

Free Radical Scavenging Capacity and Antibacterial Activity of Wild *Cirsium creticum* from Turkey

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ABSTRACT

Background: In recent years, there is increasing interest the using of herbal extracts derived from plants in medicine and as a dietary supplements. **Objective:** We aimed the determination of the antioxidant and antibacterial activity of *Cirsium creticum* (Lam) d'Urv. subsp. *creticum* included *Asteraceae* family which is wild plant species in Trakya region. **Methods:** Crude n-hexane, diethyl ether, ethylacetate and methanol extracts from whole plants was used the determination of antiradical and antibacterial activity in *C. creticum* and compared in terms of extracts efficiency. **Results:** EC₅₀ values of DPPH activity in different solvents revealed significant differences similar to TPC content. The lowest inhibitory effect was found to be Gram negative for *E. coli*, despite the highest inhibitory effect against Gram positive *S. aureus*. **Conclusion:** Antiradical and antimicrobial activity results presented that *C. creticum*, could be evaluate in food supplements and pharmaceutical industry as natural compound, and future investigations will be aimed the isolations of biologically active compounds to define individual active components.

Key words: *Cirsium creticum*, Antiradical activity, Antimicrobial activity, *E. coli*, *S. aureus*.

INTRODUCTION

The role of the free radicals and the reactive oxygen species (ROS) in the pathogenesis of human diseases, including cancer, aging, atherosclerosis, neurological damage, has been recognized. Antioxidants, are vital substances which possess the ability to protect the body from possible harms caused by the free radical induced oxidative stress which damage the cells in our bodies.¹ A balance between free radicals and antioxidants is necessary for proper physiological function.² If the free radicals overwhelm the body's ability to regulate them, a condition known as oxidative stress ensues.³

It is known that many taxa, belonging to the genus *Cirsium* Mill. (Koygocuren), have gained a rising interest in scientific area due to some pharmacologic activities. Generally, *Cirsium* seeds, stems and flowers have been

used for the treatment of some illnesses such as varicose veins, haemorrhoids, peptic ulcer, cough and bronchitis and as remedy against mushroom poisoning as traditionally.^{4,6} An ethnobotanical study on useful and edible plants reported that peeling stems of *C. creticum* can be eaten as raw vegetable or cooked.⁷ *C. creticum* (Asteraceae) which is wild plant species in Trakya region of Turkey. In the literature there is no study on the biological activity of *C. creticum* plants. In this study, firstly, total phenolic content, antiradical activity and antimicrobial activity of the *C. creticum* which is wild plant species in Trakya region have been examined. The extracts of the *C. creticum* with high biological activity will be selected for isolation studies. Therefore, this will be a distinctive study in terms of its being the first in the literature.

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MATERIALS & METHODS

Plant material and extractions

C. creticum which is wild plant species in Trakya region, was collected in June 2016. The specimens (NGBB 7230) were identified by Dr. Cabi at Namik Kemal University, Faculty of Science, Department of Biology. Air dried samples were used for *n*-hexane, diethyl ether, EtOAc, MeOH solvent extractions. The extractions were repeated two times, supernatants were combined, and solvents were evaporated under vacuum using a rotary evaporator.

Total phenolics content (TPC)

The total phenolic content (TPC) in plant extracts was determined by a colorimetric method with Folin & Ciocalteu's phenol (FCP) reagent.⁸ Absorbance of supernatants was recorded at 725 nm (Hitachi UV/Vis). Total phenolic content was expressed as mg catechol equivalents (CAE).

Antiradical activity against DPPH

The scavenging effect of free radical scavenging was assayed by DPPH (2,2-diphenyl-1-picrylhydrazyl) method against DPPH radical was monitored at 517 nm as described by Amarowicz *et al.* (2002).⁹

Determination of the antibacterial activity

The antibacterial activity was tested against four bacteria strains, which were *Staphylococcus aureus* (ATCC 43300), *Escherichia coli* (ATCC 35218), *Bacillus subtilis* (NRRL NRS-744) and *Pseudomonas aeruginosa* (ATCC 27853). Bacterial suspensions in the logarithmic growth phase were diluted according to the McFarland 0.5 turbidity standard in Mueller-Hinton Broth and then 100 μ l from this suspension spread to agar plate surface plate. Plant extracts were solved in 1 ml water and 100 μ l added to each well which were opened on the surface of the agar. Gram positive bacteria Penicillin (10IU/ml) and for the other bacteria Gentamicin (10 μ g/ml) are tested as control. Zone diameters are measured after 37°C incubation.

RESULTS & DISCUSSION

Total phenolic content (TPC)

TPC of *C. creticum* extracts ranged between 1.33 mg CAE/g and 47.90 mg CAE/g extract. The highest TPC value was found in methanolic extract of *C. creticum* (47.90 mg CAE/g) (Table 1). Our findings was similar to Nazaruk *et al.* (2008)¹⁰ results, who determined the different *Cirsium* species TPC between 43.73 and 178.27 mg/g. As shown in Figure 1, DPPH radical scavenging activities of extracts were concentration dependent

Table 1: Total Phenolic Content and EC₅₀ values of DPPH activity of *C. creticum* extracts

Extract	Total Phenolic content (μ g catechol Eq / mg extract)	EC ₅₀ values of DPPH activity
MeOH	47.90 \pm 0.49	0.24 mg/assay
EtOAc	15.29 \pm 1.82	1.42 mg/assay
Diethyl Ether	19.55 \pm 0.16	1.71 mg/assay
n-hexane	1.33 \pm 0.04	2.05 mg/assay

*The EC₅₀ value, defined as the amount of antioxidant necessary to inactivate 50% of initial DPPH•, was estimated based on the plot.

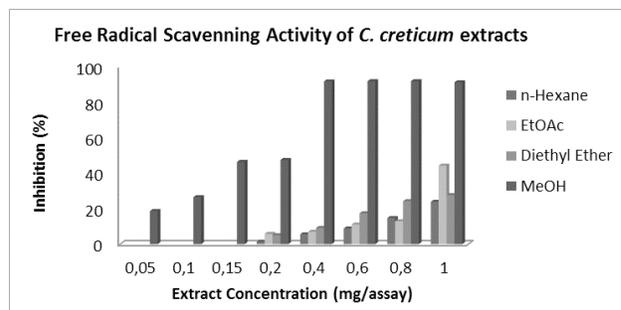


Figure 1: DPPH radical scavenging activity of *C. creticum* extracts.

and methanol extracts showed the highest activities (91.65%). DPPH activities of hexane, ethyl acetate, diethyl ether and extracts was determined as 5.53 %, 6.93 %, 9.11 % at the same concentration respectively. EC₅₀ values of DPPH activity in different solvents revealed significant differences similar to TPC content. This could be due to the differences in extractability of different solvents and it may be due to poor solubility of phenolics of this plant in hexane. These results in confirmed with the earlier researches that reported the solvent with methanolic extract showing relatively higher antioxidant ability.¹¹ This could probably be because of their higher polarity and better solubility for phenolic components present in plant materials.

Antibacterial Activity

In recent years, many types of microorganisms with active infectious diseases have become resistant to antibiotics and also, for most of uses because of high toxic effect of antimicrobial drugs it is limited. According to Table 2, the lowest inhibitory effect was found to be Gram negative for *E. coli* (ATCC 35218), despite the highest inhibitory effect against Gram positive *S. aureus* (ATCC 43300). However, plant extracts showed antimicrobial activity against *Pseudomonas aeruginosa* close to Gentamicin. Karou *et al.* (2012)¹² revealed that *E. coli* CIP 105182, which was found to be the most resistant

Table 2: Antibacterial activity of *C. creticum*

Bacteria	Extract zone	Penicilin	Gentamicin
<i>Staphylococcus aureus</i>	21 mm	33mm	-
<i>Bacillus subtilis</i>	17 mm	30mm	-
<i>Escherichia coli</i>	13 mm	-	17mm
<i>Pseudomonas aeruginosa</i>	14 mm	-	15mm

bacterial strain and was only inhibited by *S. longepedunculata* among five medicinal plant extracts.

CONCLUSION

Results presented that future investigations will be aimed the isolations of biologically active compounds such as flavonoids from *C. creticum*, as natural compounds for evaluation in pharmaceutical industry. Because of their antimicrobial activity they can be used as topical preparation or by food preservation.

ACKNOWLEDGMENT

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CONFLICT OF INTEREST

None

ABBREVIATION USED

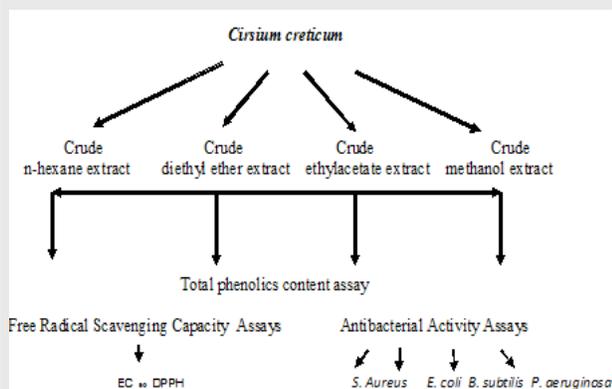
EC₅₀: The amount of antioxidant necessary to inactivate 50% of initial DPPH; DPPH: 2,2-diphenyl-1-picryl-

hydrazyl; TPC: Total phenolic content; ROS: Reactive oxygen species; EtOAc: Ethyl acetate; MeOH: Methanol; FCP: Folin & Ciocalteu's phenol reagent; CAE: Catechol Equivalents.

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PICTORIAL ABSTRACT



SUMMARY

- Cirsium creticum* (Asteraceae) is wild plant species.
- Crude extracts of plants obtained n-hexane, ether, EtOAc, MeOH extraction methods.
- The content of total phenolic was determined using Folin & Ciocalteu's phenol reagent and free radical scavenging activity was assayed by DPPH method.
- The antibacterial activity was determined against four bacteria strains, which were *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa*
- It is presented that *C. creticum* can be used as natural compounds for evaluation in pharmaceutical industry or by food preservation.

ABOUT AUTHORS



Temine Sabudak: Professor at Department of Chemistry, Faculty of Art and Sciences, University of Namik Kemal in Turkey. She is working on the chromatographic separation, purification and determination of chemical structures of organic compounds from natural products. Additionally her expert area is organic synthesis, spectroscopy phytochemistry.



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Evren Cabi: Associate Professor at Department of Biology, Namik Kemal University Faculty of Art and Sciences. His areas of research interest include 'Plant Systematics and Phylogeny', 'Ecology' and 'Conservational Biology'. He is author of over 100 peer-reviewed publications and 1 book chapter and 1 book. He collected the plant material from the natural flora of Turkey and identified them for this study

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