrobial activity of various extracts of *C. cankiriense*. Both the ethyl acetate and methanol extracts of this species inhibited the growth of 13 bacteria and the MIC values of the ethyl acetate extracts were determined as 250 µg/ml for *E. coli* and 62.5 µg/ml for *S. aureus*. According to the results, all examined *Centaurea* species showed weaker antibacterial activity than the ethyl acetate extract of *C. cankiriense*. *C. bornmuelleri*, *C. hubermorathii* and *C. schiskinii* did not show any effect on *E. coli* and *B. cereus* in the micro-dilution assay (Sarker et al., 2005). However, our results demonstrated that some studied *Centaurea* species were active against *E. coli* and *B. cereus*.

The antibacterial effect of the methanolic extract of dry heads of *C. diffusa* was much lower than some of the studied *Centaurea* species (Skliar et al., 2005). Guven et al. (2005) found a significant antimicrobial effect of five *Centaurea* species (*C. ptosomipappoides*, *C. odyseii*, *C. ptosomipappa*, *C. amonicola* and *C. kurdica*) on some microorganisms, including *S. aureus* and *B. cereus*. Extracts of *C. appendicigera* and *C. helenioides* did not display antimicrobial activity on *E. coli* and the results were reported by Buruk et al. (2006).

Ugur et al. (2010) reported that the chloroform and ethyl alcohol extracts of *C. cariensis* subsp. *niveo-tomentosa* exhibited strong antibacterial activities on many resistant bacteria, especially *Staphylococcus* strains. In a previous study, the methanolic extract of *C. polyclada* had a lower sensitivity than some other studied *Centaurea* species (especially, *C. cariensis* subsp. *maculiceps* and *C. cariensis* subsp. *microlepis*) on *B. cereus* and *S. aureus*

(Granger et al., 2009). The antibacterial effects of some Yemeni medicinal plants were investigated and the methanolic extract of *C. pseudosinaica* displayed a potent antibacterial effect on *S. aureus* (Montana et al., 2010). The methanol extract of *C. ainetensis* flowers showed efficacy against 88% of the tested microorganisms. In the same study, *C. erengoides* exhibited a low antibacterial effect on the microorganisms (Barbour et al., 2004).

Some authors focused on the antimicrobial activity of the essential oil of *Centaurea* species. For example, Yayli et al. (2009) investigated the antimicrobial effect of the essential oils from two *Centaurea* species (*C. appendicigera* and *C. helenioides*) and demonstrated a moderate antibacterial activity on Gram-positive and Gram-negative. Similar results were obtained for the essential oils of *C. sessilis* and *C. armena* (Yayli et al., 2005). The essential oil of *C. aladagensis* had an antibacterial effect against seven human pathogenic microorganisms (Kose et al., 2007) However, the essential oils of *C. nicaeensis* and *C. parlatoris* showed low activity on fourteen selected microorganisms (Senatore et al., 2008).

## CONCLUSIONS

In general, the present study revealed that eight *Centaurea* extracts (*C. balsamita*, *C. calolepis*, *C. cariensis* subsp. *maculiceps*, *C. cariensis* subsp. *microlepis*, *C. kotschy* var. *kotschy*, *C. solstitialis* subsp. *solstitialis*, *C. urvillei* subsp. *urvillei* and *C. virgata*) possess antibacterial activity against several tested microorganisms. Therefore, the extracts can be used as a source of natural antibiotics. Antibiotic-resistant bacteria are a serious problem and these extracts point to potential novel drugs for treating infectious diseases. Further studies should be performed in order to determine the active components of *Centaurea* species.

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