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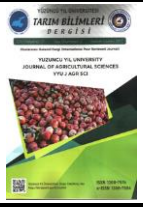

Araştırma Makalesi (Research Article)

Antimicrobial and Antioxidant effect of *Ficaria verna* Huds.

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Abstract: *Ficaria verna* Huds. is a plant belonging to the Ranunculaceae family, known as mole grass and celandine among the people. It is known to have anti-inflammatory and anti-haemorrhagic pharmaceutical effects. In this study, it was aimed to determine the antimicrobial effect of different concentrations of *F. verna* extracts obtained from methanol, ethanol and chloroform and the antioxidant activity of different concentrations of the extract obtained from methanol. In the results obtained, the best antimicrobial effect (17-20 mm) against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus megaterium*, *Salmonella thypii* and *Candida albicans* was determined in the methanol extract of *F. verna* at a concentration of 1000 µg. It was observed that the scavenging effect of the DPPH radical of *F. verna* increased depending on increasing concentrations.

Ficaria verna Huds.'nin Antimikrobiyal ve Antioksidan Etkisi

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Anahtar kelimeler
Antimikrobiyal etki,
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Ranunculus ficaria,
Tıbbi bitki.

Öz: *Ficaria verna* Huds. halk arasında köstebek otu ve kırlangıç otu adıyla bilinen ve Ranunculaceae familyasına ait bir bitkidir. Antienflamatuar ve antihemorajik gibi farmasötik etkilerinin olduğu bilinmektedir. Halk arasında hemoroide karşı kullanılmaktadır. Bu çalışmada *F. verna*'nın metanol, etanol ve kloroformdan elde edilen ekstraklarının farklı konsantrasyonlarının antimikrobiyal etkisi ile metanoldan elde edilen ekstresinin farklı konsantrasyonlarının antioksidan aktivitesinin belirlenmesi amaçlanmıştır. Elde edilen sonuçlarda *F. verna*'nın metanol ekstresi 1000 µg konsantrasonda *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus megaterium*, *Salmonella thypii* ve *Candida albicans*'a karşı en iyi antimikrobiyal etki (17-20mm) göstermiştir. *F. verna*'nın DPPH radikalinin süpürücü etkisinin artan konsantrasyonlara bağlı arttığı gözlemlenmiştir.

1. Introduction

Plants are used in different ways depending on the disease since ancient times and are considered as natural medicines (Durdevic et al., 2013). Mankind was discovered this healing effect of plants long ago and continues to use it today (Altay et al., 2015; Ozaslan and Oguzkan, 2018). Especially, with the increasing number of diseases, side effects of drugs and the lack of adequate response in treatment, the use of natural medicines of herbal origin is increasing (Doğan and Avcı, 2018). Herbal preparations are

known to have pharmacological effects such as anticancer, antimicrobial, antioxidant, anti-diarrhea, analgesic and wound healing (Karahan and Ilçim 2008; Karahan et al., 2016; Ozaslan and Oguzkan, 2018; Karahan et al., 2019;). For this reason, it is very important to examine herbal preparations and to investigate their medical effects.

Various species belonging to the Ranunculaceae family are used as spices and herbal medicine (Malik et al., 2017). These species are known to be used in conditions such as cancer, cardiac dysfunctions, various inflammation and severe hemostasis (Darshan and Doreswamy, 2004; Salem, 2005; Dewick, 2009). In addition, some species of this genus are known to have biological activities such as antibacterial, antiviral and antiprotozoal (Kaya et al., 2010). *Ficaria verna* Huds., which belongs to the Ranunculaceae family, is used in folk medicine for anti-inflammatory and anti-haemorrhagic effects. Especially, tuberous and dry root parts of the plant are used in treatment (Neag et al., 2017).

In this study, it was aimed to determine the antimicrobial effect of different concentrations of of the flower and leaf parts of *F. verna* extracts obtained from methanol, ethanol and chloroform and the antioxidant activity of different concentrations of the extract obtained from methanol.

2. Materials and Methods

2.1. Obtaining and preparation of plant material

F. verna (syn. *Ranunculus ficaria* L.) was purchased commercially from a local herbalist in 2020. The taxonomic identification of plant material was determined by using the Flora of Turkey (Davis, 1965; The Plant List, 2021); it was performed by Prof Dr. Şemsettin Civelek who is a systematic-botanic specialist from Fırat University. The plant material was pulverized. 0.5 grams of sample was taken. Each sample was kept in an orbital shaker at 100 rpm for 72 hours to obtain an extract using 100 ml 96% methanol, ethanol and chloroform solvents. It was then filtered using Whatman filter paper.

2.2. Determination of antimicrobial effect

2.2.1. Test microorganisms

In this study; *Staphylococcus aureus* ATCC25923, *Escherichia coli* ATCC25322, *Klebsiella pneumoniae* ATCC 700603, *Bacillus megaterium* DSM32, *Salmonella thypii* ve *Candida albicans* FMC17 microorganisms were used. Microorganism cultures were obtained from Fırat University, Faculty of Science, Department of Biology, Microbiology Laboratory culture collection.

2.2.2. Preparation of microorganism cultures and testing for antimicrobial effect

The antimicrobial activity of extracts of *F. verna* obtained from ethanol, chloroform and methanol solvents were determined according to the disk diffusion method (Erecevit Sönmez et al., 2019). Bacteria strains (*Staphylococcus aureus* ATCC25923, *Escherichia coli* ATCC25322, *Klebsiella pneumoniae* ATCC 700603, *Bacillus megaterium* DSM32, *Salmonella thypii*) were inoculated into Nutrient Broth (Difco) for 24 hours at 35 ± 1°C and yeast strains (*Candida albicans* FMC17) were incubated in Malt Extract Broth (Difco) for 48 hours at 25 ± 1°C. The culture of the prepared bacteria and yeast broth, respectively; was inoculated into Müeller Hinton Agar and Sabouraud Dextrose Agar at a rate of 1% (10^6 bacteria ml^{-1}, 10^4 yeast ml^{-1}). Then, after shaking well, 25 ml was placed in sterile petri dishes of 9 cm diameter. A homogeneous distribution of the medium was achieved. 6 mm diameter antimicrobial discs (Oxoid), each impregnated with different extracts of 100 µl (500 µg) and 200 µl (1000 µg), were lightly placed on the solidified agar medium. After the petri dishes prepared in this way were kept at 4°C for 1.5-2 hours, the plates inoculated with bacteria were incubated at 37 ± 0.1°C for 24 hours, and the plates inoculated with yeast at 25 ± 0.1°C for 72 hours. As controls, different standard discs were used for bacteria (Streptomycin sulphate 10 µg $disc^{-1}$) and yeasts (Nystatin 30 µg $disc^{-1}$). Dimethyl sulfoxide (DMSO) was used for negative control. Zones of inhibition were measured in mm.

2.3. Determination of antioxidant effect

Antioxidant activity was determined by the free radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sharma and Bhat, 2009; Dimitrova et al., 2010). DPPH solution was prepared to be 0.004% in methanol. Serial concentrations (1.25, 2.5, 5, 10 mg ml^{-1}) of plant extracts were prepared by dissolving in methanol. 30 µl of plant extract was added on 3 ml of DPPH solution. It was left in the dark for 30 minutes at room temperature. Then, reading was done at 517 nm in the spectrophotometer. The antioxidant activity was repeated three times. Butylated hydroxyanisole (BHA) and methanol were used as controls. The antioxidant activity was calculated by the formula below. AbsControl = Absorbance of DPPH-methanol solution, AbsSample = Absorbance of plant extract.

% DPPH inhibition = [(AbsControl - AbsSample) / AbsControl] x 100 (1)

2.4. Statistical analysis

The statistical analysis of the study was made according to the kruskal wallis test.

3. Results

3.1. Antimicrobial effect

Antimicrobial activity results of *F. verna* extracts obtained from methanol, ethanol and chloroform against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus megaterium*, *Salmonella thypii* and *Candida albicans* at different concentrations are given in Table 1.

It was determined that the chloroform extract of *F. verna* at 500µg and 1000µg concentrations did not show antimicrobial effect against all the microorganisms used. (Table 1).

While ethanol extract of *F. verna* at 1000µg concentration was formed the zone 16 mm against *E. coli*, 11mm against *K. pneumoniae*, 14 mm against *S. aureus*, 10 mm against *S. thypii* and 14 mm against *C. albicans*, it was determined that it did not show an antimicrobial effect at a concentration of 500 µg (Table 1).

It was observed that the methanol extract of *F. verna* at 1000µg concentration formed an inhibition zone (17-21 mm) in different ratios against *E. coli*, *K. pneumoniae*, *S. aureus*, *S. thypii* and *C. albicans* however it was determined that it did not show an antimicrobial effect in methanol extract at 500µg concentration (Table 1).

Table 1. Antimicrobial activity of different concentrations of *F. verna* (mm)

	E. coli	*K. pneumoniae*	*S. aureus*	*S. thypii*	*C. albicans*
R.F-C (500µg)	-	-	-	-	-
R.F-C (1000µg)	-	-	-	-	-
R.F-E (500µg)	-	-	-	-	-
R.F-E (1000µg)	16	11	14	10	14
R.F-M (500µg)	-	-	-	-	-
R.F-M (1000µg)	20	17	21	18	18
Control	12	10	10	30	12

R.F-C: Chloroform extract of *F. verna;* R.F-E: Ethanol extract of *F. verna*; R.F-M: Methanol extract of *F. verna*.

3.2. Antioxidant effect

The percentage of inhibition of DPPH radical in different concentrations of the methanol extract of *F. verna* is shown in Table 2.

According to the results, it was determined that the DPPH radical scavenging effect of the methanol extract of *F. verna* increased depend on increasing concentrations (Table 2).

Table 2. Percent inhibition of the DPPH radical of *F. verna*

BHA	91.70
MetOH	1.56
1.25 mg ml^{-1}	20.90
2.5 mg ml^{-1}	42.38
5 mg ml^{-1}	81.79
10 mg ml^{-1}	95.98

BHT: Butylated hydroxyanisole; MetOH: Methanol.
Values are means ±S.D.n:3, $p<0.05$ importantly dissimilar with Kruskal Wallis's test.

4. Discussion and Conclusion

In previous studies, the antimicrobial and antioxidant effects of different species of plant extracts belonging to the Ranunculaceae family were determined. Antimicrobial activities of hexane, ethyl acetate, methanol and aqueous extract of *R. sprunerianus* and *R. marginatus* var. *trachycarpus* against some microorganisms were tested. In the results obtained, it was determined that the antimicrobial effects of these species against *S. faecalis, S. aureus, S. epidermidis*, *B. subtilis*, *S. typhimurium*, *P. aeruginosa*, *E. aerogenes* and *E. coli* ranged between 128 and 256 µg mL^{-1}. (Kaya et al., 2010). It was reported in studies that the inhibition zones of *Ranunculus arvensis* obtained by using different solvents against *E. coli*, *E. aerogenes*, *B. bronchiseptica*, *K. pneumoniae*, *M. luteus* and *S. anginosus* are 7 mm. It was determined that the same species did not have a significant antifungal effect against *A. niger*, *A. flavus*, *A. fumigates*, *F. solani* and *Mucor* species (Bhatti et al., 2015). When the results of this study were compared with species such as *R. sprunerianus*, *R. marginatus* var. *trachycarpus* and *Ranunculus arvensis* in the literature, it was determined that the antimictobial effect of *F. verna* is different depending on the solvents and the microorganisms used. It was observed that the methanol extract of *F. verna* has a better antimicrobial effect than chloroform and ethanol extracts.

Antioxidant activities of *A. hupehensis*, *A. spicata*, *C. europaea*, *H. foetidus*, *A. vulparia*, *T. altissimus* and *C. racemosa* were determined as 0.325, 0.251, 0.195, 0.172, 0.109, 0.103 and 0.156 TE g^{-1}, respectively. Studies were reported that *R. ficaria* has 80.9% scavenging effect of DPPH radical at a concentration of 1 mg ml^{-1} (Barla et al., 2014). In studies, the scavenging effect of the DPPH radical of different concentrations (50-500 µg ml^{-1}) of the roots of *Ranunculus sceleratus* was found to be between 57.50% ± 2.88 and 21.89% ± 0.75 (Serag et al., 2020). The scavenging effect of the DPPH radical of hexane, ethyl acetate, methanol and aqueous extracts of *R. marginatus* var. *trachycarpus* were found to be 10.50% ± 0.30, 22.34% ± 0.33, 76.58% ± 0.98 and 45.50% ± 0.50, respectively. DPPH radical scavenging effect of *R. sprunerianus* hexane, ethyl acetate, methanol and aqueous extracts was found to be 27.60% ±0.06, 37.20% ±0.09, 85.34% ± 0.33 and 61.09% ± 0.29, respectively (Kaya et al., 2010). The IC50 value of the DPPH radical scavenging effect of *R. ficaria* extract obtained from glycerol-ethanol and hydroalcoholic solvents was calculated as 1.9 and 243.4 µl, respectively (Neag et al., 2017). When the results of this study are compared with *A. hupehensis*, *A. spicata*, *C. europaea*, *H. foetidus*, *A. vulparia*, *T. altissimus*, *C. racemosa*, *R. sceleratus*, *R. marginatus* var. *trachycarpus* and *R. sprunerianus*, It was determined that the antioxidant effect of *F. verna* is higher.

In this study, the antimicrobial and antioxidant effects of *F.verna* were investigated. In the results obtained, it was found that antimicrobial and antioxidant effects increased depending on the increase in concentration. As a result, we think that *F.verna* can be used as an antioxidant and antimicrobial agent in pharmacological studies.

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