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Investigation of Antimicrobial, Antibiofilm and Antiurease Activities of *Teucrium polium* L. Extracts

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ABSTRACT

Today, antimicrobial resistance and difficulties in treating infectious diseases and economic losses have increased scientists' interest in herbal medicines. *Teucrium polium* L. is widely used as a medicinal plant in traditional medicine, and therefore, determining the biological activities of this plant is important. This study aimed to reveal the antimicrobial, antibiofilm and antiurease activities of *T. polium* commonly known as "Acıyavşan" and these effects of *T. polium* petroleum ether, chloroform, methanol extracts on 6 standard pathogenic microorganisms (*Escherichia coli* ATCC 11229, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* ATCC 14028, *Klebsiella pneumoniae* ATCC 4352, *Staphylococcus aureus* ATCC 6538 and *Candida albicans* ATCC 10231). Antimicrobial activity potential of plant extracts was detected by the Agar well diffusion method and Minimal inhibitory concentration (MIC)s were determined for extracts with antimicrobial activity. Additionally, the antibiofilm effect of *T. polium* extracts was assessed by crystal violet staining. Antiurease activity was determined spectrophotometrically. *T. polium* petroleum ether and methanol extracts had antimicrobial activities against *C. albicans* (0.47 mg/ml, 7.5 mg/ml) and *S. aureus* (1.88 mg/ml, 0.94 mg/ml). In addition, all of the *T. polium* extracts showed antimicrobial activity against *P. aeruginosa* and antibiofilm activity against *E. coli, P. aeruginosa* and *S. aureus*. The highest antiurease activity of *T. polium* was found as 62.23±2.33 µg/mL in the methanol extract. Consequently, *T. polium* extracts exhibits significant biological activities against various microorganisms that are human pathogens. The high potential of *T. polium* may shed light for future studies in the discovery of new compounds that can stop or slow down the growth of pathogenic microorganisms.

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Introduction

Since their discovery, antibiotics have been the most common strategy used in the therapy of various human infections. However, their inappropriate and the misuse of antibiotics could lead to the emergence of antimicrobial-resistant bacteria over time which has posed challenges for public health and the treatment of diseases all over the world (Barancheshme and Munir,2019; Nicolosi et al., 2023; Seyirt,2023; Güngör,2024).

Biofilm can be defined as a microbial community that is irreversibly embedded in a matrix of extracellular polymeric substance (EPS) produced by microorganisms irreversibly attached to any surface or to each other (Sadekuzzaman et al. ,2015; Li et al., 2024, Türkoğlu et.al, 2025). Antibiotic resistance is fortified by biofilm formation, which is frequently observed in hospital and industry enviroments since it enhances tolerance of bacteria (Li et al., 2024). Biofilm formation caused by microorganisms is very important in the development of infections and antibiotic resistance (Li et al., 2024).

The nature of the biofilm structure and the physiological properties of microorganisms in the biofilm create a natural resistance to antimicrobial agents. Biofilm bacteria are known to be up to 1000 times more resistant to antibiotics than their planktonic forms (Li et al., 2024).

Furthermore, *Helicobacter pylori* (*H. pylori*) which infects significant part of the world's population and has a higher prevalence in developing countries causes chronic inflammation and is closely related with peptic ulcer, gastric adenocarcinoma and MALT (mucosa-associated lymphoid tissue) lymphoma. Urease produced by *H. pylori* catalyzes the hydrolysis of urea, which is also known to possess toxic effects on host cells by converting the ammonia produced into some derivatives and complicates treatment. Therefore, in recent years, urease enzyme has been accepted as the target of new drug research in the treatment of *H. pylori*. (Lomartire and Gonçalves, 2023). It has been reported that many medicinal plants can break the resistance of *H.pylori* by showing anti-urease activity through some secondary metabolites. Consequently, there is a need to investigate and discover new herbal sources with antiurease activity for the treatment of *H. pylori* infections. In order to overcome the antibiotic resistance together with biofilm formation of pathogenic microorganisms and cope with the antiurease activity of *H. pylori*, attempts for discovery of new and effective compounds is crucial (Lomartire and Gonçalves, 2023; Ersoy et al., 2023). Accordingly, the interest of scientists on herbal medicines has increased.

Teucrium polium is perennial plant belonging to the genus Teucrium, found in Lamiaceae family, *T. polium* is distributed in the rocky and sandy regions of the Mediterranean, in different parts of Europe, in the north of Africa and in Southwest Asia, but in Türkiye it is distributed in shrubs and maquis, steppes, fields, water banks, rocks and arid slopes (Davis, 1982). The stems of the perennial plant can grow up to 10-40 cm in length, and are white or gray, white or gray and hairy, lying flat or erect on the soil (Belmekki et al., 2013). Leaves are oblong, narrow, inverted ovate, with toothed margins towards the tip and curved inwards. Flowers of *T. polium* bloom in June-September have very short stalks and their petals are linear, notched or entire. The petals are whitish and the seeds are 2 mm in size. The visual characteristic of the plant is the structure of the hairy stem and leaves rather than the flowers (Belmekki et al,2013; Davis 1982).

T. polium. has traditionally been utilized in the therapy of various ailments such as gastrointestinal disorders, inflammation, eczema, urinary tract infection, diabetes, bronchitis, cough, sore throat and rheumatic diseases with its antispasmodic, antidiarrheal, anticonvulsant and antiulcerative effects (Polat et al., 2024; Ghavam and Markabi, 2024; Ersoy, 2023; Rahmouni et al., 2021). It has been shown that *T. polium*, named as 'felty germander' has a wide geographical distribution in Türkiye, and has been reported possesses many medicinal effects such as anti-rheumatic, blood pressure lowering, and when the leaves are chewed raw, it relieves pain, especially abdominal pain in a short time, and this plant can be used as free radical scavengers and natural antioxidant (Toplan et al., 2022; Candela et al., 2021).

Since *T.polium* contains vital components that have antioxidant effects and potential as a natural medicine, in this study we aimed to investigate the antimicrobial, antibiofilm and antiurease activities of petroleum ether, chloroform and methanol extracts of *T. polium* on various pathogenic microorganisms.

Materials and Methods

Plant Idenfication

The aerial parts of *Teucrium polium* were collected from the Kahramanmaraş province, Türkiye. Identification of the plant was done by Dr. Ismail Senkardeş from Marmara University, Faculty of Pharmacy, Department of Pharmaceutical Botany and *T. polium* was enrolled to the herbarium of Marmara University (MARE-18081).

Plant Extracts

First of all, the plant collected from Kahramanmaraş at 2023 and identified aerial parts of *T. polium* were dried at room temperature in the shadow and powdered by grinding at Renas Plant Grinder. Then, petroleum ether, chloroform, methanol extracts were obtained from 100 grams of powdered sample in 500ml of each solvent for 72 hours sequentially by maceration method. After maceration, the liquid part was filtered through filter paper. The solvents of the filtrates were evaporated in rotary evaporator (Heidolph Instruments GmbH & Co. KG, Germany) and lyophilizer (Faithful Liyofilizatör FSF-10N-50C, China), then crude extracts were obtained. The extracts were stored at 4 °C until the day of the study (Polat et al., 2024).

Antimicrobial Activity

Agar Well Diffusion Method

The bacteria used in this study were *Escherichia coli* ATCC 11229, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* ATCC 14028, *Klebsiella pneumoniae* ATCC 4352 and *Staphylococcus aureus* ATCC 6538. Additionally, as a yeast strain *Candida albicans* ATCC 10231 was used. Microorganism suspensions were prepared in 0.85% physiological saline solution (PSS) from colonies grown on Tryptic Soy Agar (TSA) media for bacteria and Sabouraud Dextrose Agar (SDA) media for the yeast. Bacterial suspensions were adjusted to a concentration of 10^8 cfu/ml and yeast suspension to a concentration of 10^6 cfu/ml according to Mc Farland 0.5 standard turbidity. These suspensions were taken with a sterile swab and spread on the surface of Mueller Hinton Agar (MHA) medium for bacteria and SDA medium for yeast. Then, by using sterile punches on the media, wells with a diameter of 5 mm were prepared. After that, 50 µl of the extracts dissolved in solvents (petroleum ether, chloroform, methanol) were placed in the wells. In addition, meropenem (10 µg/well) for bacteria, amphotericin B (100 µg/well) for yeasts were used as positive control and solvents together with PSS (Physiological saline solution) were used as negative control. Petri dishes were incubated at 37°C for 18-24 hours for bacterial growth and at 35°C for 24-48 hours for yeast growth and growth inhibition zones were measured in mm. Experiments were done in triplicate (CLSI, 2006; Perez, 1990; Rex et al., 1993).

Minimal Inhibitory Concentration (MIC) for Bacteria

The MIC values were determined in accordance with Clinical and Laboratory Standards Institute (CLSI) standards, where the bacterial suspensions were prepared according to Mc Farland 0.5 turbidity (1-2 × 10^8 cfu/ml) from colonies of overnight bacterial culture and diluted to a final inoculum concentration of 5 × 10^5 cfu/ml. Then, 100 µl of Cation-Adjusted Mueller Hinton Broth (CAMHB) was placed to each well of sterile U-bottom microdilution plate.

After that, 100µl of each extract were poured in the first wells (7,5mg/ml) and serial dilutions were done respectively. Next, 5µl of bacterial suspensions were added to the wells containing the extract and incubated at 37°C for 18 hours. Also, CAMHB and solvents were utilized as negative control while meropenem was utilized as positive control in the same plate. At the end of incubation, the lowest sample concentrations without growth were determined as MIC (CLSI, 2020).

Minimal Inhibitory Concentration (MIC) for Yeast

100µl of RPMI-1640 medium was placed in each of the wells of U-based microdilution plate. Dilutions of the *T. polium* extracts were prepared in U-based microdilution plates in RPMI-1640 medium. After that, 100µl of each extract were poured in the first wells and serial dilutions were done respectively. The tested yeast was prepared from colonies grown on SDA medium and the yeast suspension was made by using RPMI-1640 according to McFarland's turbidity of 0.5 (1×10^6-5×10^6 cfu/ml). Then, it was diluted with RPMI-1640 to 0.5-2.5×10^3 cfu/ml and placed in the wells. After that, the prepared plate was incubated at 35°C for 24-48 hours. At the end of the 24 hours of incubation, the plate was evaluated and incubated again and evaluated again at 48 hours. The lowest concentration without growth was considered as MIC value. RPMI-1640 and solvents were utilised as negative control and amphotericin B as positive control (CLSI, 2012; Berkow, 2020).

Antibiofilm Activity

The crystal violet staining method was applied to determine the antibacterial effect of the extracts. All of the bacterial strains were cultured in 5 ml 1% Tryptic Soy Broth-glucose medium and incubated at 37 °C for 24 hours. Bacterial suspensions equivalent to McFarland turbidity standard of 0.5 in TSB-glucose were prepared from the cultures. C. albicans yeast strain was cultered in RPMI-1640-glucose medium and incubated at 37°C for 48 hours. (McFarland value for *C. albicans* is 0.5 (1×10^6-5×10^6 yeast/ml)). The prepared microbial suspensions were diluted with 1% TSB-glucose medium to a final concentration of 5×10^5 cfu/ml and 180 µl of them were inserted into the wells of the flat bottom microplate. According to the MIC values of the samples against each strain tested, the final concentrations of the samples in the wells were 1/2, 1/10, and 1/100 times the MIC value in cation adjusted MHB and RPMI-1640 medium. Then 20 µl of each sample was added to the respective wells containing bacteria on the plate. The prepared plates were incubated at 37ºC for 24 hours and after incubation, the wells were washed twice with 250 µl PSS after carefully emptying the wells with the help of an automatic micropipette. Later, 200 µl of 99% methanol was included to the wells and left for 15 minutes, then the wells were emptied and the microplate was left to dry. After that, 200 µl of 1% crystal violet was added to the wells and kept for 5 minutes, the excess dye was washed away with water and the microplate was left to dry. Additionally, 200 µl of 95% ethanol was placed into the wells and left for 30 minutes and optical density values was measured at 595 nm on a microplate reader at the end of the time. The experiments were performed in 3 replicates and the arithmetic averages of the optical density (OD) values of each strain incubated with *T. polium* extracts (petroleum ether, chloroform, methanol) of various concentrations were taken. The optical density values of the negative and positive control wells were measured and averaged according to the method applied. The positive control OD value of each strain was taken as 100% and the % value corresponding to the OD values of the wells containing different concentrations of sample were determined according to this value. The absorbance values of the wells containing the sample were subtracted from the absorbance value of the negative control. The change in biofilm formation rates of sample microorganisms at different concentrations were calculated by the following formula (Dosler and Karaaslan, 2014; Türkoğlu et.al, 2025).

Biofilm change rate = [OD]D/[OD]C × 100

[OD]D = OD value of wells containing sample at different concentrations
[OD]C = OD value of positive control well

Anti-urease Activity

Briefly, 1 mL of the extracts or standards were taken and 15 µL urea (0.08 g/mL), 485 µL phosphate buffer and 2.5 mL R1 (120 mM phosphate buffer, 60 mM sodium salicylate, 5 mM sodium nitroprusside, 1 mM EDTA and 5 mg/L urease) was mixed and kept at 37 ºC for 5 min and then 2.5 mL R2 (120 mM phosphate buffer, 400 mM sodium hydroxide and 10 mM sodium hypochlorite) was mixed. This mixture was kept at 25 ºC for 10 min and the absorbance was measured at 625 nm against the reference solution. After adding 500 µL buffer solution and 2.5 mL R1 to the reference, R2 was added without waiting and then immediately used. Here, thiourea was used as control, where it contained all reagents without sample. The percentage of the urease inhibition was calculated according to the following formula (Ahmed and Mir, 2014):

% Urease Inhibition = [(Ac - As)/Ac] × 100

Ac: Absorbance of the control
As: Absorbance of the sample

Results

All of the *T. polium* extracts showed antimicrobial activity against *P. aeruginosa* ATCC 27853; petroleum ether and methanol extracs of *T. polium* were effective against *S. aureus* ATCC 6538 and *C. albicans* ATCC 10231(Table 1).

Petroleum ether extract of *T. polium* reduced the biofilm growth of *E. coli* ATCC 11229 by 99.4% at MIC/2 concentration and methanol extract of *T. polium* reduced the biofilm growth of *E. coli* ATCC 11229 by 98.7% at MIC/2 concentration. In addition, methanol extract of *T. polium* reduced the biofilm growth of *P. aeruginosa* ATCC 27853 by 89.5% and *S.aureus* ATCC 6538 by 78.4% at MIC/100 concentration. It was also observed that the chloroform extract of *T.polium* reduced the biofilm growth of *P. aeruginosa* ATCC 27853 by 88.9% at MIC/2 concentration (Table 2).

When the anti-urease activities of the plant extracts were evaluated comparatively, it was observed that the methanol extract of T. polium (62.23±2.33) showed higher activity than the other extracts (Table 3). Thiourea (70.05±0.007) was used as control and when compared with the extracts, the plant extracts showed lower enzyme inhibition than the control.

Table 1. Antimicrobial activities of petroleum ether, methanol and chloroform extracts of *Teucrium polium*

Extracts	1		2		3		4		5		6	
	ZD (mm)	MIC (mg/ml)	ZD (mm)	MIC (mg/ml)	ZD (mm)	MIC (mg/ml)	ZD (mm)	MIC (mg/ml)	ZD (mm)	MIC (mg/ml)	ZD (mm)	MIC (mg/ml)
Petroleum Ether	9.74	0.47	0.00	-	23.87	7.5	0.00	-	0.00	-	13.46	1.88
Methanol	14.37	7.5	0.00	-	19.18	7.5	0.00	-	0.00	-	11.62	0.94
Cloroform	0.00	-	0.00	-	19.51	7.5	0.00	-	0.00	-	0.00	-
Meropenem	23.42	0.06	20.33	2.00	30.60	0.25	19.23	4.25	19.02	4.25	-	-
Amphotericin B	-	-	-	-	-	-	-	-	-	-	15.78	1

1: *Staphylococcus aureus* ATCC 6538; 2: *Escherichia coli* ATCC 11229; 3: *Pseudomonas aeruginosa* ATCC 27853; 4: *Klebsiella pneumoniae* ATCC 4352; 5: *Salmonella typhimurium* ATCC 14028; 6: *Candida albicans* ATCC 10231; ZD: Zone diameter, MIC: Minimum inhibitory concentration, -: Not tested

Table 2. Biofilm formation in the presence of *Teucrium polium* extracts (%)

M	Petroleum Ether Extract			Methanol Extract			Chloroform Extract		
	MIC/2	MIC/10	MIC/100	MIC//2	MIC//10	MIC//100	MIC//2	MIC//10	MIC//100
1	70.3±0.15	56.3±0.23	59.7±0.25	42.2±0.11	70.9±0.42	21.6±0.11	22.0±0.15	41.2±0.15	78.5±0.30
2	34.5±0.15	53.5±0.13	14.8±0.05	2.8±0.06	16.6±0.09	10.5±0.11	11.1±0.1	13.8±0.12	11.7±0.1
3	0.6±0.07	11.0±0.05	10.9±0.05	1.3±0.05	12.3±0.11	28.2±0.15	14.5±0.06	31.3±0.10	21.8±0.11
4	13.8±0.01	41.1±0.04	107.4±0.07	34.8±0.07	42.6±0.11	49.4±0.13	41.6±0.1	66.2±0.04	48.0±0.07
5	21.3±0.06	24.1±0.1	47.3±0.1	48.7±0.19	75.0±0.22	101.4±0.07	49.8±0.07	54.4±0.07	93.8±0.1
6	16.8±0.1	26.6±0.1	52.1±0.11	8.9±0.03	72.4±0.12	86.8±0.05	98.7±0.13	92.4±0.07	136.3±0.20
7	70.4±0.16	85.9±0.09	100.7±0.13	99.7±0.13	93.0±0.18	90.8±0.21	101.1±0.20	88.2±0.15	103.8±0.21

M: Microorganism; 1: *S. aureus* ATCC 1; 2: *P. aeruginosa* ATCC 27853; 3: *E. coli* ATCC 11229; 4: *K. pneumonia* ATCC 4352; 5: *S. typhimurium* ATCC 14028; 6: *P. aeruginosa* PAO1; 7: *C. albicans* ATCC 10231

Table 3. Anti-urease activity of *Teucrium polium* extracts

Extracts	Inhibition of urease enzyme (%) (12.5 µg/mL)
Petroleum ether	10.32±1.2
Chloroform	48.25±1.52
Methanol	62.23±2.33
Thiourea	70.05±0.007

Discussion

In recent years, the widespread increasing use of antibiotics throughout the world has led to significant increase in antibiotic-resistant microorganisms and encouraged scientists to search for new and more effective drugs (Tanwar et al, 2014). In this context, plants are considered as a potential source of antimicrobial agents due to their richness in alkaloids, anthraquinones, saponins, terpenoids, tannins and polyphenols (Subramani et al, 2017).

Teucrium polium has been used in traditional medicine for many years since its antidiabetic, anti-inflammatory, antispasmodic and antioxidant effects. Phytochemical compounds isolated from the aerial parts of *T. polium* were analysed by Bahramikia et al (2012). In their study, more than 130 molecules were identified, of which terpenoids (60%) predominated.

Alreshidi et al. (2020) reported that, cirsimaritin, luteolin, rutin, salvigenin, apigenin, cirsiliol,and eupatorin were the main compounds detected from *T. polium* roots, aboveground parts and inflorescences.

Alreshidi et al. (2020) showed that the methanolic extract of *T. polium* is a rich source of unidentified bioactive molecules in the genus Teucrium obtained using HR-LCMS. The researchers reported that the methanolic extract of *T. polium* has antimicrobial activity against *P.aeruginosa , S. aureus, C. albicans*. The findings of Alreshidi et al. (2020) were found to be compatible with the findings of our study. According to the findings of the same researchers, the methanolic extract of *T. polium* inhibited *P.aeruginosa* PAO1 at a concentration of 100 µg/ml.

In addition, Toplan et al (2022) have shown that methanolic and chloroform extract of *T. polium* have antifungal effect against various Candida strains. In our study methanolic extract of *T. polium* was *effective* against *C.albicans* ATCC 10231, while chloroform extract was not effective. This could be due to fact that the place where the flowers were picked were different from each other. While Toplan et al (2022) have used *T. polium* from Ağrı (Doğubeyazıt), the plant of the present study was collected from Kahramanmaraş province.

Demiryapan et al. (2020) determined that the most effective values of essential oil on bacteria were 1,563µg/ml on *P. aeruginosa*, 6,25 µg/ml on *S.aureus* and 12,5µg/ml on *Salmonella enteritidis*. All extracts of *T. polium* in our study, which showed antimicrobial activity in the agar well diffusion method, were found to be effective against *P. aeruginosa*, while petroleum ether and methanol extracts of our plant were found to be effective against *C. albicans* and *S. aureus*. Therefore, the findings of our study were consistent with that of Demiryapan et al. (2020).

Ben Othman et al (2017) investigated the antimicrobial effects of essential oil, ethanol and aqueous extracts of *T. polium* against microorganisms and the results showed that the essential oil had the highest antimicrobial activity and was most effective against *Citrobacter freundii, Proteus mirabilis*, and *S. aureus*.

In our study, the most effective values of *T. polium* extract on bacteria were determined to have both antibacterial and antifungal effects, as 7.5 mg/ml in petroleum ether, methanol and chloroform solvents on *P. aeruginosa*, 9.74 mg/ml in petroleum ether and 14.37 mg/ml in methanol on *S. aureus*, 1.875 mg/ml in petroleum ether and 0.9375 mg/ml in methanol on *C. albicans*.

Elmasri et al (2014) showed that the methanol extract prepared from the aerial parts of *T.polium* collected from Egypt inhibited the biofilm formed by *S.aureus.*

Also, Alarjani and Skalicky (2021) reported that essential oil of *T.polium* showed strong effect affect against the biofilm formation of drug-resistant *S.aureus* strains .

The antibiofilm results of our study are in line with the existing literature mentioned above since we have found that all extracts of *T.polium* exhibited antibiofilm effects. The most significant ones were against *E. coli* ATCC 11229, *P. aeruginosa* ATCC 27853 and *S.aureus* ATCC6538 strains.

Urease inhibitory activity is crucial for its antiulcer effects (Ersoy et al., 2023). Additionally, the urease inhibition activity is very important as it has antiulcer properties. As it is known, regular human can synthesis around 10 kg of urea in a year and this created amount takes approximately four years till it becomes completely degraded. With the existance of urease enzyme this hydrolysis reaction can take place 10000 times faster. Ammonia the main end compound of this reaction, increases the stomach pH levels thus creating a suitable environment for *Helicobacter pylori* colonization, which can subsequently cause peptic ulcers, gastric ulcers, and eventually stomach cancers (Ersoy et al., 2023). In their study Ersoy et al. (2023) observed antiurease properties of *T. polium* ethanol extract.

It is known that various Teucrium species, especially *T. polium*, are widely used against gastrointestinal complaints such as stomach pain, gastritis, and also have beneficial effects since their digestive properties (Ersoy et al, 2023). Furthermore, *T. polium* 's antiulcerative activity has been by specified in in vivo studies, which deducted that the plant is highly effective (Mehrabani, et a.2009; Ersoy et al, 2023).

Mehrabani, et al (2009) reported the healing effects of *T. polium* (300 mg/kg body weight/day) in the repair of indomethacin-induced gastric ulcer in rats. According to the investigators healing properties of the plant on indomethacin induced stomach ulceration in rat. could be attributed to their antioxidant activity along with the ability to modulate the mucin secretion. Additionally, the antiulcer efects can be due to all efective plant compounds such as tannins and/or flavonoids.

In the present study methanol extract of *T. polium* was found to show higher antiurease activity than the other extracts.

Studies have shown that herbal agents possess antibacterial, antibiofilm, and antioxidant potential and produce various metabolites. Plant extracts and pure phenolic compounds are known to be therapeutic components or agents that control diseases in humans. This study demonstrated the antimicrobial, antiurease, and antibiofilm potential of *T. polium* plant extracts.

Conclusion

In conclusion, our study demonstrated that *T. polium* plant extracts possess antimicrobial, antibiofilm, and antiurease activity against pathogenic microorganisms. *T. polium* extracts were found to have antimicrobial and antibiofilm activity, against both Gram-positive and Gram-negative bacteria.

Therefore, we believe that the results obtained will contribute to the prevention and treatment of infectious diseases by creating new approaches, reducing the use of antibiotics and obtaining complementary natural products.

Declarations

Ethical Approval Certificate

This study does not require an ethical approval certificate as it does not involve in vivo procedures.

Author Contribution Statement

PR: Project administration, writing, supervision, methodology, investigation, conceptualization, review and editing

SŞ: Project administration, writing, methodology, investigation

ER: Experimental design, methodology, formal analysis, supervision

IŞ: Collecting and identifying the plant

TT: Methodology, investigation, supervision

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Conflict of Interest

The authors declare no conflict of interest.

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