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Research Article

INVESTIGATION OF ANTIMICROBIAL PROPERTIES OF ENDEMIC *Hypecoum trullatum* Å.E.Dahl Plant IN IN-VİTRO CONDITIONS

Seda Başkan[1,a,*], Ali Savaş Bülbül[2,b]

[1]*Kahramanmaraş Sütçü İmam University, Faculty of Arts and Science, Dept. of Biology, Kahramanmaraş, Turkey*
[2]*Bayburt University, Faculty of Applied Sciences, Dept. of Emergency Aid and Disaster Management, Bayburt, Turkey*

**Corresponding Author:*
E-mail: sedambaskan@gmail.com

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a: ORCID 0000-0001-5542-3009, b: ORCID 0000-0002-2200-7348

ABSTRACT. In this study, the antimicrobial activity of the plant *Hypecoum trullatum* Å.E.Dahl, which is an endemic species in our country, was investigated. In the study, plant extracts dissolved with methanol were used. These extracts were obtained by agar well diffusion method with *Salmonella infantis*, *Listeria innocua*, *Enterococcus durans*, *Salmonella typhimurium*, *Staphylococcus epidermidi*s (DSMZ 20044), *Eschrichia coli* (ATCC 25922), *Saratia marrescens* (ATCC 13048), *Enterococcus faecalis* (ATCC 29212) antimicrobial activities were studied. . Studies were supported by Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBK). As a result, it was observed that the plant extract inhibited the growth of *Salmonella infantis*, *Listeria innocua*, *Enterococcus durans*, *Staphylococcus epidermidis* (DSMZ 20044), *Eschrichia coli* (ATCC 25922), *Saratia marrescens* (ATCC 13048) microorganisms, while *Salmonella typhimurium*, *Enterococcus faecalis* (ATCC 292) microorganisms were observed to inhibit the growth of microorganisms observed to be inhibited.

Keywords: *MIK, MKB*

INTRODUCTION

Humanity has used plants as a treatment method since ancient times. In our country, there are many plants that are used as 'medicinal plants' among the people. According to the research conducted by the World Health Organization (WHO) on 91 countries, medicinal plants used for therapeutic purposes are around 20,000 (1). The properties of plants that kill microorganisms and are important for health have begun to be investigated in the laboratory environment. Scientists have defined many medicinal plants and the effect of many plants has been scientifically proven [1, 2].

The biological activities of the plants are in the essential oil part. antimicrobial activity; It depends on the type, composition and concentration of the plant, the type and load of the target microorganism, the composition of the food, and the processing and storage conditions [3].

In this study, the antimicrobial activity of *Hypecoum trullatum* Å.E.Dahl, an endemic plant species grown in Turkey, was investigated. The plant is popularly known as 'hashidrellezotu'. *Hypecoum trullatum* is a member of the poppy family (Papaveraceae). In their research, it was determined that the Isoquinoline alkaloids of the Papaveraceae family have a rich chemical content in terms of denaporphine, protopine, protoberberine and proaporphine [4].

There are varieties of plants of the genus *Hypecoum*. These are *Hypecoum dimidiatum, Hypecoum pendulum, Hypecoum procumbens, Hypecoum pseudograndiflorum, Hypecoum torulosum, Hypecoum trullatum* plants.

In the study, it was observed that the plant *Hypecoum trullatum* Å.E.Dahl has antimicrobial activity. Antimicrobial activity was supported by MIC and MBK tests. It has been determined that the results show parallelism.

MATERIALS AND METHODS

Material

The identification and supply of the plant sample was provided by Kahramanmaraş Sütçü İmam University, Department of Herbal and Animal Production, Dr. Yusuf Ziya Kocabaş. The *Hypecoum trullatum* Å.E.Dahl plant used in the study was collected from Kahramanmaraş, Kapıçam recreation area and the plant species was identified. Bacterial strains used for antimicrobial test on plant sample (*Salmonella infantis, Listeria innocua, Enterococcus durans, Salmonella typhimurium, Staphylococcus epidermidis* (DSMZ 20044), *Eschrichia coli* (ATCC 25922), *Saratia marrescens* (ATCC 13048), *Enterococcus faecalis* from Kahramanmaraş University, Enterococcusfaecalis) has been done.

Method

Preparation of Plant Samples

Hypecoum trullatum Å.E.Dahl was left to dry for ten days at room temperature under sterile conditions in a laboratory environment. The aerial parts of the dried plant sample were ground using a Commercial blender. Extraction was carried out in a soxholet device with a methanol solvent in the range of 6-8 hours using a ratio of 1/10 (w/v) from the ground plant sample. The prepared extract was mixed with distilled water and worked at a single concentration of 25 mg/mL in triplicate. The zone diameters were measured and the arithmetic averages of the results were taken.

Antimicrobial Activity

AgarWell Diffusion Method

The test microorganisms to be treated with the plant concentration were incubated in Nutrientbroth (NB) broth at 37ºC for 24 hours in a shaking incubator. Test microorganisms were prepared with a value of 0.5 in the Mcfarland device. The prepared microorganisms were inoculated on Mueller Hilton Agar (MHA) with a sterile swab under aseptic conditions. Plant concentrations were added to the corresponding wells on MHA, as well as the negative control Dimethyl Sulfoxide (DMSO) and Penicillin as the positive control. After sowing, the petri dishes were left in the 37ºC incubator for 18-24 hours, and the zone diameters were measured and recorded at the end of the period.

Minimum Inhibitor Concentration (MIC)

The activity of plant extracts against microorganisms was tested with the agar well diffusion method, and the Minimum Inhibition Concentration (MIC) test was applied on 96-well microplates to determine the lowest concentrations of these plant samples in microorganisms. Plant sample and bacterial strains were added to the relevant wells in the microplates and the microplate was left to incubate in an oven at 37ºC for 18-24 hours.

On the microplates, the 7th well was selected as the positive control and the 8th control as the negative control. MIC concentration was established as 50mg/mL, 25mg/mL, 12.5mg/mL, 6.25mg/mL, 3.125mg/mL, 1.5625mg/mL. At the end of the incubation process, the microplate was read in the microplate reader at 550 nm.

Minimum Bactericidal Concentration (MBK)

After the MIC test, the concentration that inhibited the growth of plant concentrations on microorganisms and killed 100% was measured as MBK value. Samples were taken with the help of sterile loops from the relevant wells on the microplates on which MIC test was applied, and they were cultivated on MHA, which is solid medium. The cultivated petri dishes were kept in an oven at 37ºC for one day.

RESULTS AND DISCUSSION

Antimicrobial Activity results

In the study; antimicrobial activity was applied at a single concentration of 25 g/mL in 3 repetitions. Antibiotics were used as positive control and DMSO was used as negative control.

Table 1. *Measurements of the inhibition zones of the extracts of Hypecoumtrullatum against microorganisms (mm). * -- inhibition zone diameter not observed. * well diameters are not removed (8mm).*

	25 g/mL bitki konsantrasyonu	**Pozitif kontrol (Penisilin) (mg\mL)**	**Negatif kontrol (DMSO)**
Salmonella infantis	12	23	--
Listeria innocua	12	29	--
Enterococcus durans	10	10	--
Salmonella typhimurium	--	--	--
***Staphylococcus epidermidis* (DSMZ 20044),**	11	--	--
***Eschrichia coli* (ATCC 25922),**	10	19	--
***Saratia marrescens* (ATCC 13048)**	16	--	--
***Enterococcus faecalis*(ATCC 29212)**	--	--	--

After the antimicrobial test study, it was observed that the *Hypecoum trullatum* plant showed more activity than other bacterial strains by creating an inhibition diameter of 16 mm in the microorganism of the plant *Saratia marrescens* (ATCC 13048), while it was observed that the *Enterococcus durans* bacterial strain showed the least activity among all bacterial strains. Among all bacterial strains, no effect was found on *Enterococcus faecalis* and *Salmonella typhimurium* bacterial strains.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBK) Activity Results

The minimum inhibitory concentration (MIC) of the plants affecting the antimicrobial activity was determined by the agar well diffusion method. In order to determine the minimum inhibitory concentration, measurements were made at 550 nm in the spectrophotometer. Minimum bactericidal concentrations (MBC) were determined to stop the growth of the studied bacteria by 100%.

Table 2. *MIC and MBK results of Hypecoum trullatum*

	MİK	**MBK**
Salmonella infantis	3,125mg\mL	6,25mg\mL
Listeria innocua	3,125mg\mL	6,25mg\mL
Enterococcus durans	6,25mg\mL	12,5 mg\mL
Salmonella typhimurium	25 mg\mL	50 mg\mL
Staphylococcus epidermidis **(DSMZ 20044),**	12,5 mg\mL	25 mg\mL
Eschrichia coli **(ATCC 25922),**	3,125 mg\mL	1,5625 mg\mL
Saratia marrescens **(ATCC 13048)**	12,5 mg\mL	25 mg\mL
Enterococcus faecalis**(ATCC 29212)**	3,125 mg\mL	6,25 mg\mL

CONCLUSION

The antibiotic effect of essential oils in plants has been the most researched topic over the years. Essential oils are highly active against bacteria, viruses and protozoa. The antimicrobial effect of essential oils varies according to the type of plant and ecological conditions.

In this study, plant extracts were obtained by using the sun-facing parts of the *Hypecoum trullatum* plant (flower, leaf and branch) and the antimicrobial properties of these extracts were investigated. *Escherichia coli*, *Salmonella infantis*, *Enterococcus faecalis*, *Listeria innocua*, *Salmonella typhimurium*, *Staphylococcus epidermidis*, *Saratia marrescens*, *Enterococcus durans* bacteria strains were used in the investigation of antimicrobial activity. As a result of the antimicrobial activity test of *Hypecoum trullatum*, it was observed that *Saratia marrescens* bacterium formed the most zones, while it showed a zone diameter of 10-12 mm inhibition against *Eschrichia coli*, *Staphylococcus epidermidis*, *Enterococcus durans*, *Listeria innocua*, *Salmonella infantis* bacterial strains, and *Enterococcus* fac. strains did not show any effect. It was observed that the MIC and MBK results were in the same parallel line with the antimicrobial results of the plant.

In literature studies, antibacterial activity of *Argemonemexicana* L. plant was examined. *Argemone* is a genus of the Papaveraceae family. When the in-vitro antibacterial activity (regions of inhibitor diameters) was examined, N-demethyloxysanguinarine (4 g/mL) versus *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae, Pseudomonas aeruginosa* (17.30±0.03) mm, (15.8±0.12) mm, (18.72±0.18)) mm, (17.32±0.06) mm zone diameters were reached (Bhattacharjee, I). *Hypecoum* is a genus of the Papaveraceae family. As a result of the antimicrobial activity test of the *Hypecoum trullatum* plant, it was observed that *Saratia marrescens* bacterium forms a 16 mm zone, while it shows any zone diameter between 10-12 mm inhibition against *Eschrichia coli, Staphylococcus epidermidis, Enterococcus durans*, *Listeria*

innocua, Salmonella infantis bacterial strains, and *Enterococcus facillus* bacteria. showed no effect. The common bacterial strain of the studies is Eschichiacoli. The effect of *Argemonemexicana* L. plant belonging to Papaveraceae family on *Eschichia coli* bacteria is more sensitive than *Hypecoum trullatum* plant. While it has been observed that the *Hypecoum trullatum* plant has an effect on antimicrobial activity, much more comprehensive studies will be needed to reveal this. When the literature studies are examined, our study reveals a unique study examining the antimicrobial properties of the methanol extract of the plant *Hypecoum trullatum*, which is an endemic species.

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