



ANTIMICROBIAL, ANTIBIOFILM, ANTI-QUORUM SENSING AND ANTIOXIDANT ACTIVITIES OF SOME EDIBLE ASTERACEAE MEMBERS

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ABSTRACT

In this study, antimicrobial, antioxidant and anti-quorum sensing activities of *S. oleraceus*, *T. scaturiginosum*, *T. bithynicum* and *L. tuberosus* were investigated. *T. scaturiginosum* and *T. bithynicum* were effective in terms of antimicrobial activity. The highest antibiofilm inhibition was shown by 33.30% aqueous extract of *S. oleraceus* on *P. aeruginosa* ATCC 27853. While *S. oleraceus* aqueous extract showed 35.32% violacein inhibition, *T. bithynicum* had a zone diameter of 13 mm for quorum sensing inhibition. *L. tuberosus* ethanol extract was found remarkable with its 52.5% anti-swarming activity. The most effective plant in terms of antioxidant activity was determined as *L. tuberosus*. This value is IC₅₀ 4.36 mg/mL for DPPH and 67.64% for FTC activity. It has been shown that these edible plants may be suitable candidates for reducing microorganismal resistance and using them in the treatment of diseases.

Keywords: Antimicrobial, antibiofilm, anti-quorum sensing, antioxidant, edible plants

BAZI YENİLEBİLİR ASTERACEAE ÜYELERİNİN ANTİMİKROBİYAL, ANTİBİYOFİLM, ANTI-QUORUM SENSİNG VE ANTIOKSİDAN AKTİVİTELERİ

ÖZ

Bu çalışmada, *S. oleraceus*, *T. scaturiginosum*, *T. bithynicum* ve *L. tuberosus*'un antimikrobiyal, antioksidan ve anti-quorum sensing aktivitesi araştırılmıştır. *T. scaturiginosum* ve *T. bithynicum* antimikrobiyal aktivite açısından etkili bulunmuştur. En yüksek antibiyofilm inhibisyonunu *P. aeruginosa* ATCC 27853 üzerinde *S. oleraceus*'un %33.30'luk sulu ekstraktı göstermiştir. *S. oleraceus* sulu ekstraktı %35.32 viyolasin inhibisyonu gösterirken, *T. bithynicum* quorum sensing inhibisyonu için 13 mm'lik zon çapı

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göstermiştir. *L. tuberosus* etanol ekstraktı, %52.5 anti-swarming aktivitesi ile dikkat çekici bulunmuştur. Antioksidan aktivite açısından en etkili bitki *L. tuberosus* olarak tespit edilmiştir. Bu değer DPPH için 4.36 mg/mL IC₅₀ ve FTC aktivitesi için %67.64'tür. Elde edilen bulgular yenilebilir bitkilerin mikroorganizma direncinin azaltılması ve hastalıkların tedavisinde kullanılması için uygun adaylar olabileceği göstermiştir.

Anahtar kelimeler: Antimikrobiyal, antibiyofilm, anti-quorum sensing, antioksidan, yenilebilir bitkiler

INTRODUCTION

Infectious diseases pose an important health problem for both society and individuals. One of the major reasons for this problem is the rise of acquired bacterial resistance to antibiotics, a major worldwide health threat facing the world today (Hemeg et al., 2020). As antibiotic resistance increases over time, attention is increasing for plants used as herbal remedies in traditional medicine, which have the potential to contain biologically active compounds with strong antibacterial and antifungal properties (Erfan and Marouf, 2019; Singh et al., 2023).

Medicinal plants have been exploited for centuries, laying the construction for modern pharmacology. Over the centuries, humans have employed these plants in the cure of varied illnesses, contributing significantly to the development of contemporary medical practices (Pammi et al., 2023). Additionally, medicinal plants, grains, vegetables, and fruits contain bioactive organic chemical compounds. These compounds play important defensive roles both in metabolic and genetic dysfunction of the host, as well as in infections and chronic diseases. Revealing the chemical content of medicinal plants has enabled the detection of various bioactive compounds such as saponins, phenols, alkaloids, tannins, anthraquinone, lignin, organic acids, volatile oils, and polysaccharides (Lambo et al., 2024). Phytochemicals have many therapeutic potentials as antioxidant, antimicrobial, anticancer, anti-inflammatory, immune enhancing, neuropharmacological agents. (Ugboko et al., 2020). The Asteraceae family, typically known as the sunflower family, is one of the largest flowering plants. Many members of the Asteraceae family have been cultivated for edible and medicinal purposes for over 3000 years. It is most common in arid and semi-arid subtropical regions but is known and distributed worldwide.

These plants demonstrate a broad activity, including, hepatoprotective, antioxidant, antimicrobial, and anti-inflammatory properties (Garcia-Oliveira et al., 2021). *Sonchus oleraceus* L. (Eşek halvesi), *Taraxacum scaturiginosum* G. HAGL. (Meyram göbeği), *Taraxacum bithynicum* DC. (Sarı çiçekli ot), and *Leontodon tuberosus* L. (Yumaklı ot) are the Asteraceae members that can be edible plants of the Muğla region and are also used in conventional treatment for the remedy of different ailments. Based on current knowledge, there are a few studies on the antimicrobial and antioxidant activities of *S. oleraceus*, *T. scaturiginosum*, *T. bithynicum*, and *L. tuberosus* there is no research on their antibiofilm and quorum-sensing inhibitory effects, including violacein production, swarming inhibition. This study aims to investigate the antimicrobial, antioxidant, antibiofilm, and quorum-sensing inhibitory effects of ethanol and aqueous extracts from four plants that grow in the Muğla region and are used as food, focusing on their impact on clinically important microorganisms.

MATERIALS AND METHODS

Preparation of Plant Extracts

Plants subject to study: *Sonchus oleraceus* L., *Taraxacum scaturiginosum* G. HAGL., *Taraxacum bithynicum* DC., and *Leontodon tuberosus* L. These species, belonging to the Asteraceae family, were collected from Muğla the southwestern region of Türkiye. The plant materials were identified by Assistant Professor Mehtap Dönmez Şahin from Uşak University. The plants were dried and subjected to extraction processes. A Soxhlet device was used for ethanol extract and a lyophilizer was used for aqueous extract.

Microbial Strains

Staphylococcus aureus ATCC 25923, *Micrococcus luteus* NRLL B-4375, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa*

ATCC 27853, *Candida albicans* ATCC 10239, and multiresistant strains of *Pseudomonas aeruginosa*, *Pseudomonas fluorescens* and various species of *Staphylococcus*, *Chromobacterium violaceum* CV 12472,

Chromobacterium violaceum CV026, and *Pseudomonas aeruginosa* PA01. Microbial strains used in this study were given at Table 1.

Table 1. Microbial strains and growth conditions

Test strains	Growth conditions
Gram positive	Nutrient Broth (NB)
<i>Bacillus subtilis</i> ATCC 6633	37°C, 24 h
<i>Micrococcus luteus</i> NRRL B-4375	
<i>Staphylococcus aureus</i> ATCC 25923	
<i>Staphylococcus aureus</i> MU 38	
<i>Staphylococcus aureus</i> MU 40	
<i>Staphylococcus</i> MU 46	
<i>Staphylococcus</i> MU 47	
<i>Staphylococcus epidermidis</i> MU 30	
Gram negative	Nutrient Broth (NB)
<i>Escherichia coli</i> ATCC 25922	30°C, 24 h
<i>Pseudomonas aeruginosa</i> ATCC 27853	
<i>Pseudomonas aeruginosa</i> MU 187	
<i>Pseudomonas aeruginosa</i> MU 188	
<i>Pseudomonas aeruginosa</i> MU 189	
<i>Pseudomonas fluorescens</i> MU 180	
<i>Pseudomonas fluorescens</i> MU 181	
<i>Pseudomonas aeruginosa</i> PA01	
<i>Chromobacterium violaceum</i> CV026	Luria Bertani (LB) Broth 30°C, 24-48 h
<i>Chromobacterium violaceum</i> CV 12472	Luria Bertani (LB) Broth 30°C, 24 h
Yeast	Saboraud Dextrose Broth'da (SDB) 30°C, 24-48 h
<i>C. albicans</i> ATCC 10239	

Determination of Antimicrobial Activity

Disc diffusion method (Bauer et al., 1966; Collins et al., 1995; Murray et al., 1995) and minimum inhibition concentration (MIC) (CLSI, 2006) were used for antimicrobial activity. In the disc diffusion test, discs impregnated with plant extracts (20 µL) were placed on plates containing bacteria (100 µL) spread on Mueller-Hinton agar. For MIC, plant extracts in the range of 20-125 mg/mL were added to the bacteria inoculated in Mueller Hinton Broth. The experiment was performed in 96-well microplates. The bacterial density for both tests was 5×10^5 colony forming units (CFU)/mL. As a result of appropriate incubation conditions, the inhibition zone (mm) and MIC were measured.

Antibiofilm Activity

Biofilm inhibition of MIC and sub-MIC values of plant extracts on bacteria were measured. Briefly, 1% of overnight cultures (OD adjusted to 0.4 at

600 nm) of test pathogens were added into 200 µL of fresh TSB medium and cultivated in the presence/absence of extract. The wells containing only TSB served as control. After two days of incubation conditions at the appropriate temperature, bacteria were evacuated from the wells. The remaining bacteria were subsequently stained with 0.1% crystal violet solution for 10 min at room temperature. The wells were washed to remove the crystal violet solution. 95% ethanol was added to the wells for Gram-negative bacteria and *C. albicans*, and 33% acetic acid was added to the wells for Gram-positive bacteria. Measuring was made at 550 nm on a microreader (Merritt et al., 2005). Antibiofilm activity was calculated with the following formula:

$$\text{Biofilm inhibition (\%)} = \frac{\text{OD 550 control} - \text{OD550 sample}}{\text{OD 550 control}} \times 100$$

Anti-Quorum sensing activity

Violacein inhibition assay using C. violaceum CV12472
C. violaceum CV12472 was inoculated into LB broth in microplate wells and added plant extracts. It was incubated at 37°C for 24 hours. Sub-MIC concentrations of the extracts were read in a microreader to examine violacein inhibition (Tamfu et al., 2020). Violacein pigment production inhibition percentage was calculated by the following formula:

$$\text{Violacein inhibition (\%)} = \frac{\text{OD 585 control} - \text{OD585 sample}}{\text{OD 585 control}} \times 100$$

Bioassay for Quorum sensing inhibition (QSI) activity using CV026

The method of Koh & Tham (2011) is slightly modified. *C. violaceum* CV026 and C₆HSL were added to Molten Soft Top Agar, mixed and poured onto LBA. Wells were made in the solidified medium. Sub-MIC concentrations of plant extracts were put in each well. The plates were incubated at 30°C for 3 days. Halo formation on the purple-colored medium was evaluated as Quorum sensing inhibition and zone diameters were measured in mm.

Swarming motility inhibition on Pseudomonas aeruginosa PA01

Plant extracts were added to plates containing 1% peptone, 0.5% NaCl, 0.5% agar and 0.5% D-glucose and mixed. Thereupon, central point-inoculated of *P. aeruginosa* PA01 was performed. The plates were incubated at 37°C for 18-24 hours. Inhibition of swarming motility was determined by measuring swarm zones (Packiavathy et al., 2012).

Antioxidant Activity

Antioxidant activity of plant extracts was determined according to two methods: DPPH (1,1-diphenyl-2-picryl hydrazyl) (Yamasaki et al., 1994) and FTC (Ferric thiocyanate) (Kikuzaki and Nakatani, 1993). For DPPH radical scavenging activity, extracts were added to the DPPH solution. It was incubated for 30 minutes and its absorbance was measured at 517 nm. DPPH radical scavenging activity was calculated as IC₅₀. Standards were ascorbic acid and BHT. In FTC, the solution consisting of ethanol, plant extracts,

linoleic acid and buffer was incubated at 40°C. Then, some amount of this solution was taken, ethanol, ammonium thiocyanate and ferric chloride were added, left for 3 minutes and measurements were made at 500 nm. This was repeated at 24-hour intervals until the maximum value of the control was reached.

RESULTS AND DISCUSSION**Antimicrobial Activity**

The present investigation determined the antimicrobial activity of aqueous and ethanol extracts of *S. oleraceus*, *T. scaturiginosum*, *T. bithynicum*, and *L. tuberosus* plants against various microorganisms. Antimicrobial activity was performed by two methods: disc diffusion and minimum inhibition concentration. The findings are exhibited in Table 2. According to the disc diffusion method, it was determined that aqueous extracts showed limited activity against the test microorganisms, and ethanol extracts did not show an inhibition zone. However, it is noteworthy that all of the aqueous extracts are especially active against *S. aureus* MU 47 and the highest activity belongs to *T. bithynicum* (14 mm zone diameter). When looking at the MIC, it is understood that plant extracts generally inhibit test microorganisms in the concentration range of 5-20 mg/mL (Table 2). In particular, it was determined that the alcohol extract of *T. scaturiginosum* inhibited *S. aureus* ATCC 25923 with a concentration as low as 5 mg/mL. Finally, in terms of antimicrobial activity, it can be said that not all extracts are active against fungi, but are more effective against gram-positive bacteria than gram-negative bacteria. In this context, it was determined that all extracts used in our study had more effective antimicrobial potential against gram-positive bacteria. Modern science utilizes plants as probable sources of medicines to treat or prevent illnesses. Antimicrobial resistance, highlighted as a global health crisis by the WHO, demands concerted action from nations and societies alike. The rise of multidrug-resistant microbes (MDR) has triggered socioeconomic turmoil and exacerbated public health challenges worldwide. In developing regions, these resilient microorganisms undermine the effectiveness of many affordable antimicrobial treatments (Arslan,

2023). Given this escalating threat, exploring medicinal plants as potential sources of new antimicrobial drugs is imperative to combat these MDR strains (Kebede et al., 2021). The study reveals that extracts from numerous medicinal plants exhibit promising antimicrobial properties, suggesting a fertile ground for discovering novel treatments against diseases caused by pathogenic microbes.

In particular, the inhibition activity of *T. bithynicum* and *T. scaturiginosum* against different strains of *S. aureus* reveals the potential of the plants extracts (Table 2). These findings echo previous research advocating for plant-based therapeutic agents as viable substitutes, alternatives, or complementary treatments for infectious diseases (Seğmenoğlu and Sevindik, 2022; Naz et al., 2022; Machado et al., 2023).

Table 2. Antimicrobial activity of extracts

Microorganisms	Extracts	<i>S.oleraceus</i>		<i>T. scaturiginosum</i>		<i>T.bithynicum</i>		<i>L.tuberosus</i>	
		DD (mm)	MIC (mg/mL)	DD (mm)	MIC (mg/mL)	DD (mm)	MIC (mg/mL)	DD (mm)	MIC (mg/mL)
<i>B.subtilis</i> ATCC 6633	Aqueous	-	-	-	-	-	-	-	-
	Ethanol	-	20	-	10	-	-	-	-
<i>M.luteus</i> NRRL B-4375	Aqueous	-	-	-	-	-	-	-	-
	Ethanol	-	10	-	10	-	-	-	-
<i>S.aureus</i> ATCC 25923	Aqueous	11	20	-	-	-	-	-	-
	Ethanol	-	20	-	5	-	10	-	-
<i>S.aureus</i> MU 38	Aqueous	-	-	-	-	9	-	8	-
	Ethanol	-	20	-	10	-	10	-	-
<i>S.aureus</i> MU 40	Aqueous	-	-	-	-	9	-	-	-
	Ethanol	-	-	-	-	-	-	-	-
<i>S.aureus</i> MU 46	Aqueous	10	20	-	-	-	-	-	-
	Ethanol	-	-	-	10	-	10	-	-
<i>S.aureus</i> MU 47	Aqueous	10	-	12	20	14	20	11	-
	Ethanol	-	-	-	-	-	20	-	-
<i>S.epidermidis</i> MU 30	Aqueous	-	-	8	-	9	-	8	-
	Ethanol	-	20	-	-	-	-	-	-
<i>E.coli</i> ATCC 25922	Aqueous	-	20	-	-	-	-	-	-
	Ethanol	-	20	-	-	-	-	-	-
<i>P.aeruginosa</i> ATCC 27853	Aqueous	-	-	-	-	-	-	9	-
	Ethanol	-	-	-	-	-	-	-	-
<i>P.aeruginosa</i> MU 187	Aqueous	-	-	-	-	-	-	-	-
	Ethanol	-	-	-	-	-	-	-	-
<i>P.aeruginosa</i> MU 188	Aqueous	-	-	-	-	-	-	-	-
	Ethanol	-	20	-	-	-	-	-	-
<i>P.aeruginosa</i> MU 189	Aqueous	-	20	-	-	-	-	-	-
	Ethanol	-	20	-	20	-	-	-	-
<i>P.fluorescens</i> MU 180	Aqueous	-	-	7	-	7	-	-	-
	Ethanol	-	-	-	-	-	-	-	-
<i>P.fluorescens</i> MU 181	Aqueous	-	-	-	-	-	-	-	-
	Ethanol	-	10	-	20	-	-	-	-
<i>C. albicans</i> ATCC 10239	Aqueous	-	-	-	-	-	-	-	-
	Ethanol	-	-	-	-	-	-	-	-

(-) No inhibition, (DD) Disc diffusion, (MIC) Minimum inhibition concentration

Antibiofilm activity

In the study, sub-MIC antibiofilm effects of all plant extracts against test strains were investigated. The obtained results are shown in Table 3. According to the findings, among all of

the extracts, the aqueous extract of *S. oleraceus* was observed to inhibit biofilm formation *P.aeruginosa* ATCC 27853 with 33.3 % (at 10 mg/mL concentration).

Table 3. Antibiofilm activity of extracts

Microorganisms	Extracts	<i>S.oleraceus</i>	<i>T. scaturiginosum</i>	<i>T.bithynicum</i>	<i>L.tuberosus</i>
		Concentration (mg/mL) / Inhibition (%)			
<i>B.subtilis</i> ATCC 6633	Aqueous	-	-	-	-
	Ethanol	-	10 (7.19)	-	-
<i>M.luteus</i> NRRL B-4375	Aqueous	-	-	-	-
	Ethanol	-	10 (11.87)	-	-
<i>S.aureus</i> ATCC 25923	Aqueous	-	-	-	-
	Ethanol	-	-	-	-
<i>S.aureus</i> MU 38	Aqueous	-	-	10 (21.2)	-
	Ethanol	-	10 (13.08)	5 (14.39) 2,5 (5.24)	-
<i>S.aureus</i> MU 40	Aqueous	-	-	-	-
	Ethanol	-	-	-	-
<i>S.aureus</i> MU 46	Aqueous	-	-	-	-
	Ethanol	-	-	-	-
<i>S.aureus</i> MU 47	Aqueous	-	10 (32.51) 5 (15.4)	10 (9.65)	-
	Ethanol	-	-	-	-
<i>S.epidermidis</i> MU 30	Aqueous	-	-	-	-
	Ethanol	-	-	-	-
<i>E.coli</i> ATCC 25922	Aqueous	10 (9.95)	-	-	-
	Ethanol	-	-	-	-
<i>P.aeruginosa</i> ATCC 27853	Aqueous	10 (33.3) 5 (15.51)	-	-	-
	Ethanol	20 (17.51)	-	-	-
<i>P.aeruginosa</i> MU 187	Aqueous	-	-	-	-
	Ethanol	-	-	-	-
<i>P.aeruginosa</i> MU 188	Aqueous	-	-	-	-
	Ethanol	-	-	-	-
<i>P.aeruginosa</i> MU 189	Aqueous	10 (23.91) 5 (10.17)	-	-	-
	Ethanol	20 (6.89)	20 (26.20) 10 (10.34)	20 (6.20)	-
<i>P.fluorescens</i> MU 180	Aqueous	-	-	-	-
	Ethanol	-	-	-	-
<i>P.fluorescens</i> MU 181	Aqueous	10 (11.51)	-	10 (23.5) 5 (8.88)	-
	Ethanol	-	-	-	-
<i>C. albicans</i> ATCC 10239	Aqueous	-	-	-	-
	Ethanol	-	-	-	20 (11.47)

(-) No inhibition

Microorganisms, such as bacteria and fungi, are implicated in nearly 87% of healthcare-associated infections affecting humans. The virulence of these microorganisms stems from various mechanisms, including the modulation of biofilm production, crucial for colonization and infection development. Biofilm formation contributes significantly to approximately 80% of infections caused by bacteria and fungus in human tissue, causing persistent infections, especially in chronic wounds. Given the looming threat of antimicrobial resistance, which could potentially

cause 10 million deaths annually by 2050, the urgency to develop novel antibiofilm therapies alongside conventional antibiotics has never been more critical (Lopes et al., 2021).

In current work, the antibiofilm activity of plants frequently used in traditional medicine and nutrition was demonstrated and especially the activity of *S. oleraceus* aqueous extract revealed that this plant has a potential in the production of new therapeutic for diseases. There is no study on the antibiofilm activity of *S. oleraceus* in the literature,

so it is not possible to make a reliable comparison. However, this study significantly contributes to the existing literature and underscores its importance for future investigations.

Anti-Quorum sensing activity

Quorum sensing (QS), a density-dependent mechanism in gram-negative bacteria mediated by autoinducers like AHLs, orchestrates gene expression, including virulence factors crucial for pathogenesis. Targeting QS with natural and herbal compounds has emerged as a strategy to control bacterial virulence (Li et al., 2022). In this study, the anti-QS inhibition activity of plants was determined by three different methods: Violacein inhibition, QS inhibition and Swarming motility.

C. violaceum CV12472, a gram-negative bacterium, produces purple violacein pigment indicative of

QS activity and protects its membrane against oxidative stress. The purple violacein produced can be measured and reflects the quorum-sensing process in bacteria. Prior to violacein inhibition, the MIC values of the extracts were determined against strain *C. violaceum* CV12472 and the ability of the extracts to inhibit violacein production was measured at sub-MIC concentrations. The results obtained are reported in Table 4. MIC values varied between 1.25 mg/mL and >25 mg/mL for all extracts. The sub-MIC concentrations were used to determine the percent violacein inhibition of the extracts. The most active *S. oleraceus* aqueous extract showed 35.32% inhibition at a concentration of 2.5 mg/mL. Also, all extracts of *S. oleraceus* and alcohol extract of *T. scaturiginosum* are two herbs that inhibit violacein.

Table 4. Anti-quorum sensing activity by extracts

Plant extracts		MIC (mg/mL)	VI (%) / Con (mg/mL)	MIC (mg/mL)	QSI (mm) / Con (mg/mL)	SI (%)
<i>S. oleraceus</i>	Aqueous	5	35.32 / 2.5 20.32 / 1.25	100	-	19
	Ethanol	25	5.54 / 12.5	2	-	4.8
<i>T. scaturiginosum</i>	Aqueous	>25	-	100	-	-
	Ethanol	12.5	14.26 / 10	2	-	-
	Aqueous	>25	-	100	-	19
<i>T. bithynicum</i>	Ethanol	>25	-	0.8	13 / 0.75 11 / 0.50 9 / 0.25	9.5
	Aqueous	10	-	100	-	-
<i>L. tuberosus</i>	Ethanol	>25	-	2	12 / 1 10 / 0.5	52.5

*(VI) Violacein inhibition, (Con) Concentration, (QSI) Quorum sensing inhibition, (SI) Swarming inhibition, (-) No inhibition

The mutant strain *C. violaceum* CV026, supplemented with external AHL, served to detect QS impairments through quorum sensing inhibition zones. These bacteria can be used to find impairments in quorum sensing by determining quorum sensing inhibition zones. In test plates with purple lawn colour produced by activated *C. violaceum* CV026 bacteria, the formation of a cream or yellowish halo around the well was an indicator of QS inhibition. The quorum sensing inhibition zones were determined at sub-MIC concentrations and

diameters were measured in millimetres (Table 4). Only ethanol extract of *T. bithynicum* and *L. tuberosus* exhibited anti-QS activity. *T. bithynicum* ethanol extract had the highest QS inhibition zone diameter (13 mm) at the low concentration of 0.75 mg/mL.

During bacterial communication, small signaling molecules produced inside cells diffuse into the extracellular space. Thus, it mediates the behavior of colonies. Phenotypic factors regulated during this process include bacterial motility. The

motility of microorganisms is associated with QS-mediated biofilm formation. *P. aeruginosa* PA01 uses swarming motility to invade and colonize surfaces. We examined the anti-QS potential of extracts from four plants against QS-dependent swarming motility in this strain. The swarming motility of *P. aeruginosa* PA01 strain was evaluated at a concentration of 100 mg/mL and the results are shown in Table 4. The results showed that the PA01 pathogen inhibited swarming behavior to different levels among test plants, except for *T. scaturiginosum*. Contrary to the fact that the aqueous extract of *L. tuberosus* showed no anti-swarming activity, the ethanol extract of this plant showed the highest level of inhibition in the migration of PA01, with 52%.

Antioxidant Activity

The concept of oxidative stress, a relatively recent focus in medical sciences over the past three decades, is intricately involved in the pathophysiology of prevalent diseases such as diabetes, hypertension, pre-eclampsia, atherosclerosis, acute renal failure, Alzheimer's, and Parkinson's disease. Cells utilize oxygen in metabolic processes, generating reactive oxygen

species (ROS) that can be harmful. Normally, the production and elimination of ROS are balanced; however, when this equilibrium is disrupted typically due to an excess of pro-oxidants or a deficiency in antioxidants oxidative stress occurs. Elevated ROS levels within cells profoundly impact cellular function, contributing to cellular dysfunction, accelerated aging, and disease progression. Antioxidants play a crucial role in biological cells by neutralizing free radicals, which are highly reactive molecules that can inflict damage on living organisms. (Munteanu et al., 2021).

This study used DPPH free radical scavenging and Ferric thiocyanate (FTC) method for antioxidant activity. Table 5 gives information about the antioxidant activity values of all extracts. Test plants showed radical scavenging activity in various ranges. The aqueous extract of *L. tuberosus* showed the best radical scavenging activity with an IC₅₀ value of 4.36±0.58 mg/mL. However, the antioxidative capacity of all extracts was weaker than the standard substances. The IC₅₀ values of BHT and α-tocopherol are 0.487 mg/mL and 1.75 mg/mL, respectively.

Table 5. Antioxidant activity of extracts

Plant extracts		DPPH	FTC
		IC ₅₀ (mg/mL)	Inhibition %
<i>S. oleraceus</i>	Aqueous	63.47±2.45	54.76±1.72
	Ethanol	56.67±1.18	27.55±0.63
<i>T. scaturiginosum</i>	Aqueous	46.32±0.94	62.72±2.80
	Ethanol	28.88±0.22	19.91±1.02
<i>T. bithynicum</i>	Aqueous	6.96±0.55	64.27±2.15
	Ethanol	44.3±1.14	28.46±0.44
<i>L. tuberosus</i>	Aqueous	4.36±0.58	67.64±2.08
	Ethanol	43.54±1.22	22.88±0.34
Standards	BHT	0.487±0.014	63.36±2.52
	α-tocopherol	1.75±0.04	39.96±1.65
	Ascorbic acid	NT	77.67±2.81

(NT) Not tested

FTC is a method by which the amount of peroxide in the initial stages of lipid peroxidation is measured. FTC test results displayed that aqueous extracts were more effective than alcohol extracts, especially the aqueous extract of *L. tuberosus* showed the highest inhibition with 67.64%. Moreover, it appears that the aqueous

extract of *L. tuberosus* has more effective antioxidant potential in preventing lipid peroxidation chain reactions and free radical scavenging. In the literature, data on the antioxidant activity of natural plant extracts can be found (Benitez et al., 2023; Christou et al., 2024; Abd-El-Aziz et al., 2024). The results

obtained are consistent with each other. Therefore, it is thought that it would be appropriate to include these plants used as food in diets as antioxidant agents.

CONCLUSION

There is limited knowledge and research on wild species traditionally consumed as food. In conclusion, the antimicrobial and anti-quorum sensing activities of edible plants grown in the Muğla region and included in local diets are promising as important antioxidant agents, especially in reducing the damage caused by reactive oxygen species. Therefore, it has emerged that these plants and the compounds they contain, which traditionally have an important potential, can be evaluated in a broader perspective.

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AUTHOR CONTRIBUTIONS

Özgür Ceylan: Material collection, organization and data analysis of all studies; Aysel Uğur: Antimicrobial, antibiofilm and antioxidant activities; Nurdan Saraç: Antimicrobial, antibiofilm and antioxidant activities; Büşra Eroğlu Arslan: Anti-Quorum sensing activity and writing

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