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Antimicrobial Activity of *Paronychia kurdica* Boiss. subsp. *kurdica* var. *kurdica* cultivated in Medicinal and Aromatic Plants Garden of Inonu University Faculty of Pharmacy

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ABSTRACT: It was aimed to examine the antimicrobial activities of the aqueous-methanol extract of *Paronychia kurdica* Boiss. subsp. *kurdica* var. *kurdica* on Gram-positive and Gram-negative bacteria and fungal microorganisms. The plant taxon is collected from Medicinal and Aromatic Plants Garden of Inonu University Faculty of Pharmacy. The aerial parts were powdered and extracted with methanol. The samples were tested at a concentration of 800-1,56 µg/ml against the standard culture collections of Gram negative bacteria (*Acinetobacter baumannii, Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*), Gram positive bacteria (*Enterococcus faecalis, Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus* (MRSA)) and *Candida* species fungal strains *(C. albicans* and *C. glabrata*). *P.kurdica* extract was found to be more effective against Gram-positive bacteria than Gram-negative bacteria. Its effect on our fungal strains was found to be similar to Gram-positive bacteria. Globally, this paper is the first antimicrobial activity report of *Paronychia kurdica* Boiss. subsp. *kurdica* var. *kurdica*.

Keywords: Antimicrobial activity, Malatya, *Paronychia kurdica* Boiss. subsp. *kurdica* var. *kurdica*

1 INTRODUCTION

In tropical countries, the rate of deaths from infection is approximately half compared to other causes of death. Infection-related diseases and deaths are increasing day by day in developed countries. This situation necessitates new searches for the prevention and treatment of infectious diseases. Secondary metabolites, which are biologically active chemical compounds such as flavonoids, alkaloids, terpenoids, tannins, etc. synthesized by plants, are extensively employed in the management of infectious illnesses. The fact that pathogenic microorganisms in humans inactivate drugs over time, failing in the treatment of infectious diseases, creates a need for antimicrobials in the form of a vicious cycle. Although many pathogenic microorganisms have developed resistance to drugs today, there are also many plants with antimicrobial effects, causing researchers to prefer plants in the search for antimicrobial agents. The therapeutic effects of

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plants, which have significant potential in the fight against microorganisms, arise from the synergistic effect of many substances in their composition, rather than a single active substance isolated from its content. Phyto compounds provide a more effective treatment by counteracting the resistance of microorganisms that are difficult to kill with a single antibiotic. Antimicrobial action is influenced by the kind and load of the target microorganism, the content of the food, the conditions during processing and storage, and the species, composition, and concentration of the plant. The chemical compositions of some medicinal plants that prevent microbial growth, either in combination with traditional antimicrobials or individually, may vary depending on geographical conditions, collection conditions, time of acquisition, and growth conditions. This situation directs researchers to investigate the inhibitory effect compositions of natural antimicrobial agents obtained from plant extracts [1-8].

The genus *Paronychia* Mill., which is in the Illecebraceae family in the Flora of Turkey, has been assigned to the Paronychioideae subfamily of the Caryophyllaceae family in recent years. The genus, represented by 117 species in the world and 45 taxa belonging to 32 species in Türkiye, has native range from temperate and subtropical America, Macaronesia, Mediterranean to Iran and northeastern tropical Africa. The subshrub *P. kurdica* Boiss. subsp. *kurdica* var. *kurdica* taxon is native to Iran, Iraq, Lebanon-Syria, Transcaucasia, and Türkiye. It is mainly found in temperate biomes [9-14].

P. kurdica, which has been used traditionally for many years in some rural regions of Turkey, has therapeutic effects such as hypoglycemic, diuretic, and cancer suppressant. Also, it is used in kidney stone treatment [15]. *P. kurdica* known under the name Haşişılselulet in Midyat district of Mardin locally used as infusion in the treatment of wounds and gallbladder [16], in the treatment of warts by eating with salt in Hizan district of Bitlis [17], and in Amasya, the aerial parts are made into mush and mixed with vinegar and garlic, and then soaked in a cloth against bloodshot eyes, placed on the head against headache [18].

Various usage forms of some species of *Paronychia* are recorded in traditional folk medicine in Turkey. In Eskişehir, the aerial parts of the *P. amani* Chaudhri species are crushed when fresh and externally used for the treatment of ingrown and inflamed wounds. In Mersin, the decoction prepared from the aerial parts of the *P. argentea* Lam. (1-2 glasses per day, 3 days) is used internally for kidney stones [18]. *P. mughlaei* Chaudhri known under the name dolamaotu in Muğla is used against boil and felon [19].

In phytochemical studies of *Paronychia* species, especially saturated fatty acids, sterols, essential oils, terpenes, tannins, saponins, phenolic compounds and flavonoids were found [15, 20-26]. In biological activity studies conducted on *Paronychia* species, antioxidant, antimicrobial, antiviral and anti-inflammatory effects were reported [15, 19, 23-24, 27].

In this research, it was aimed to investigate the antimicrobial activities of the aqueous-methanol extract of the *P. kurdica* subsp. *kurdica* var. *kurdica* taxon, which was discovered to be least researched, on Gram-positive and Gram-negative bacteria and fungal microorganisms.

2 MATERIAL AND METHOD

2.1 Plant Material

P. kurdica subsp. *kurdica* var. *kurdica* taxon cultivated in Medicinal and Aromatic Plants Garden of Inonu University Faculty of Pharmacy was collected by Dr. Narin Sadıkoğlu during the flowering period, in April 2021. The specimen is kept in the Herbarium of Inonu University Faculty of Pharmacy (INUE) with the voucher number NS/2021/030.

2.2 Microbial Strains

Gram positive bacterial strains; *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213 and Methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 43300.

Gram negative bacterial strains; *Acinetobacter baumannii* ATCC 19606, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603 and *Pseudomonas aeruginosa* ATCC 27853.

Fungal strains*: Candida glabrata* ATCC 90030, *Candida albicans* ATCC 14053.

2.3 Extraction and Fractionation

The aerial parts were dried in the shade and at room temperature. The powder drug (47.6704 g) was extracted with methanol on a magnetic stirrer for 8 hours by shaking. The obtained extracts were concentrated with the help of rotavapor under low pressure (200 mbar) and 40 °C. This process was repeated 4 times and the efficiency was calculated.

The resulting dry extract was dissolved in 100 mL of 90% methanol and subjected to liquid-liquid extraction with 100 mL of hexane. This process was terminated with the end of the substance transition to the hexane phase, and thus lipophilic impurities were removed from the extract. The amount of 90% methanol extract obtained was recorded.

2.4 Antimicrobial Activity

By the recommendations of the Clinical Laboratory Standards Institute (CLSI), the samples were tested at different concentrations of 800 (for samples) and 1,56 µg/mL (for antibiotics) against standard culture collections of the most commonly isolated strains among society and

hospital-acquired infectious agents using a method of serial dilutions using sterile 96-well microplate readers (PLT microtiterplate ESP) [28].

To create the stock solution, 10 mg of the material was dissolved in 1000 µL of dimethyl sulfoxide. To the test wells, 100 µL of Müller-Hinton Broth (Merck 110293, USA) was added. We took 100 µL of our material's stock solution, performed serial dilutions from the first to the tenth well, and used the final two wells as control groups. A turbidity threshold of Mc Farland 0.5 was used to create 10 µL of bacterial suspensions, which were then added to all samples, including the control wells [29]. Thermo USA's PST 60HL orbital shaker was utilized for five minutes to combine our material with the microorganisms. The microplate lid was shut, and it was incubated for eighteen to twenty hours at 35 ºC. A sterile plastic loop was used to streak the culture from each well onto a Müller-Hinton Agar plate, and the plate was then incubated under the same conditions to monitor the development of bacteria. The substance's minimal inhibitory concentration (MIC) was established as a pre-dilution of growth [30-32]. Using Sabouraud Dextrose Broth and Agar (oxoid CM0147, CM0041, USA) under identical conditions, antifungal activity was assessed. Furthermore, under identical testing settings as our substances, reference drugs for every category of bacteria and fungus were examined in our investigation [33].

3 RESULT

3.1 Yield

The amount of crude methanol (100%) extract produced from the powdered plant is 4.6603 g (11.37 g/g), whereas 90% methanol extract taken from the crude extract after the lipophilic contaminants were eliminated and used for antimicrobial activity is 3.4391 g.

3.2 Antimicrobial Effect

The antibacterial and antifungal activity of *P. kurdica* subsp. *kurdica* var. *kurdica* extract was determined in the range of MIC values of 50-400 µg/mL. The antimicrobial activity of our extract varies between bacterial and fungal species. *P. kurdica* extract was found to be more effective against Gram-positive bacteria (*S. aureus*, *E. faecalis*, *S. aureus* MRSA) than Gram-negative bacteria (*E. coli*, *K. pneumoniae*, *P. aeruginosa*, *A. baumannii*). Its effect on our fungal strains (*C. glabrata*, *C. albicans*) was found to be similar to Gram-positive bacteria (Table 1).

4 DISCUSSION

It has been reported that *Paronychia argentea* extract, the most studied species, protects kidney endothelial cells against oxidative damage [26]. In addition, the protective activity of the methanol extract of this species against tobacco mosaic virus (TMV) infection in tomatoes has been proven

Table 1. The antimicrobial activity of *P.kurdica* subsp. *kurdica* var. *kurdica* extract varies between bacterial and fungal species.

	S.aureus	***E.faecalis***	***E.coli***	***P.aeruginosa***	***C.albicans***	***C.glabrata***	***A.baumannii***	***K.pneumoniae***	**MRSA**
P.kurdica	**50**	**50**	**200**	**400**	**100**	**100**	**200**	**200**	**200**
Control	**Growth**	**Growth**	**Growth**	**Growth**	**Growth**	**Growth**	**Growth**	**Growth**	**Growth**
Ampicillin	**1.56**	**1.56**	**3.12**					**1.56**	
Amikacin				**1.56**			**3.12**	**1.56**	
Ciprofloxacin			**1.56**					**1.56**	
Ciprofloxacin					**6.25**	**3.12**			
Vankomicin									**3.12**
Tigecycline							**1.56**		

agriculturally [25]. It has some therapeutic effects such as hypoglycemic, diuretic, cancer suppressant, and treatment of kidney stones and heartaches. It is used widely in traditional medicine, particularly in Middle Eastern countries such as Jordan, Israel and Palestine, in the treatment of urinary systems and diabetes [15, 21, 34-35].

Antimicrobial effect of *P. argentea* species grown both in the field (ex vitro) and in the laboratory (in vitro) with Cobalt (Co), copper (Cu) or lead (Pb) heavy metals in Jordan, against Gram-positive bacteria *Listeria monocytogen* and *S. aureus*, Gram-negative bacteria *Coronobacter sakazakii* and *Salmonella typhimurum* and fungus *Calvularia lunata* has been examined. As a result, it has been proven that *P. argentea* strain growing in medium with 0.3 mg/L of supplement Cu in extracts showing similar antimicrobial activity showed maximum inhibition on *S. aureus* (30.0 mm inhibition zone) and then on *C. lunata* (30.0 mm inhibition zone) [36].

The antibacterial activity of chloroform, ethanol and distilled water extracts of *P. argentea* species collected from Gaza Strip-Palestine, against *E. coli*, *K. pneumoniae*, *Morganella morganii*, Methicillin-sensitive *S. aureus* (MSSA) and Methicillin-resistant *S. aureus* (MRSA) bacteria has been investigated. It has been shown that for chloroform, ethanol, and aqueous extracts, the average diameter of the

inhibitory zones against the studied bacteria varied between 9–14 mm, 13–19 mm, and 13–20 mm, respectively. The aqueous extract has a value of 4.17 mg/mL against *K. pneumoniae* and *MRSA* species, while the mean minimum inhibitory concentration (MIC) values were between 4.17-33.33 mg/mL and 1.04-2.08 mg/mL for the chloroform and ethanol extracts, respectively [24].

The antimicrobial activities of aqueous and ethanol extracts of *P. argentea* plant collected from the West Bank region of Palestine was evaluated against *P. aeruginosa, P. aeruginosa* (clinical isolate), *E. coli, Proteus mirabilis* and *K. pneumoniae* by agar well diffusion method. It has been reported that the ethanol extracts examined showed higher MIC values, ranging from 1.56 to 50 mg/ml, compared to aqueous extracts [37].

Crude saponin extracts of the aerial part of *P. argentea* collected from near Marrakech, Morocco, was examined for antimicrobial activity against gram-negative bacteria *E. coli, K. pneumoniae*; gram-positive bacteria *Bacillus cereus, Micrococcus luteus, S. aureus,* and fungi *C. albicans, C. glabrata, C. krusei* and *C. parapsilosis*. The agar disc diffusion method was used to assess antibacterial activity, and the microdilution method was used to assess the minimum inhibitory concentration and antibiotic synergistic interaction. With an inhibitory zone of 11.10±0.35 mm, *M. luteus* was the most susceptible gram-positive bacterium to *P. argentea* extract. Moreover, the extract had minimal antibacterial activity on *E. coli* and no effect on *K. pneumoniae*. The extract showed an inhibition zone ranging from 9.40 to 13.07 mm on *Candida* strains. The MIC values of *P. argentea* extract were 8, 16 and 16 mg/mL for *M. luteus, B. cereus*, and *S. aureus*, respectively [38].

The antimicrobial activities of aqueous and methanol extracts of *P. mughlaei* species collected from Muğla were evaluated at concentrations of 1, 2.5, 5 and 10% by the agar well diffusion method, against Gram-negative bacteria *Aeromonas hydrophila, E. coli, K. pneumoniae, M. morganii, P. mirabilis, P. aeruginosa, S. typhimurium* and *Yersinia enterocolitica*, Gram-positive bacteria *B. brevis, B. cereus, B. subtilis, L. monocytogenes* and *S.s aureus* and fungi *C. albicans* and *Saccharomyces cerevisiae*. As a result, methanol extract showed weak antimicrobial activity only on *A. hydrophila, B. brevis, B. cereus, B. subtilis*, and water extract showed weak antimicrobial activity on *B. brevis* [19].

Aqueous extract of *P. kurdica* subsp. *kurdica*, collected from Bingöl-Elazığ area displayed beneficial effects on nipple papillomatosis in cattle, when administered

orally or subcutaneously injection, while its application as ointment is not very successful [15].

Antibiotic resistance has been accelerated in the past few years due to the extensive use of antibiotics to boost production in animal husbandry and agriculture, and to treat infectious diseases. The current state of affairs has rendered the creation of novel antibiotics necessary. Nevertheless, challenges encountered in the process of creating novel pharmaceuticals have prompted scientists to explore the potential of plants possessing inherent antibacterial characteristics. Our work focused on exploring the antibacterial characteristics of *Paronychia* species, which have not been previously investigated. The efficacy of our extract has been specifically demonstrated against Gram positive bacteria and fungal strains. However, as this is the first study conducted on this specific species, there hasn't been any previous research on the antibacterial qualities of other members of the same genus as our species.

Consequently, there is a substantial disparity in the MIC values between the ones we obtained in our study and the MIC values of the antibiotics currently used as reference medications (1.56 µg/ml). Nevertheless, the absence of any impediment that would restrict the use of plants with natural qualities, from which we derive extracts, effectively nullifies this drawback.

As a result, it seems that the genus has not been researched much, except for *P. argentea*, and more detailed studies need to be done on *P. kurdica* compared to other *Paronychia* species. The antimicrobial effect mechanism of the plant extract should be investigated by analyzing its components. It becomes necessary to find new antimicrobial agents due to both the side effects of antimicrobial agents and the resistance mechanism developed by microorganisms against them. Studies that determine the antimicrobial activities of plants in order to find new alternatives to treat diseases or reduce possible side effects, which are among today's popular research topics, are one of the best alternatives in terms of both cost effectiveness and easy availability of plants.

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6 AUTHOR CONTRIBUTIONS

Hypothesis: N.S.; Design: N.S.; Literature review: Z.T., N.S.; Data Collection: N.S., Z.T.; Analysis and/or interpretation: S.G., Z.T.; Manuscript writing: N.S., Z.T., S.G.

7 CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

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