



Determination of Antimicrobial and Antioxidant Activity of *Alchemilla alpina* L.

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ABSTRACT

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Alchemilla genus, which belongs to the Rosaceae family, is a medicinal plant used for various purposes among the people. Species of this genus are known in Turkish folk medicine as lion claw or hazelnut grass. Especially, they are used mainly women's illnesses, in gastritis, anti-inflammatory, as carminative, and in the treatment of wound. Besides the antimutagenic effect of *Alchemilla alpina* L., its above-ground parts are used for antimycotic purposes in the form of tea or oral care water. In this study, it has been aimed to determine the antimicrobial effect of the above-ground parts of *Alchemilla alpina* extracts obtained from methanol, ethanol and chloroform and the antioxidant activity of different concentrations of the extract obtained from methanol. The antimicrobial activity of methanol, ethanol and chloroform extracts of the above-ground parts of *A. alpina* has been determined according to disk diffusion method. In the results obtained have been showed that these extracts inhibited the growth of some bacteria (*Staphylococcus aureus* ATCC25923, *Escherichia coli* ATCC25322, *Klebsiella pneumoniae* ATCC700603, *Bacillus megaterium* DSM32) and yeasts (*Candida albicans* FMC17 and *Candida glabrata* ATCC66032) at different rates (8-23 mm). The antioxidant activity of different concentrations (1.25, 2.5, 5 and 10 mg/ml) of the above-ground parts of *A. alpina* extract obtained from methanol has been determined according to the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity method. In the results obtained, it has been observed that the effect of removing DPPH radical of *A. alpina* increased depending on increasing concentrations.

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Introduction

Medicines from natural products have been used in folk medicine for centuries. Because they are known to have fewer side effects than synthetic drugs (Banerjee et al., 2018). Especially in recent years, due to the increase in the side effects of the drugs used in treatment, patients are turning to herbal products that are more natural and have less risk in terms of side effects (Ege and Elmastaş, 2020). The use of plants in folk medicine based back much earlier than modern medicine. According to the World Health Organization, 80% of the world's population still relies on plant-based medicines. (Uritu et al., 2018).

It is a medicinal plant belonging to the genus *Alchemilla* L. Rosaceae, especially known as lion paw or nut grass in our country (Ozbek et al., 2017; Acet and Özcan, 2018). It is known that these species have many biological effects such as antioxidant, antimicrobial,

diuretic, tonic, reproductive disorders, cell regenerative and antidiabetic and they are used in many regions for this purpose (Viegi et al., 2003; Altundag and Öztürk, 2011; Kaya and Artuvan, 2016; Ozbek et al., 2017). Especially, they are used mainly women's illnesses, in gastritis, anti-inflammatory, as carminative, and in the treatment of wound. Besides the antimutagenic effect of *Alchemilla alpina* L., its above-ground parts are used for antimycotic purposes in the form of tea or oral care water.

In this study, it has been aimed to determine the antimicrobial effect of the above-ground parts of *Alchemilla alpina* extracts obtained from methanol, ethanol and chloroform and the antioxidant activity of different concentrations of the extract obtained from methanol.

Materials and Methods

Obtaining and Preparation of Plant Material

A. alpina was purchased commercially from a local herbalist in 2020. 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.

Determination of Antimicrobial Effect

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.

Preparation of microorganism cultures and testing for antimicrobial

The antimicrobial activity of extracts of *A. alpina* obtained from ethanol, chloroform and methanol solvents were determined according to the disc diffusion method (Collins and Lyne, 2004). 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 $35 \pm 1^\circ\text{C}$ and yeast strains (*Candida albicans* FMC17) were incubated in Malt Extract Broth (Difco) for 48 hours at $25 \pm 1^\circ\text{C}$. The culture of the prepared bacteria and yeast broth, respectively; was inoculated into Müller Hinton Agar and Sabouraud Dextrose Agar at a rate of 1% (10^6 bacteria / ml, 10^4 yeast / ml). 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 extracts of 100 μl (500 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 $37 \pm 0.1^\circ\text{C}$ for 24 hours, and the plates inoculated with yeast at $25 \pm 0.1^\circ\text{C}$ for 72 hours. As controls, different standard discs were used for bacteria (Streptomycin sulphate 10 μg / disc) and yeasts (Nystatin 30 μg / disc). Dimethyl sulfoxide (DMSO) was used for negative control. Zones of inhibition were measured in mm.

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) 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:

$$\text{DPPH inhibition}(\%) = \frac{(\text{AbsControl}-\text{AbsSample})}{\text{AbsControl}} \times 100 \quad (1)$$

AbsControl = Absorbance of DPPH-methanol solution,
AbsSample = Absorbance of plant extract.

Statistical Analysis

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

Results and Discussion

Antimicrobial Effect

Natural remedies are used in the treatment of diseases in many developing countries. In this sense, plants are natural products that are beneficial for human health in the fight against infections (Onbaşı et al., 2020). For this purpose, the antimicrobial effect of different extracts of *A. alpina* was determined in this study.

The antimicrobial effects of methanol, ethanol and chloroform extracts of *A. alpina* against *E. coli* were determined as 18mm, 15mm and 8mm, respectively (Table 1). While the methanol and ethanol extracts of *A. alpina* formed an inhibition zone (20-15mm) against *K. pneumoniae* at different rates, chloroform extract did not create an inhibition zone (Table 1). It was been determined that *A. alpina* extracts obtained from different solvents show antimicrobial effect against *C. albicans* at different rates (22-10mm) (Table 1). The ethanol extract of *A. alpina* showed the highest antimicrobial effect (20mm) against *C. glabrata* (Table 1). While methanol (23mm) and ethanol (15mm) extracts of *A. alpina* showed antimicrobial activity against *S. aureus*, chloroform extract did not show antimicrobial activity (Table 1). *A. alpina* extracts obtained from methanol, ethanol and chloroform solvents formed 20mm, 15mm and 8mm inhibition zones against *B. megaterium*, respectively (Table 1).

In previous studies, *Alchemilla ellenbergiana* Rothm. ethanol extract showed an 18 mm inhibition zone against *Candida albicans*, and ethyl acetate and methanol extracts showed 13mm and 12mm inhibition against methicillin resistant *S. aureus* (MRSA), respectively. Against *K. pneumoniae*, ethanol and ethyl acetate extracts showed an equal inhibition zone of 11mm (Acet and Özcan, 2018). It was determined that *Alchemilla glabra* Neygenf showed antimicrobial activity at different rates (12-22mm) against some strains of *E. coli* and *S. aureus*. In the same study, it formed 22-17mm inhibition zone against *P. vulgaris* at different concentrations, 14-16mm inhibition zone against *C. albicans* and 13-15mm inhibition zone against *K. pneumoniae* (Denev et al., 2014). *Alchemilla vulgaris* L. extracts showed an inhibition rate of 86.98% against *Phytophthora infestans* and 81.57% against *Macrophomina phaseolina*, 63.11% against *Alternaria alternata* and 45.20% against *Cylindrocarpon destructans*. (Özbek et al., 2021). In Australia and Iceland, ethanol extract from the aerial parts of *A. alpina* and petroleum ether extracts of the roots of *A. faeroensis* and *A. vulgaris* plants were found to be active against *Plasmodium falciparum*. (İlgün et al.,

2014). It was determined that the extracts of *A. mollis* in different solvents showed antimicrobial activity at different rates against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Klebsiella pneumoniae* microorganisms, but did not prevent the development of *Candida albicans* (Kaya et al., 2009). The inhibition zones of the methanol extract of *Alchemilla mollis* (Buser) Rothm. against *E. coli*, *S. typhimurium*, *S. aures*, *S. enteriditis* and *C. albicans* were reported as 12.16 ± 1.04 , 10 ± 1 , 18.66 ± 0.57 , 22 ± 1 and 15 ± 1 mm, respectively. (Şeker Karatoprak et al., 2017). *A. acutifolius* and *A. officinalis* were found to be effective at 50-400 µg/mL concentrations against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Candida albicans*, *Candida krusei* and *Candida glabrata*. (Mohammed et al., 2021a). The minimum inhibitory

concentrations of the methanol, ethanol and dichloromethane extract of *S. marianum* against *S. aureus*, *S. aureus* MRSA, *E. coli*, *P. aeruginosa*, *C. glabrata*, *C. albicans* are calculated between 25-800 µg/mL (Mohammed et al., 2019). The minimum inhibitory concentration values of *M. longifolia* subsp *longifolia* collected from different localities were determined between 50 µg/mL and 800 µg/mL against some microorganisms (*S. aureus*, *E. faecalis*, *E. coli*, *P. aeruginosa*, *C. albicans*, *C. tropicalis*) (Sevindik et al., 2017). In the results obtained, it is seen that when the antimicrobial effect of *A. alpina* against pathogenic microorganisms is compared with similar species and different plant species, the results show differences. Because the antimicrobial effect varies depending on the microorganisms used, the solvent, the plant species used and their habitats.

Table 1. Antimicrobial effect of ethanol methanol and chloroform extracts of *A. alpina* (mm)

	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>C. albicans</i>	<i>C. glabrata</i>	<i>S. aureus</i>	<i>B. megaterium</i>
<i>A. alpina</i> -M	18	20	20	18	23	20
<i>A. alpina</i> -E	15	15	22	20	15	15
<i>A. alpina</i> -C	8	-	10	11	-	8
Control	11	18	10	23	15	10

A. alpina-M: Methanol extract of *A. alpina*; *A. alpina* -E: Ethanol extract of *A. alpina*; *A. alpina* -C: Chloroform extract of *A. alpina*

Table 2. Percent inhibition of the DPPH radical of *A. alpina*

Concentrations	Percent inhibition
BHA	84.5±0.257
MetOH	1.5±0.724
1.25 mg/ml	45.4±0.440
2.5 mg/ml	67.8±0.978
5 mg/ml	84.8±1.348
10 mg/ml	94.4±1.301

Antioxidant Effect

Antioxidants are compounds that can eliminate the harmful effects of free radicals. For this reason, in order to minimize the harmful effects of synthetic antioxidants used today, the tendency towards natural antioxidants is increasing (Çelik and Ayran, 2020). Especially for this purpose, researches on the natural antioxidant capacities of plants are increasing. Therefore, in this study, the antioxidant effect of the aerial parts of *A. alpina* was determined.

The results of the DPPH radical scavenging effect of different concentrations of the methanol extract of the aerial parts of *A. alpina* are shown in Table 2.

The percentage of inhibition of the DPPH radical scavenging effect of the methanol extract of *A. alpina* was found to be in the range of 45.4 ± 0.440 - $94.4 \pm 1.301\%$, depending on the varying concentrations (Table 2).

In one study, it was determined that the antioxidant effects of water and methanol extracts of *Alchemilla mollis* Buser were increased up to a certain point, but still low (Uçar Sözmen et al., 2020). The highest DPPH radical scavenging effect of *Alchemilla ellenbergiana* Rothm. was found in the ethanol extract (243.6 µg/ml), and the methanol extract (243.1 µg/ml) was found to be very close. The lowest DPPH radical scavenging activity was also determined in the hexane extract (7.1 µg/ml) (Acet and Ozcan, 2018). It was reported that the antioxidant activities

of methanol extracts of *Alchemilla* species were high. (Usta et al., 2013). The antioxidant effect of *Alchemilla glabra* was determined using various methods. In the results obtained, ORAC, TRAP and HORAC were calculated as 1337 ± 68 µmol TE/g, 1815 ± 38 µmol TE/g, and 1999 ± 70 µmol GAE/g, respectively (Denev et al., 2014). It was determined that *Allcemilla sericata* Rchb. was 58.9% scavenging effect on DPPH radical and Iron(III) ion reducing antioxidant power (FRAP) was 770.8 µmol Fe II/g (Murathan, 2018). The IC50 values of the DPPH radical scavenging effect of 70% methanol and water extract of *Alchemilla mollis* were calculated as 0.21 ± 0.001 mg/ml and 0.24 ± 0.002 mg/ml, respectively (Şeker Karatoprak et al., 2017). The percentage inhibition of the DPPH radical scavenging effect of the leaf extract of *Alchemilla vulgaris* (L.) was reported as $71.8 \pm 4.1\%$ (Oktyabrsky et al., 2009). The TAS value of the ethanol extract obtained from the flower parts of *Datura stramonium* was determined as 7.559 ± 0.224 mmol/L and the TOS value was determined as 10.711 ± 0.243 µmol/L (Mohammed et al., 2021b). It has been reported that the antioxidant effect of different concentrations of *M. longifolia* subsp. *longifolia* is between $92.94 \pm 1.83\%$ and $48.46 \pm 1.32\%$. TAS values of ethanol extract of *M. longifolia* subsp. *longifolia* collected from different localities were determined in the 1.809 ± 0.07 - 3.628 ± 0.234 mmol/L range and TOS values of the same species

were detected in the 11.058 ± 0.610 - 14.077 ± 0.634 range $\mu\text{mol/L}$ (Sevindik et al., 2017). TAS values of *A. acutifolius* and *A. officinalis* were reported to be 6.238 ± 0.032 and 7.449 ± 0.088 , respectively, and TOS values were reported to be as 13.892 ± 0.162 and 18.607 ± 0.352 , respectively (Mohammed et al., 2021). The TAS value of the ethanol extract of *Thymbra spicata* L. was 8.399 ± 0.102 and the TOS value was 6.530 ± 0.115 . (Mohammed et al., 2020). TAS and TOS values of ethanol extract of *Rosa canina* were determined as 4.602 ± 0.215 mmol/L and 6.294 ± 0.191 $\mu\text{mol/L}$ (Pehlivan et al., 2018). When the results were compared with the results in the literature, it was determined that the antioxidant effect of *A. alpina* was higher at 10 mg/ml.

Generally, sometimes the variability of the results may vary depending on the harvest time of the plant, ecological conditions, plant species, concentration and solvents used.

Conclusion

In this study, antimicrobial effect and antioxidant effect of *A. alpina* against some microorganisms were determined. It was determined that the methanol extract of *A. alpina* showed the best antimicrobial effect against *S. aureus*. In addition, it was determined that methanol extract had the highest antioxidant activity at 10 mg/ml. The fact that the biological effects of *Alchemilla* species are low in the literature and that *A. alpina* is not included in the studies makes this study important. This study the results obtained will contribute to the literature and that the biological effects of *A. alpina* may be important.

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Data of the study is presented and the study is placed in 4th International Conference on Physical Chemistry and Functional Materials Congress Abstract book (08-09 April 2021, pp. 40)

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