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# The Antibacterial Activities of *Cardopatium corymbosum* (L.) PERS. Against Mastitis Pathogens and its Antioxidant Activities

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**ABSTRACT**
*Mastitis is a common disease in dairy farms and causes serious financial loss. The use of unconscious drugs used for the treatment of infections causes the increase of antibiotic resistant bacteria. A major cause of mastitis is microorganisms. For this reason, this research focuses on antibacterial effects of Cardopatium corymbosum (L.) Pers. extracts against mastitis pathogens and its antioxidant capacity. In our research, two Staphylococcus aureus and five Coagulase Negative Staphylococcus were used for experiments. Plant material collected from Alaşehir, Manisa in Turkey. The plant extracts were tested by disc diffusion assay for antibacterial activity and antioxidant activity determined by DPPH (2,2-diphenyl-1-picrylhydrazyl) method. The ethanol extract of plant showed inhibition effect on S. aureus-17 pathogen while the aqueous extracts on S. aureus-17 and CNS-36 pathogens. Minimum inhibitory concentrations values of extracts are 6500 µg/mL. In the antioxidant study, it was determined that the methanol extract had a higher DPPH radical scavenging activity than the ethanol and aqueous extracts and antioxidant activity of this plant was high. As a result, the our studies indicate that the extracts of Cardopatium corymbosum have significant antioxidant activities.*
**Keywords:** *Cardopatium corymbosum*, Mastitis, Antibacterial activity, Antioxidant activity, DPPH

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## INTRODUCTION

One of the important diseases in dairy cattle breeding is mastitis. Mastitis is determined by the pathological changes in the breast tissue and the increase in somatic cells. Physical and chemical changes occur in milk as a result of mastitis [1]. Fungus, algae, viruses and especially bacteria are causing mastitis The main bacterial strains causing this disease are *Streptococcus* and *Staphylococcus* species. *Staphylococcus aureus* and *Escherichia coli* are important pathogens in cow mastitis [2]. Coagulase negative *Staphyloccocci* are minor mastitis pathogens compared with *Staphylococcus aureus* [3]. Antibiotic is frequently used for the treatment and protection of mastitis. The use of unconscious antibiotics causes the development of resistant strains [4].

Most of the plants which are members of the Asteraceae family have pharmacological effects such as antifungal, antibacterial, antitumor, antihelminthic, antifungal and anti-inflammatory. Secondary metabolites, such as diterpenes, sesquiterpenes and flavanoids have therapeutic effects [5]. The majority of sesquiterpenes have antibacterial activity [6] .

*Cardopatium corymbosum* (L.) Pers. is a species in the Asteraceae family. Distribution of *Cardopatium corymbosum* species in the world: Italy, Sicily, Macedonia, Greece, Crete, East Aegean Islands, Rhodes, Turkey, Lebanon, Syria, Cyprus [7]. According to Flora of Turkey the distribution of species in Turkey: Tekirdag, Canakkale, Kocaeli, Manisa, Konya, Denizli, Antalya, Mersin, Adana, Hatay [8] .

These species helps to reduce painful urinary excretion, kills intestinal parasites, removes the freckle when mixed with other ingredients [9] .But medicinal effects are based on more ethnobotanical research than experiment. There is not enough research on this species.

Antioxidants play an important role in body defense against reactive oxygen species that form as by-products during cell respiration [5]. The Alphabet of Galen: pharmacy from antiquity to the middle ages: a

critical edition of the latin text with english translation and commentary. University of Toronto Press., Canada.

Natural antioxidants are needed because synthetic antioxidants cause cancer and for this reason researches are being carried out in order to reveal the antioxidant activities in plants [10]. For this reason we are conducted to antibacterial and antioxidant activity research on *Cardopatium corymbosum*.

## MATERIAL AND METODS

### Plant material

*Cardopatium corymbosum* (L.) Pers. was collected from Alaşehir of Turkey. In the identification of this plant was used the Flora of Turkey and the East Aegean Islands [8].

### Microorganisms

The bacteria obtained from previous studies by Dr. Zafer Cantekin, University of Mustafa Kemal (Project number: 1101M0103; Ethics committee number: 2010/02-30:12). Seven bacteria were used in these research. Two of them were *S. aureus* and five of them were Coagulase Negative *Staphylococcus* (CNS). Identification of all mastisis pathogens was carried out using biochemical tests with conventional culture methods [11].

### Preparation of plant material

The aerial parts of plant were washed several times with running and sterile distilled water. Then plant material was air-dried. These samples were powdered in a laboratory blender. All materials were stocked at room temperature and they were stored at 4˚C until for analysis.

### Preparation of extracts

The air dried and powdered aerial parts of the plant (50 g) were extracted with ethanol, methanol and water solvents using the Soxhlet. After solvents have been evaporate, extracts (300 mg/mL) were kept in small sterile opac bottles under refrigerated conditions until used.

### Cultivation

Mastisis pathogens were used as a source of microorganisms. The bacteria were cultivated in Mueller-Hinton Broth for 24 hour at 37˚C (MHB; Merck).

### Determination of antibacterial activity

The antibacterial activities of extracts were determinated by disc diffusion assay. The ethanol, methanol and aqueous extracts of aerial parts of plants (300 mg/mL) were individually tested against mastitis pathogens by disk diffusion assay. The bacteria were incubated on Mueller- Hinton Agar plates (MHA, Merck) for 24 hour at 37 ˚C. The cultures set to 0.5 McFarland. Diameters of inhibition zones around the discs were measured after 24 h. The inhibition zones were recorded as mm for all materials. Ampicillin (10 µg) used as positive control. Methanol, ethanol and aqueous extracs used as negative control [12].

### Determination of minimum inhibitory concentration (MIC)

Another antibacterial activity test is MIC in our research. The broth dilution assay was made according to CLSI standarts [13,14].The MIC values were taken as the lowest concentration that inhibit growths of bacteria after incubation. The final concentrations of extracts are 13000, 6500, 3250, 1625 and 812.5 µg/mL.

### Determination of antioxidant activity

The non-enzymatic antioxidant activity was determined using DPPH (2,2-diphenyl-1-picrylhydrazyl) as the free radical. DPPH was used to determine the free radical scavenging activity of the extracts in the study. Plant extract (0.1 mL) was mixed with 0.1 mM DPPH in methanol. After incubation for 30 minutes, the extract absorbance was measured at 517 nm using spectrophotometric methods. Methanolic DPPH solution was used as control and methanol was used as blank sample. In addition to trolox was used as reference antioxidant. Percent inhibition of DPPH radical was calculated according to formula [15].

## RESULTS

Results of antibacterial activities of different extracts are given in Table 1. It was observed that methanol extracts showed no inhibitory effect on any mastisis pathogens (*S. aureus*-17, *S. aureus*-18, CNS-22, CNS-32, CNS-33, CNS-36 ve CNS-37). It was determined that the ethanol extracts have an inhibitory effect on *S. aureus*-17 pathogen, the aqueous extracts on *S. aureus*-17 and CNS-36 pathogens.

MIC values of *Cardopatium corymbosum* are shown in Table 2. In this research *S. aureus*-17 showed lowest sensitivity to ethanol extract of plant (6500 µg/mL).

In the antioxidant activity study, it was determined that the methanol extract had a higher DPPH radical scavenging activity than the ethanol and aqueous extracts. The DPPH radical scavenging activites are methanol, ethanol and aqueous extracts, respectively (Table 3). The methanol extract showed 82.1 % inhibition at 300 mg/mL concentration and trolox equivalent value was 2.1 mM/g DW.

**Table 1: Antibacterial activities of *Cardopatium corymbosum* various extracts aganist mastitis pathogens**

| Bacteria | Inhibition zone diameters of extracts (mm) | | | |
|---|---|---|---|---|
| | Ethanol | Methanol | Aqueous | A |
| *S. aureus*-17 | 8 | (-) | 7 | 18 |
| *S. aureus*-18 | (-) | (-) | (-) | 12 |
| CNS-22 | (-) | (-) | (-) | - |
| CNS-32 | (-) | (-) | (-) | 10 |
| CNS-33 | (-) | (-) | (-) | 8 |
| CNS-36 | (-) | (-) | 8 | (-) |
| CNS-37 | (-) | (-) | (-) | (-) |

CNS: Coagulase negative *Staphylococci*; A: Ampicillin (-) : Zone did not occur

**Table 2: Minimum inhibitory concentrations of *Cardopatium corymbosum* various extracts (µg/mL)**

| Bacteria | Extracts | | |
|---|---|---|---|
| | Ethanol | Methanol | Aqueous |
| *S. aureus*-17 | 6500 | (nt) | - |
| *S. aureus*-18 | (nt) | (nt) | (nt) |
| CNS-22 | (nt) | (nt) | (nt) |
| CNS-32 | (nt) | (nt) | (nt) |
| CNS-33 | (nt) | (nt) | (nt) |
| CNS-36 | (nt) | (nt) | - |
| CNS-37 | (nt) | (nt) | (nt) |

CNS: Coagulase negative Staphylococci; (nt): Not tested; (-): inhibition did not occur

**Table 3: Antioxidant activities of *Cardopatium corymbosum* extracts (300 mg/mL)**

| DPPH Scavenging Activity (%) | Extract | | |
|---|---|---|---|
| | Ethanol | Methanol | Aqueous |
| Activity | 71.3 | 82.1 | 80.9 |
| TE (mM / g DW) | 1.8 | 2.1 | 2.1 |

TE: Trolox equivalent (mM / g DW) ; DW: Dry weight

## DISCUSSION

There are usually ethnobotanical studies on *Cardopatium corymbosum*. In the ethnobotanic questionnaires made among the people, it was stated that the parts of *Cardopatium corymbosum* were crushed and then they were used against in cow as ointment. In an ethnobotanical research carried out by Tuzlacı and Aymaz [16] it was determined that the preparations obtained by decoction method of *Cardopatium corymbosum* roots were used in cows mastitis treatment by adding cow's cattles to food and water.

In the ethnobotanical study *Cardopatium corymboum* was reported to be effective against mastitis. For this reason we decided to do this research.

In present research, it was determined that *Cardopatium corymbosum*methanol extracts was not inhibition effects on all test pathogens. But ethanol extracts were effective on *S. aureus*-17 and aqueous extracts were showed inhibition effects on *S. aureus*-17 and CNS-36. In a research conducted by Okmen *et al.* [3] the same mastitis pathogens were used. According to this research, methanol, ethanol and ethyl acetate extracts of *Piper nigrum* L. were inhibited *S.aureus*-18, CNS-32 and CNS-36. But it was showed that this extracts were not effective on *S. aureus*-17, CNS-22 and CNS-33.

Hambaba *et al*. [17] investigated the antibacterial and hemostatic activities of the methanolic, dichloromethane and eteropetrolic extracts of the *Cardopatium corymbosum* aerial parts and found that all the extracts were resistant to some of the bacterial strains (*Staphylococcus aureus* ATTC 25922, *Escherichia coli* ATTC 25922, *Staphylococcus* spp., *Proteus vulgaris* and *Klebsiaella pneumoniae*) it has been determined that the most sensitive bacteria is *Proteus vulgaris* and methanolic extracts have haemostatic activity.

In a study conducted by Ilçim *et al.* [17] it was observed that *Crocus chrysanthus* (Herb.) Herb., *Rumex scutatus* L. and *Asphodelus aestivus* L. was not effective on *Staphylococcus aureus* but *Myrtus communis* subsp. *communis* L. and *Eugenia caryophyllata* Thunb.was effective.

The need for herbal medicine has been increasing day by day due to the decrease in the level of the effect of synthetic drugs in recent times. For this reason, alternative treatment methods have been recently used against diseases.

Antioxidants prevent damages caused by reactive oxygen (ROT) that are formed as a result of various physical and chemical events [18]. Plants are natural antioxidant source. There are not antioxidant studies on *Cardopatium corymbosum.* For this reason, this study was carried out by us.

In our antioxidant study, it was determined that the methanol extract had a higher DPPH radical scavenging activity than the ethanol and aqueous extracts. The methanol extract showed 82.1 % inhibition at 300 mg/mL concentration.

Our results showed that antibacterial activities of *Cardopatium corymbosum* aerial parts were low but plant extracts have very high antioxidant activity.

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