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THE ANTIMICROBIAL ACTIVITY OF *HYPERICUM PERFORATUM* L. FLOWER EXTRACT AGAINST FOOD PATHOGENS AND ITS NON-ENZYMATIC ANTIOXIDANT ACTIVITY

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ABSTRACT. Foodborne pathogens pose a significant hazard to food safety. Most cases of foodborne illnesses are caused by bacterial pathogens that have infiltrated the food chain at some point, from farm to kitchen. According to the World Health Organization (WHO), approximately one-third of individuals in developed countries are affected by foodborne pathogens each year. Although there are studies on *Hypericum perforatum* L. in the literature, research in Turkey remains limited. Therefore, the aim is to contribute to the literature by studying *H. perforatum* samples from the Yaraş region of Muğla province in Turkey. This study specifically aims to investigate the antimicrobial activities against foodborne pathogens and the antioxidant activity of *H. perforatum* in Muğla. The *in vitro* antimicrobial activities of flower components from plants grown in Mugla were evaluated using the disc diffusion method and broth dilution test. Additionally, the extracts underwent ABTS (2,2′-azinobis-(3-ethyl benzothiazoline-6-sulfonic acid)) free-radical testing to evaluate their antioxidant activity. The extract exhibited a maximum inhibition zone of 16 mm against *Staphylococcus aureus* and *Listeria monocytogenes*. Notably, *S. aureus* and *L. monocytogenes* demonstrated the lowest sensitivity to *H. perforatum* methanol extract (1625 µg/mL). The methanol extract displayed moderate antioxidant activity, with a 53% ABTS radical scavenging capacity. Consequently, the extracts of *H. perforatum* exhibited both antimicrobial and antioxidant potential.

Keywords: food pathogen, *Hypericum*, antimicrobial activity, antioxidant activity.

INTRODUCTION

Free radicals are atoms or molecules carrying unpaired electrons, highly reactive and capable of rapidly engaging in exchange reactions that destabilize other molecules and generate many more free radicals (MANDAL *et al.*, 2009). In biological systems, free radicals are often derived from oxygen, nitrogen, and sulfur molecules. These free radicals are components of molecular groups referred to as reactive oxygen species (ROS), reactive nitrogen species (RNS), and reactive sulfur species (RSS) (VAJRAGUPTA *et al.*, 2004). If not neutralized, free radicals can cause damage to all cellular macromolecules, including proteins, carbohydrates,

and nucleic acids (NGUYEN *et al*., 2017). Their destructive effects alter the physiological functioning of the cell (YOUNG and WOODSIDE, 2001). The accumulation of free radicals initiates various neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, muscular dystrophy, and atherosclerosis (SINGH and JIALAL, 2006).

Neutralizing free radicals is made possible through antioxidants. The mechanistic definitions of antioxidants generally focus on their ability to act as hydrogen donors or electron donors (LÜ *et al*., 2010). Synthetic antioxidants are chemically synthesized compounds that do not naturally occur in nature and are added to foods as preservatives to help prevent lipid oxidation (ATTA *et al*., 2017). Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tert-butylhydroquinone (TBHQ) are widely used as antioxidants in the food industry. However, synthetic antioxidants such as BHA, BHT, n-propyl gallate (PG), chemical precursors, and toxic solvents, lead to the formation of hazardous by-products with a potential health risk (FLIEGER *et al*., 2021).

With the increasing consumer demand for natural antioxidants, research continues to discover non-toxic and non-carcinogenic alternatives from natural sources that can meet consumer demands and serve as alternatives to synthetic antioxidants (BANDONIENE *et al*., 2002). Nature-identical antioxidants (tocopherols, phospholipids, carotenoids, ascorbic acid, and their esters) are recommended due to their safe and reliable purity, relatively low cost, and easy availability. They are generally recognized as safe (POKORNY, 2007). Compared to synthetic antioxidants, natural antioxidants derived from plants are considered more acceptable, reliable, and safer. This appeal addresses a nature-conscious society that seeks natural remedies for improving health and preventing diseases (FIRUZI *et al*., 2011).

Plant-derived antioxidants have been shown to function as single and triple oxygen quenchers, free radical scavengers, peroxide decomposers, enzyme inhibitors, and synergists (MANDAL *et al*., 2009). Flavonoids, tannins, and other phenolic compounds found in plant-based foods are also potential antioxidants (RECORD *et al*., 2001; AMAROWICZ and PEGG, 2019).

Medicinal plants produce secondary metabolites for defensive purposes, many of which have antimicrobial properties, and are still commonly used in traditional medicine (KONGKHAM *et al*., 2020). Recognizing their potential, the World Health Organization (WHO) advocates for the thorough investigation of these plants to gain deeper insights into their properties, safety, and efficacy (NASCIMENTO *et al*., 2000).

Hypericum perforatum L. is a plant with yellow flowers and is naturally found in various locations around the world including West Asia, Europe, and North Africa (WEBER *et al*., 2008). *H. perforatum* is distributed in many geographical regions in Turkey such as Marmara, Aegean, Mediterranean, Black Sea, Eastern and Southeastern Anatolia (GÜNER and ÖZHATAY, 2000). It grows on roadsides, forest edges, neglected uncultivated fields, meadows, and rocky and stony places (KAÇAR and AZKAN, 2005). Flowering time is between June and September. *Hypericum* L. belongs to the Hypericaceae family. *Hypericum* has 98 species and 119 taxa, 49 of which are endemic. The flowers contain five petals with many stamens protruding (CEYLAN *et al*., 2005). The plant is the Mediterranean element (BİNGÖL *et a*l., 2011). *H. perforatum* is a well-known medicinal plant that has been in use for many years (DI CARLO *et al*., 2001). *H. perforatum* is a plant known as St. John's wort. Antioxidant and antimicrobial activity, and other biological properties of the plant have been reported in the literature (JAKOVLJEVIĆ *et al*., 2000; DI CARLO *et al*., 2001; POPOVIĆ *et al*., 2002; LUO *et al*., 2004; RADULOVIĆ *et al*., 2007; SPITELLER *et al*., 2008).

Extracts from *H. perforatum* are known to contain compounds from six major natural product groups: naphtodianthrones, acylphloroglucinols, flavonol glycosides, biflavones, proanthocyanidins, and phenylpropanes (GREESON *et al*., 2001; GUDZIC *et al*., 2001; PETRAKIS *et al*., 2005; SAROGLOU *et al*., 2007; SUNTAR *et al*., 2010). Additionally, it has been reported that hypericin and hyperforin compounds found in *H. perforatum* are promising in treating diseases (BREYER *et al*., 2007; LINDE, 2009). Flavonoids are particularly interesting due to their antioxidative properties, i.e. excellent radical scavenging ability (BREYER *et al*., 2007).

There are numerous studies in the literature on the biological activities of plants. The plants from *Hypericum* genus are distributed worldwide and in Turkey. *H. perforatum* is a spontaneously growing plant in almost all of Turkey (ASLAN, 2012). Although there are studies on *Hypericum* species in the literature, research in Turkey remains limited. Therefore, the aim is to contribute to the literature by studying *Hypericum* samples from the Yaraş region of Muğla province in Turkey. This study specifically aims to investigate the antimicrobial activities against foodborne pathogens and the antioxidant activity of *H. perforatum* in Muğla.

MATERIALS AND METHODS

Plant material and extraction

In May 2014, *H. perforatum* flowers were collected from the Yaraş region of Muğla at an altitude of approximately 700 meters above sea level. The coordinates of the region are 37.178211 latitude and 28.464499 longitude. The identification of this plant was carried out by Dr. Olcay Ceylan, and the plant specimen has been preserved in the Herbarium of the Department of Biology, Mugla Sitki Kocman University, with Herbarium No: MUH5692. The plant's identification was performed using the Flora of Turkey (DAVIS, 1988).

The flowers of the plant underwent a thorough washing process, including two rinses in running water and one rinse in sterile water. Following this, the materials were air-dried. Subsequently, they were pulverized using a blender and prepared for the study. The resulting samples were stored at room temperature until the initial preparation, after which they were transferred to a temperature-controlled environment at 4 ˚C for subsequent analysis.

To extract the plant samples, 30 grams of air-dried and powdered flowers were subjected to methanol (Merck) extraction using the Soxhlet apparatus (Isotex). 300 mL methanol was added to the Soxhlet apparatus, and the last concentration was adjusted to 100 mg/mL. Then all experiments were conducted over a 4-hour period. After obtaining the extracts, they were evaporated (Heidolph) and then transferred into sterile amber bottles with their respective solvents (10 mL) to prevent the extract from drying out. These bottles were stored in a refrigerator until used in the study.

Organisms and cultivation

This study focused on the investigation of foodborne pathogenic organisms, specifically *Bacillus subtilis* RSKK245, *Staphylococcus aureus* RSKK2392, *Salmonella* Typhimurium RSKK19, *Enterococcus faecalis* ATCC8093, *Escherichia coli* ATCC11229, *Listeria monocytogenes* ATCC7644, *Yersinia enterocolitica* NCTC11174, and *Candida albicans* RSKK02029. The strains used in this study were obtained from well-established institutions, including ATCC (American Type Culture Collection, USA), NCTC (National Type Culture Collection), and RSKK (Refik Saydam National Type Culture Collection, Turkey).

To conduct the tests, bacteria were cultured in Mueller-Hinton Broth (MHB)medium (Merck) and incubated at 37˚C for 24 hours, whereas *C. albicans* was cultured in Sabouraud Dextrose Broth (Merck) and incubated at 30˚C for 48 hours.

Measurement of antimicrobial activity

The antimicrobial activity of flower extract was assessed using the Kirby-Bauer method (BAUER *et al*., 1966). Methanol was employed as the organic solvent in this study. The extract was applied in a concentration and quantity of 35 µL of 100 mg/mL.

The bacterial and *C. albicans* cultures were adjusted to the 0.5 McFarland standard to achieve consistent turbidity. All experiments were conducted in triplicates, and the results are presented as the mean values. Bacterial cultures were incubated in a 37˚C for 24 hours, while *C. albicans* cultures were incubated for 24 hours in a 30˚C. After the incubation period, the

zones of inhibition around the discs were recorded. Methanol served as the negative control in the study, whereas the positive controls were antibiotics including tetracycline (Bioanalyse; 30 μg), nystatin (Bioanalyse; 100 μg), and penicillin (Bioanalyse; 10 μg).

Measurement of minimum inhibitory concentration (MIC)

An method for evaluating antimicrobial activity involves the Minimum Inhibitory Concentration (MIC) test. MIC is defined as the lowest concentration of the extract that effectively inhibits the growth of bacteria and fungi following an incubation period. The broth dilution test was conducted in accordance with the procedures outlined in the Clinical and the Laboratory Standards Institute (CLSI) standards (CLSI, 2003; CLSI, 2006).

A growth control tube without extract and a sterile control tube without bacterial inoculation were prepared for the study. All cultures were activated in Nutrient Broth (NB) (9 mL) at 37°C for 18 hours. The turbidity of the inoculums was adjusted to the McFarland 0.5 standard. For this test, the final concentrations of the extract used were 6500, 3250, 1625, 812.5, and 406.25 μg/mL. These concentrations were employed to determine the MIC values for the respective plant extract against the tested microorganisms.

Active cultures (100 μL) were inoculated into tubes containing MHB (4.5 mL), and then 0.5 mL of the extract was added. Subsequently, all tubes were incubated at 37°C for 24 hours. At the end of the incubation period, concentrations that inhibited the growth of microorganisms in the tubes were observed. The concentration at which there was a 90% or greater reduction compared to controls was recorded as the MIC value.

Measurement of non-enzymatic antioxidant capacity

The experiments utilized an improved 2,2′-azinobis-(3-ethyl benzothiazoline-6-sulfonic acid (ABTS) (Merck) radical decolorization assay (RE *et al*., 1999). Stock solutions included 7 mM ABTS•+ and 2.45 mM potassium persulfate (Merck). The working solution was prepared by equally mixing these stocks and allowing them to react for 12 hours at room temperature in the dark. This solution was then diluted by adding 10 μL of methanol to 1 mL of the ABTS•+ solution. Absorbance was measured at 734 nm using a spectrophotometer (Optizen), 15 minutes after mixing 10 μL of methanol extract (99.9%) with the ABTS•+ solution. Trolox (Sigma-Aldrich) served as the reference standard, and the results are expressed as mM Trolox equivalents (TE)/g dry weight.

Essential oil isolation and analysis

In this study, 40 grams of the dried plant was taken and distilled in 1000 mL of distilled water by boiling in water with the Clevenger Distillation System for 4 hours. The resulting essential oil was dissolved with hexane. The extract was also dried with $MgSO_4$. A yield of 0.03% (w/w) essential oil was obtained from the dried plant material on a dry basis. The essential oil has been supplied to the GC-MS system. The GC-MS analysis of the essential oil was performed using an Agilent Inert MS Detector 5975 & 6890 GCMS, equipped with a DB35 –MS column (30 m x 0.25 mm x 0.25 μM).

Temperature program:

Oven temperature	60°C	10 min
4 °C/min	220	10 min
1 °C/min	240	0

Gas flow: Helium 1 mL/min

m/z : Scanned between 40-550.

Ion source temperature: 230 °C

Wiley and NIST libraries on the computer were used to identify the components (SCHWOB *et al.,* 2002; BALEA *et al.,* 2020).

Statistical analyses

In this study, the means of the activities were calculated with Excel 2016.

RESULTS

In this study, the methanol extract of *H. perforatum* was subjected to *in vitro* testing against eight foodborne pathogenic microorganisms. The results of the antimicrobial activities of the plant extract are presented in Table 1. Additionally, Table 2 presents the diameters of inhibition zones produced by the reference antibiotics against these microorganisms.

At the conclusion of the antibacterial activity studies, the diameter of the formed inhibition zones was measured in millimeters and recorded. The results revealed that the methanol extract of *H. perforatum* effectively suppressed the growth of four bacterial strains. The largest inhibition zone diameters were observed for *Staphylococcus aureus* and *Listeria monocytogenes*, measuring 16 ± 1.25 mm and 16 ± 0.47 mm, respectively. Furthermore, the methanol extract of this plant exhibited no discernible anticandidal effects against the employed yeast strain. Remarkably, the methanol extract of the flowers demonstrated significant efficacy against both *S. aureus* and *L. monocytogenes*, yielding the maximum zone of inhibition (16 mm). However, the methanol extract did not produce any inhibition zones against three bacterial strains (as shown in Table 1).

Table 1. Antimicrobial activities of *H. perforatum* flower extract (100 mg/mL)

Organisms	**Inhibition zone diameters (mm)**
Bacillus subtilis RSKK245	14 ± 0.58
Staphylococcus aureus RSKK2392	16 ± 1.25
Salmonella Typhimurium RSKK19	10 ± 0.57
Enterococcus faecalis ATCC8093	-
Escherichia coli ATCC11229	-
Listeria monocytogenes ATCC7644	16 ± 0.47
Yersinia enterocolitica NCTC11174	-
Candida albicans RSKK02029	-

(-): zone did not occur

Tetracycline (30µg), nystatin (100µg), and penicillin (10µg) antibiotics were employed as positive controls. Tetracycline exhibited a robust inhibitory effect on the growth of *Yersinia enterocolitica* (Table 2).

Table 3 displays the Minimum Inhibitory Concentrations (MICs) of *H. perforatum* flower extract, as determined utilizing the broth dilution method. Among the tested microorganisms, two bacteria exhibited the lowest sensitivity to the methanol extract of *H. perforatum*, with MIC values of 1625 µg/mL, except for *Salmonella* Typhimurium, which had a MIC value of 6500 µg/mL. The MIC value of *Bacillus subtilis* was 3250 µg/mL.

Table 4 displays the non-enzymatic antioxidant activity of the plant extract assessed using the ABTS radical scavenging method. Trolox served as the positive control, and all values were expressed in terms of the Trolox equivalent. The flower extract at a concentration of 100 mg/mL exhibited 53% inhibition. At the end of the study, the Trolox Equivalent (TE) was determined to be 0.31 mM/g DW.

The results of the chemical analysis of *H. perforatum* essential oil by using GC-MS methods are listed in Figure 1. Fifty-four components were identified, making 71.34% of total oil ingredients. The main components of *H. perforatum* oil were: 1-tetra decene (18.52%), 1-dodecanal (8.23%), β-selinene (7.66%), α-selinene (5.325%), cyclododecane (4.465%), 2-

pentadecanonane (3.46%), trans-betafarnesene (2.57%) and 2-tetra decene (2.155%). The results of the RT values of *H. perforatum* essential oil are listed in Table 5. The components include 1-tetradecene and 2-tetradecene belonging to the alkene group, and 1-dodecanal classified under the long-chain fatty acid aldehyde group. β-selinene, α-selinene, and trans-beta farnesene are part of the sesquiterpene group. Cyclododecane falls within the cycloalkane group. 2-pentadecanonane is associated with the methyl tridecyl ketone group.

Table 2. Standard antibiotics susceptibility of tested microorganisms

Organisms	**Standart antibiotics**		
	Te	**Ns**	**P**
Staphylococcus aureus RSKK2392	(N)	(N)	(N)
Bacillus subtilis RSKK245	(N)	(N)	10 ± 0.58
Listeria monocytogenes ATCC7644	(N)	(N)	10 ± 1
Enterococcus faecalis ATCC8093	(N)	(N)	(-)
Salmonella Typhimurium RSKK19	14 ± 1	(N)	(N)
Yersinia enterocolitica NCTC11174	20 ± 0.58	(N)	(N)
Escherichia coli ATCC11229	14 ± 0.58	(N)	10 ± 1
Candida albicans RSKK02029	(N)	7 ±1.53	(N)

Te: Tetracycline (30µg); Ns: Nystatin (100µg); P: Penicillin (10µg); (N): not tested

Table 3. Minimum inhibitory concentration values of *H. perforatum* flower extract

Organisms	**MIC values** (µg/mL)
Bacillus subtilis RSKK245	3250 ± 0
Staphylococcus aureus RSKK2392	1625 ± 0
Salmonella Typhimurium RSKK19	6500 ± 0
Enterococcus faecalis ATCC8093	-
Escherichia coli ATCC11229	-
Listeria monocytogenes ATCC7644	1625 ± 0
Yersinia enterocolitica NCTC11174	-
Candida albicans RSKK02029	-

(-): no inhibition

Table 4. ABTS radical scavenging capacity of *H. perforatum* flower extract

	Methanol extract (100 mg/mL)
% ABTS radical scavenging	53 ± 0
Trolox equivalent (mM/g DW)	0.31

DW: Dry weight

DISCUSSION

Medicinal plants have traditionally been employed globally in the treatment of various human ailments (CHITME *et al*., 2004). These plants have been acknowledged as abundant reservoirs of biologically active compounds, many of which have served as foundational elements for the advancement of novel pharmaceuticals (PALOMBO, 2011). In this study, *H. perforatum* flowers were selected based on their ethnomedical use.

Table 5. RT values of components of *H. perforatum*

Component No.	Essential oil components	Retention time (RT)	Component No.	Essential oil components	Retention time (RT)
1	Decane-2-methyl	11,344	28	ɣ-cadinene	33,471
2	Undecane	13,788	29	Delta-cadinene	33,529
3	Nonanal	18,573	30	α -muurolene	34,194
4	Dodecane-2 metil	21,476	31	Cis-calamenene	34,299
5	Tridecane	23,102	32	Nerolidol	34,817
6	α-longipinene	26,733	33	1,5-epoxysalvial-4(14) ene	36,018
7	α-ylangene	27,444	34	Cyclododecane	36,572
8	α-copaene	27,671	35	Caryophylllene oxide	36,741
9	β-bourbonene	28,219	36	Cis3-hexenyl benzoate	36,898
10	Hexadecane	28,417	37	Ledene	37,032
11	β-elemene	28,592	38	Salvial-4(14)-en-1-one	37,201
12	Aromadendrene	29,094	39	1-tetra decene	37,498
13	3-dodecen-1-al	29,198	40	Cyclotetradecene	37,883
14	β-funebrene	29,519	41	Spathulenol	38,443
15	Germacrene-D	29,636	42	Tricyclo undec-9ene	38,886
16	Trans-caryophylllene	29,752	43	Tetra decanoic acid	40,419
17	ɣ-muurolene	30,084	44	Cyclotetradecane	41,147
18	Trans -beta farnesene	30,452	45	2-pentadecanonane	41,952
19	β-himachelene	31,017	46	Alloaromadendrene	42,762
20	α-humulene	31,204	47	2 tetra decene	43,135
21	1-dodecanal	31,862	48	Benzybenzoate	44,207
22	α-amorphene	31,961	49	Hexadecanoic acid	45,554
23	α-himachelene	32,171	50	Heneicosane	46,44
24	β-selinene	32,527	51	Neophytadiene	48,48
25	α-selinene	32,713	52	Tricosane	51,003
26	β-himachelene	32,836	53	Tetracosane	53,766
27	4,7-methanoazulene	32,906	54	Heptacosane	67,615

In the current study, the methanol extract of the flowers was tested against eight microorganisms to determine their antimicrobial activities. The results revealed that the methanol extract inhibited the growth of four bacteria (Table 1). The methanol extraction of the *Holarrhena antidysenterica* drug showed high activity on the pathogens above the 16 mm inhibition zone (AHMAD and AQIL, 2007). Researchers found a lot of compounds in *H. perforatum*. Numerous flavonoid compounds, including hyperoside, quercitrin, isoquercitrin, rutin, quercetin, campferol, luteolin, and myricetin are found in the aboveground portions of the plant, including the leaves, stalk, flowers, and buds (GREESON *et al*., 2001).

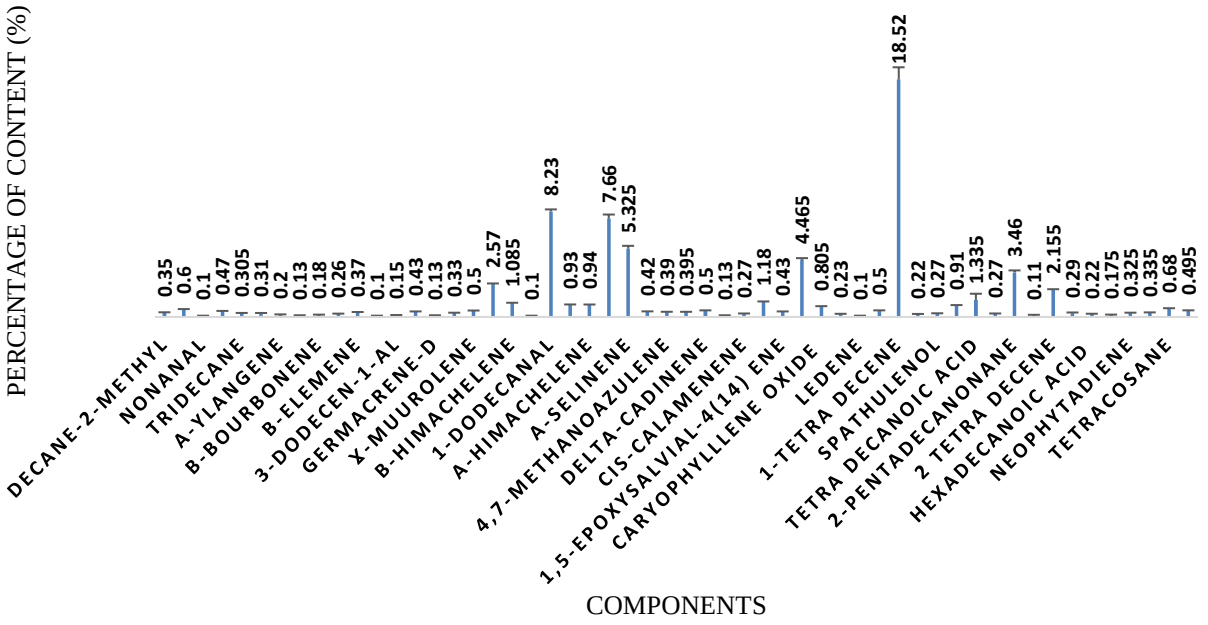

Figure 1. Chemical compounds of methanol extract of *H. perforatum*

In this study, the methanol extract of the flowers did not inhibit the growth of two Gram-negative bacteria, namely *Escherichia coli* and *Yersinia enterocolitica*. Previous studies have consistently found that Gram-positive bacteria are more susceptible to plant extracts compared to Gram-negative bacteria (PAREKH and CHANDA, 2006; GARCIA *et al*., 2008; NAZZARO *et al*., 2013; DENG *et al*., 2020), likely due to structural differences in their cell walls. Gram-positive bacteria possess a single-layered cell wall, while Gram-negative bacteria have a more complex and multilayered cell wall structure (SILHAVY *et al*., 2010).

The results of this study demonstrated that the tested plant extract exhibited high effectiveness against *S. aureus* and *Listeria monocytogenes*. In a study by Oskay et al., it was revealed that *H. perforatum* L. methanol and ethanol extracts showed high sensitivity against methicillin-resistant *S. aureus* (MRSA), making it the most susceptible organism (OSKAY *et al*., 2009). Similarly, the extract of *H. perforatum* showed an inhibitory effect of 16 mm against *Staphylococcus aureus* (KELEŞ *et al*., 2001). These findings align with the results obtained in our study.

In this study, the methanol extract exhibited a 53% inhibition of free radicals at a concentration of 100 mg/mL. These results are similar to the literature (HUCK *et al*., 2006; TATSIS *et al*., 2007). Studies have demonstrated that *H. perforatum* contains compounds exhibiting various biological activities. Among these are flavonoids and phenolic acids, which contribute to its antioxidant activity. Very few studies on the antioxidant activity of *H. perforatum* have been found in the literature. One of these is the study by Okmen and Balpınar. In their study, Okmen and Balpinar reported that the DPPH scavenging activity of *H. perforatum* flowers exhibited 32% inhibition (OKMEN and BALPINAR, 2017). Another study was conducted by GÜZEL et al. (2019), where the ABTS radical scavenging activity of *H. perforatum* was reported to be approximately 20%. The studies support the results of this study.

The components obtained from the composition study and having a high percentage belong to the alkene, long-chain fatty acid aldehyde, sesquiterpene, and methyl tridecyl ketone groups. These groups constitute 52.38% of the oil obtained in this study. Therefore, the presence of antimicrobial and antioxidant activity can be attributed to the high proportion of these groups in the plant's essential oils and their substantial effectiveness (SADDIQE *et al*., 2010; HODZIC *et al*., 2010; SHAFAGHAT, 2011; RAHNAVARD, 2015; MUNOZ-CAZARES *et al*., 2017; AYGULA and ŞERBETCI, 2020; SONMEZ *et al*., 2021; GHODRATI *et al*., 2021; JAKUBCZYK *et al*., 2021; ÇELEBİ *et al*., 2023).

The phytochemical composition of *H. perforatum* has been reported in this study. The main compounds from the methanol extract of *H. perforatum* were identified to be 1-tetra decene and 1-dodecanal as determined by GCMS (Figure 1). In many studies with other species belonging to *Hypericum*, different results have been reported (SHAROPOV *et al.*, 2010; SHAFAGHAT, 2011; JAIMAND *et al.*, 2012; PIRBALOUTI *et al.*, 2014; KÜÇÜK *et al.*, 2015; YÜCE, 2016; SCHEPETKIN *et al.*, 2020; GÜLER and OZDEMİR, 2023). However, this study supports the studies in the literature.

In studies on the essential oils of *H. perforatum* in the literature, different components have been obtained not only from various countries but also from different regions of the same country. Furthermore, the percentages of these components have been found to vary. Some components obtained in this study overlap with those in the literature but have different values (HOSNİ *et al*., 2008; ÇIRAK *et al*., 2010; DEVECİ, 2014; CARRUBBA *et al*., 2021; GÜLER, 2022). Several factors influence the quantity and composition of essential oils in plants. These factors vary depending on which part of the plant the essential oil is derived from, the species of the plant, the geographical conditions of the region where the plant is located, climate, the growth stages of the plant, and variations in extraction methods (BAYAZ, 2014).

CONCLUSION

The methanol extract of *H. perforatum* demonstrated high efficacy against *S.s aureus* and *L. monocytogenes*, highlighting its potential as a natural antimicrobial agent. It exhibited maximum inhibition against foodborne pathogens. These findings support the traditional medicinal use of this plant and suggest that certain extracts possess promising antibacterial compounds, which could be explored as potential agents in the search for new drugs. *H. perforatum* flower extract showed moderate antioxidant activity *in vitro*, potentially offering beneficial antioxidant protection against oxidative damage in the human body. Further research is needed to explore the bioactive compounds in this plant and investigate its antimicrobial and antioxidant effects. Determining the active compounds is crucial for a deeper understanding of *H. perforatum*.

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