DOI: http://dx.doi.org/10.32571/ijct.1445857 **E-ISSN: 2602-277X**

International Journal of Chemistry and Technology

http://dergipark.org.tr/ijct

Research Article

Determination of Antimicrobial, Anticarcinogenic Activity of Bioactive Components of *Hypericum Perforatum* L. Plant

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Received: 1 March 2024; Revised: 24 May 2024; Accepted: 30 May 2024

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Citation: Akkaya, O.B.; Çelik, İ.S.; Ertaş, E.; Çömlekçioğlu, N.; Aygan, A, *Int. J. Chem. Technol.* **2024**, 8(1), 73-82.

ABSTRACT

Hypericum perforatum L. (St John's wort), although primarily utilized in traditional medicine, is also frequently employed in modern therapy. Within the scope of the study, *H. perforatum* was collected from Kahramanmaraş region and dried. Extracts derived from above-ground parts were analyzed to determine their levels of total phenolic and flavonoid compounds, as well as their antioxidant, anticancer and antimicrobial properties. Following the GC-MS analysis of the extracts, a total of 18 distinct fatty acids were identified. The main fatty acid components were identified as behenic (37.90 %), linoleic (21.22 %), gamma-linolenic (15.87 %), oleic (9.45 %) and palmitic acid (7.64 %). The plant extracts were found to have a total phenolic content of 60.22 mg GAE g^{-1} and a flavonoid content of 7.68 µg QE g^{-1}, as determined through analysis. Additionally, FRAP and IC_{50} values were determined as 26.96 µg AAE g^{-1} and 0.44 µg mL^{-1}, respectively. The high phenolic and flavonoid content of the extracts indicates strong antioxidant properties. The antimicrobial activities of *H. perforatum* extracts were investigated among a total of 11 microorganisms, including 9 bacteria and 2 yeasts. It has been observed that extracts possess significant antimicrobial activity against all tested microorganisms. *H. perforatum* extracts were observed to have dose-dependent inhibition of all organisms. The highest inhibition zone was observed against *E. coli* (32 ± 2.2.2/50) among gram-negative bacteria and against *B. subtilis* (28 ± 1.1/100) among gram-positive bacteria. According to the results of cell viability analysis, a significant decrease in cell viability was observed in all cell lines tested (H1299, MCF-7, HUVEC). It was observed that *H. perforatum* extracts killed cancer cells at concentrations of 0.1 mg mL^{-1} and above on H1299, MCF-7 and HUVEC cancer cell lines.

Keywords: 1st H1299, 2nd chemical composition, 3rd biological activity, 4th DPPH, 5th fatty acids

1. INTRODUCTION

Hypericum perforatum L. (Hypericaceae) has a rich historical background as one of the most extensively researched medicinal plants, which has been used since ancient times.[1] *H. perforatum* is a member of the *Hypericum* genus, which comprises over 480 species; although native to Europe, North Africa, Western Asia and India, it is now a cosmopolitan species spread throughout the world.[2] Turkey is one of the important centers of the genus with 107 taxa identified, 49 of which are endemic.[3] In our country, *H. perforatum* is known by names such as "Sarı kantaron, Binbir delik otu, Yara otu, Kan otu, Mayasıl otu, Kuzu kıran".[4,5] Due to its wide range of applications covering wound healing in burns, gastric ulcers, disorders of the biliary tract, inflammation of the bronchial and genitourinary

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systems, colds, migraines, headaches, diabetes and skin problems; it is a highly popular traditional herbal remedy.[5-6] Although its analgesic property has been known since Dioscorides, the plant's main popularity stems from its use in the treatment of depression.[1] The rising need for raw material sourced from *H. perforatum* has resulted in the overexploitation of this species in the wild. In response, efforts have been made to cultivate the species to meet demand sustainably, aiming to boost yield, enhance disease resistance and improve adaptation to environmental conditions. These initiatives have led to the cultivation of *H. perforatum* cultivars with tailored functional attributes.[7] Although studies have been conducted in previous years to determine agricultural and quality characteristics, cultivation of *H. perforatum* is not practiced in our country, despite its medical and economic importance.[8,9] In recent years, there has been an increase in market demand for natural bioactive compounds derived from plants. Nowadays, various herbal medicines obtained through extraction from medicinal plants or other chemical and physical processes are used to improve human health. Specifically, authorization from the Italian Medicines Agency (AIFA) or the European Medicines Agency (EMA) is required to verify the quality, efficacy, and safety of each medication. *Hypericum perforatum L.* today represents a promising plant that has been studied and researched for its health-giving properties and stands out as a well-established herbal product in European society due to its historical and therapeutic uses over the past decade. Due to its widespread cultivation worldwide, *H. perforatum* is abundant in the current market, produced by various herb producers. As a result, the quality of its preparations on the market can vary considerably, influenced by factors such as the use of different subspecies and cultivars, the geographical locations where the plant is grown, the development of the plant, differences in harvest time, etc.[10]

Considering the secondary metabolites and pharmacological activities of *H. perforatum*, studies have shown that it can reduce symptoms associated with moderate depression, such as anxiety, decreased appetite and energy, insomnia, hopelessness, and suicidal thoughts.[11] Additionally, it has been reported to have other activities, including antimicrobial, antioxidant, antitumoral, anti-inflammatory, antiviral, and wound-healing properties. Research clearly demonstrates that the positive effects of *H. perforatum* are due to various bioactive components acting synergistically.[12,13,14]

This study aims to evaluate the antimicrobial, antioxidant and anticancer properties of *H. perforatum* extracts naturally grown in Kahramanmaras. Furthermore, the objective includes determining the total phenolic and flavonoid content through analytical methods and identifying the fatty acid composition via GC-MS analysis.

2.MATERIALS AND METHODS

2.1. Procurement of Plant Material and Preparation of Extracts

Samples belonging to *H. perforatum* used in the study were collected in July 2020 from the Uzunsöğüt - Türkoğlu location of Kahramanmaraş, where it naturally grows at an altitude of 950 meters. Species identifications of the samples were made according to the Flora of Turkey (Herbarium number: YZK-2383).[15] The leafy stem parts and flowers of the plant were dried in shade at room temperature and then powdered using a Waring blender grinder. Subsequently, they were stored in glass bottles to protect them from light and moisture for later use in the study.

Extraction process was performed on 10 g of powdered *H. perforatum* plant sample using 300 ml of methanol at 30 °C for 1 hour in an Ultrasonic Water Bath (USB) device to ensure dissolution of the plant content in water with sound waves. Following the extraction process, the solvent was evaporated using an evaporator and the samples were obtained in dry form. The samples were stored at -20 °C until analysis was performed.[16]

2.2. Analyzing Samples for Fat Content and Fatty Acid Composition

The extraction (Leafy stem parts and flowering part of the plant) was performed by using Soxhelet apparatus at 60 °C for 6 hours with the addition of methanol (100 ml) on to 10 g of the plant material. After elimination of the solvent in a vacuum rotary evaporator at 40 °C, the extract was stored at -20 °C for further analysis. Fatty acid analysis from samples extracted via the Soxhlet method was carried out using GC-MS, employing a validated procedure.[16] GC-MS analysis was conducted utilizing a Schimadzu GC 2025 system, with a TRCN-100 SE-54 fused silica capillary column (60 m x 0.25 mm x 0.20 µm film thickness). The electron energy used was 70 eV and the injection volume was 1 µL. The samples underwent a heating process starting at 80 °C for 2 minutes, followed by an increment of 5 °C per minute until reaching 240 °C, where they were maintained for an additional 2 minutes. Subsequently, the samples were held at 240 °C for an extra 5 minutes with an increase of 3 °C per minute. The entire analysis duration was set at 61 minutes. Injections were carried out in split mode (1:50) at 240 °C. The detector temperature was set to 250 °C. Helium served as the carrier gas with a flow rate of 30 mL min^{-1}, while gas flows for H_2 and dry air were set at 40 mL min^{-1} and 400 mL min^{-1}, respectively.

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2.3.Determination of Phenolic Compound Composition of Extracts

2.3.1. Total Phenolic Compound Analysis

The total phenolic compound content was determined using the Folin-Ciocalteu Reagent (FCR) method, following modifications based on Blainski's[17] procedure. Galllic acid (Sigma) served as the standard. The plant material was washed with water to remove soil, dried in a circulating-air oven (37 ± 2 °C) and powdered in a hammer mill. The milled roots (630 g) were extracted in 6.3 L of 7:3 acetone (v/v) by turbo-extraction. Next, the extractive solution was filtered, washed with 7.2 L of 7:3 acetone (v/v), concentrated in a rotavapor under reduced pressure and lyophilized to yield a crude extract (CE, 272 g), which was stored at −20 °C.[17] The solutions prepared were measured at 750 nm using a spectrophotometer (Perkin-Elmer Lambda EZ 150, USA). Absorbance values were then converted to milligrams of gallic acid equivalent (GAE) per gram of dry sample weight utilizing a calibration curve generated from gallic acid solutions.

2.3.2. Total Flavonoid Compound Analysis

The determination of total flavonoid compound content was performed spectrophotometrically according to Chang's[18] method. Standard solutions at different concentrations (25-200 µg mL^{-1}) were prepared by modifying the procedure of Blainski[17] and were used with quercetin (Sigma) as the standard. Ten milligrams of quercetin was dissolved in 80% ethanol and then diluted to 25, 50 and 100 µg/mL. The diluted standard solutions (0.5 mL) were separately mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1M potassium acetate and 2.8 mL of distilled water. After incubation at room temperature for 30 minutes, the absorbance of the reaction mixture was measured at 415 nm with a Shimadzu UV-160A spectrophotometer (Kyoto, Japan). The amount of 10% aluminum chloride was substituted by the same amount of distilled water in the blank. Similarly, 0.5 mL of ethanol extracts or 15 flavonoid standard solutions (100 ppm) were reacted with aluminum chloride for the determination of flavonoid content as described above. The obtained absorbance values were expressed as micrograms of quercetin equivalent per gram of dry sample weight.[18]

2.4. Determination of Antioxidant Activity

2.4.1. DPPH Radical Removal Test

The antioxidant capacity, assessing the reduction potential of free radicals, was determined through a modified version of the DPPH method originally described by Brand-Williams[19]. The samples were prepared and diluted to yield five different concentrations. The outcomes were presented in terms of IC_{50}, representing the concentration necessary to reduce 50 % of DPPH free radicals. All experiments were replicated three times, with ascorbic acid serving as the positive control.

***Antioxidant capacity**: % AA = [($A_{control}$ - A_{sample}) / $A_{control}$] x 100*

2.4.2. FRAP Test

The FRAP method, as outlined by Benzie[20], was employed for analysis. For each sample, 50 µl was transferred to 2 ml eppendorf tubes, followed by the addition of 600 µl of FRAP reagent. Absorbance readings were taken at 593 nm. Results were calculated as µmol ascorbic acid equivalent per gram of dry plant weight using a calibration curve ranging from 100 to 1000 µmol L^{-1} of ascorbic acid. The findings were expressed as µmol per gram of dry plant weight (µmol g^{-1}).

2.5. Determination of Antimicrobial Activity

The antimicrobial properties of the extracts were assessed through the agar well diffusion method, following the guidelines set by the National Committee for Clinical Laboratory Standards.[21] As test microorganisms, clinical isolates including *Escherichia coli*, Acinetobacter sp., Serratia marcescens, Pseudomonas sp., Klebsiella pneumoniae, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 29213, MRSA (Methicillin-Resistant *Staphylococcus aureus*), *Sarcina lutea* ATCC 9341, *Saccharomyces cerevisiae* and *Candida parapsilosis*, as well as standard strains, were used. The test microorganisms were inoculated onto LB (Luria-Bertani) agar plates and Sabouraud dextrose broth media 24 hours prior. Then, 0.1 mL of cultures diluted with sterile saline solution corresponding to 0.5 McFarland standard turbidity (1 x 10^8 bacteria mL^{-1} and 0.5-3 x 10^4 yeast mL^{-1}) after autoclaving and cooled to 50-55 °C were inoculated onto Mueller Hinton Agar and Sabouraud Dextrose Agar plates, and poured into Petri dishes. The solidified Petri dishes at room temperature were aseptically punctured with 6 mm diameter wells. The plant samples were dissolved in DMSO (16 mg ml^{-1}). Then 100 µL of the prepared extracts were added to these wells using a micropipette. The prepared petri dishes were kept in the refrigerator for 45 minutes. Bacterial cultures were incubated at 37 °C for 24 hours, while yeast-inoculated petri dishes were incubated at 25 °C for 2 days. The inhibition zones formed around the agar blocks were measured in mm after incubation. DMSO (50 µl) was used as the solvent control. The Minimal Inhibitory Concentration (MIC) values of plant extracts showing antimicrobial activity were assessed at various concentrations in Mueller Hinton Broth and Sabouraud Dextrose Broth.[22] MIC values were recorded as the

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lowest extract concentration in microplate wells that prevented observable growth/turbidity.

2.6.1. Cell Viability Analysis

Methanol extracts obtained from *H. perforatum* were lyophilized for 24 hours to completely remove the solvent. After the lyophilization process, the extracts were dissolved in sterile DMSO at a 1:1 ratio to prepare *H. perforatum* extracts at a concentration of 1000 mg mL^{-1}. *H. perforatum* extracts were prepared with nutrient medium dilutions at eight different concentrations ranging from 15.6 to 2000 µg mL^{-1} for addition to the cell culture. The nutrient media within which the cell cultures were incubated were aspirated in a sterile cabinet and 100 µL of nutrient media containing previously prepared extracts were added to each well onto the remaining cell monolayers. After being incubated for 24 hours at 37 ºC in a 5 % CO_2 incubator, 10 µL of Cell Viability Detection Kit (CVDK) solution was added to each well for cytotoxicity analysis. After the mixture was reincubated for an additional 3-4 hours at 37 ºC in a 5 % CO_2 incubator, colorimetric measurements were taken at 450 nm. The experiment was conducted in triplicate at different time points and the averages were calculated. The experiments were performed separately for each cell line.

3.RESULTS AND DISCUSSION

3.1.Results on Fat Content and Fatty Acid Composition of *H. Perforatum*

The oil yield obtained from the aerial parts of *H. perforatum* was determined to be 5.56 %. The oil content analysis was revealed through GC-MS measurements, and the data regarding the fatty acid composition of the samples are presented in Table 1.

Table 1. Fatty acid composition of *H.perforatum* (%).

	Carbon number	Fatty acid	Amount (%)
		Saturated Fatty Acids	
1	C12:0	Lauric Acid	0.28 ± 0.01
2	C14:0	Myristic Acid	0.93 ± 0.04
3	C16:0	Palmitic Acid	7.64 ± 0.27
4	C18:0	Stearic Acid	1.46 ± 0.08
5	C21:0	Heneicosanoic Acid	0.81 ± 0.2
6	C21:0	**Behenic Acid**	**37.9 ± 0.38**
7	C23:0	Tricosanoic Acid	0.48 ± 0.01
8	C24:0	Lignoceric Acid	0.99 ± 0.14
		Monounsaturated Fatty Acids	
9	C14:1	Myristoleic Acid	0.09 ± 0.02
10	C16:1	Palmitoleic Acid	0.32 ± 0.04
11	C18:1	**Oleic Acid**	**9.45 ± 0.06**
12	C24:1	Nervonic Acid	0.27 ± 0.22
		Polyunsaturated Fatty Acids	
13	C18:2	**Linoleic Acid**	**21.22 ± 0.25**
14	C18:3	**Gama-Linolenic Acid**	**15.87 ± 0.34**
15	C22.6	Cis-4,7,10,13,16,19-Docosahexaenoic Acid	1.11 ± 0.02
16	C20:0	Arachidonic Acid	0.20 ± 0.05
17	C20:3	Cis-8,11,14-Eicosatrienoic Acid	0.62 ± 0.18
18	C22:5	Cis-5.8.11.14.17-Eikosapentaenoic Acid	0.29 ± 0.01
Saturated Fatty Acid Fraction (SFA)			50.49
Monounsaturated Fatty Acid Ratio (MUFA)			10.13
Polyunsaturated Fatty Acid Fraction (PUFA)			39.31

The GC-MS chromatograms obtained from *H. perforatum* within the scope of the study are provided in Figure 1. According to the GC-MS chromatogram results, a total of 18 fatty acids were identified in the plant extract, comprising 8 saturated and 10 unsaturated fatty acids. Saturated fatty acids (SFA) were found to be the most abundant, with a percentage of 50.54 %. The ratio of polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA) to the total fat was determined to be 39.31 % and 10.15 %, respectively (Table 1). Behenic acid (37.90 %) and palmitic acid (7.64 %) from saturated fatty acids, oleic acid (9.45 %) from monounsaturated fatty acids and linoleic acid (21.22 %) and gamma-linolenic acid (15.87 %) from polyunsaturated fatty acids constitute the main fatty acids in the plant extract of *H. perforatum*.

Numerous studies have confirmed that unsaturated fatty acids such as linoleic acid, gamma-linolenic acid and oleic acid (PUFA) lower cholesterol levels in the blood.[23] These fatty acids prevent blood clotting within the blood vessels, reducing the risk of heart attacks and they also protect and strengthen the immune system. Additionally, they possess anticancer effects and anti-inflammatory properties.[24] It has been determined that linoleic acid (LA) (omega-6) and gamma-linolenic acid (GLA) (omega-6), essential fatty acids that animals cannot synthesize and must be obtained from external sources, are present in varying proportions and significant amounts in the studied plant material.

DOI: http://dx.doi.org/10.32571/ijct.1445857 E-ISSN: 2602-277X

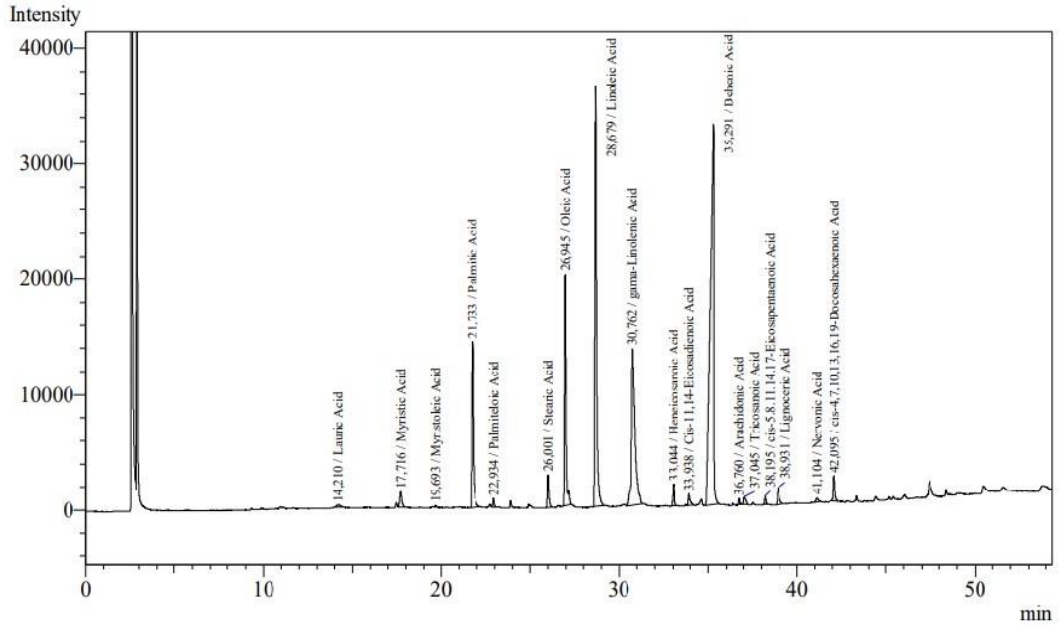

Figure 1. GC-MS chromatograms of *H. Perforatum.*

In a study by Hosni[25], it was reported that the most abundant fatty acids in *H. perforatum* are oleic acid (23.27 %), palmitic acid (17.43 %) and linoleic acid (11.21 %). Additionally, they reported that palmitic and linolenic acids are the main fatty acids in different *Hypericum* species (*H. perfoliatum*, *H. tomentosum* and *H. ericoides* ssp. *roberti*).

In a study conducted by Bakir[26], the fatty acid content of *H. perforatum* plant was examined using gas chromatography (GS-MS) and it was reported that the most abundant fatty acids were palmitic acid (8.86 %), petroselinic acid (34.23 %) and linoleic acid (44.35 %), respectively. Consistent with Bakir[26], in this study, linoleic and palmitic acids were identified as major fatty acids; however, unlike the previous study, petroselinic acid was not detected.

In another study, Stojanovic[27] compared the fatty acid profiles of *H. perforatum*, *H. maculatum* and *H. olympicum* and found that the most abundant fatty acids were linoleic (8.0 %, 29.4 %, 28.5 %), palmitic (20.3 %, 25.9 %, 23.4 %) and oleic acids (17.1 %, 24.1 %, 18.0 %), respectively. In this study, similar to the research conducted by Stojanovic[27], oleic, linoleic and palmitic acids were found to be present in high amounts; however there are differences from Stojanovic[27] in terms of the presence of behenic (37.9 %) and gamma-linolenic (15.87 %) acids, which are major fatty acids of *H. perforatum*.

In conclusion, it has been observed that the main components of *H. perforatum*, namely palmitic, oleic and linoleic acids, are consistent with data reported in the literature, while behenic and gamma-linolenic acids show variations compared to articles available in the literature. This diversity is believed to stem from various factors such as the region where the plant is grown, climatic conditions, soil quality, genetic makeup and growth factors, ultimately influencing the variety and content of fatty acids. Additionally, behenic acid (37.9 %), which was found to be the major component in this study, has been identified as the highest main constituent of the oil, contrary to other publications in the literature. This indicates that the data presented here represent a first record in the literature.

3.2. Results on Bioactivity

The results of total phenolic and flavonoid, FRAP and DPPH analyses determined in methanol extracts obtained from *H. perforatum* are given in Table 2.

Table 2. Total phenolic and flavonoid content and antioxidant activity values of *H. Perforatum.*

Total Phenolic Content (mg GAE g^{-1})	60.22 ± 0.13
Total Flavonoid Amount (mg QE g^{-1})	7.68 ± 0.32
FRAP (µg AAE g^{-1})	26.96 ± 0.85
IC_{50} Value (%DPPH) (µg mL^{-1})	0.44 ± 0.07

DOI: http://dx.doi.org/10.32571/ijct.1445857 E-ISSN: 2602-277X

In order to determine the phenolic contents of the aerial parts of *H. perforatum*, methanol extracts were analyzed spectrophotometrically. The total phenolic content was expressed as gallic acid equivalents (GAEs) and determined to be 60.22 mg GAE g^{-1} in the methanol extract. In contrast, the total flavonoid content of the plant was given as quercetin equivalents (QEs) and was determined to be 7.68 mg QE g^{-1} in the methanol extract.

The disparity in levels between endogenous antioxidants and oxidant compounds results in oxidative damage during metabolic processes.[28] Antioxidants play a crucial role in mitigating or removing the detrimental impacts of free radicals on living organisms. Nevertheless, when the body's internal antioxidants prove inadequate in combating reactive oxygen species, the utilization of supplementary antioxidants becomes paramount. Numerous medicinal plants employed in complementary medicine exhibit the potential to act as antioxidants.[29] Antioxidants are used not only in the pharmaceutical industry but also in many food and cosmetic Products.[30] The antioxidant activity of plant extracts is routinely investigated in laboratory tests. Due to the use of multiple antioxidant tests and the observed differences in the results, a single extract is usually examined with two or more tests.[31] Since it has been proven in the literature that the methanol extract has good antioxidant activity, all extracts were prepared using this solvent.[32] In this study, FRAP and DPPH tests were used to evaluate the antioxidant activity of the samples. When the DPPH radical scavenging activities of *H. perforatum* were examined in terms of IC_{50} values, they were found to be quite low (0.44 ± 0.07). *H. perforatum* exhibited an IC_{50} value quite close to that of the standard antioxidant ascorbic acid. Similar to the DPPH analysis, FRAP analyses were also prepared in the methanol extract. The reducing power capacities of the samples using the FRAP method were calculated ascorbic acid equivalents using the ascorbic acid calibration curve. As can be seen from Table 2, the results of the FRAP test were determined to be 26.96 µg AAE g^{-1}.

Valuable bioactive compounds obtained from natural sources have demonstrated good potential for use in the food industry. Numerous studies have been published on the antioxidant activity of *Hypericum* species. According to the data obtained from these studies in the literature, Napoli[33] extracted flower upper part samples of *H. perforatum* and analyzed them using high-performance liquid chromatography coupled with mass spectrometry (HPLC-DAD-MS). In the phytochemical analysis, they identified 20 metabolites, each known for its well-established biological activity. In another study, Alahmad[31] reported that extracts obtained from *H. perforatum* with the highest phenolic content (water 170.6 mg GAE g^{-1}, ethanol 64.4 mg GAE g^{-1}, methanol 93.2 mg GAE g^{-1}) and lower IC_{50} values exhibited higher antioxidant activity. Additionally, there are numerous studies demonstrating the strong antioxidant activity properties of all *Hypericum* species.[29, 35, 36] The results obtained in our study are consistent with the data obtained in previous studies. However, there may be significant differences in predicting antioxidant activity. Factors responsible for these differences include the extraction method, the test used, the manner in which results are expressed and the chemical components of the extract. Additionally, the high phenolic and flavonoid contents of the extracts play a significant role in their strong antioxidant properties. However, this study did not precisely determine which phenolic compounds are responsible for the antioxidant activity. Consequently, when taken together, the antioxidant activity data emphasize the significance of qualitative and quantitative differences in radical scavenging activity of an extract.

3.3. Results on Antimicrobial Activity

To determine the antimicrobial activities of *H. perforatum*, a total of 11 microorganisms, including 9 bacteria and 2 yeasts, were investigated. The results are presented in Table 3.

Table 3. Antimicrobial activity of *H. perforatum* extract against some microorganisms.

		Antibiotic Control	
Microorganisms	**IZ / MIC**	**Cn**	**Nys**
B. subtilis ATCC6633	28 ± 1.1/100	25	ND
S. lutea ATCC 9341NA	17 ± 0.98/25	33	ND
S .aureus ATCC 29213	24 ± 1.8/12.5	26	ND
MRSA*	23 ± 0.71/50	31	ND
*S. marcessens**	15 ± 0.90/50	33	ND
E. coli ATCC13846	32 ± 2.2/50	34	ND
Acinetobacter sp.*	18 ± 1.1.12/ 50	0	ND
Klebsiella sp.*	15 ± 0.88/50	26	ND
Pseudomonas sp.*	19 ± 0.56/25	30	ND
*C. parapsilosis**	12 ± 0.74/25	ND	14
S. cerevisia	14 ± 0.49/50	ND	22

The current study focused on the antimicrobial and antioxidative effects of *H. perforatum*. It was observed that the plant's methanol extract exhibited antimicrobial activity against all test bacteria and fungi. The samples showed similar levels of effectiveness against both gram-negative and gram-positive bacteria. The highest inhibition zone was observed against *E. coli* (32 ± 2.2/50) among the gram-negatives and against *B. subtilis* (28 ± 1.1/100) among the gram-positives. The lowest inhibition zone was detected against *S. macessens* (15 ± 0.90/50) and *Klebsiella* sp. (15 ± 0.88/50). A value of inhibition close to that of the antifungal control agent was found against the pathogenic *C. parapsilosis* (15 ± 0.88/50).

The differences observed in the study are thought to arise from variations in the growth conditions of *H. perforatum*, as well as various environmental factors, in addition to differences in the chemical composition and ratios of the extracts. In a study investigating the

DOI: http://dx.doi.org/10.32571/ijct.1445857 E-ISSN: 2602-277X

antimicrobial activity of methanol extracts of six *Hypericum* species grown in southern Brazil, the most active antimicrobial effect was reported against *S. aureus*.[37] In another study, it has been reported that *Hypericum* species exhibit antibacterial activity against both gram-positive and gram-negative organisms.[38]

Many plants worldwide have been screened by various researchers against different microorganisms using different methods. In this study, the methanolic extract of *H. perforatum* was tested against common microorganisms. As a result, it was found that these extracts were highly effective against test organisms, including clinical isolates.[11-14] It is known that plant phenolic compounds are responsible for various biological properties, including antimicrobial properties. Therefore, the antimicrobial activity of *H. perforatum* extracts is thought to be associated with the plant's phenolic and flavonoid compounds.

3.4. Results of Cell Viability Analysis

The methanol extract of *H. perforatum* was tested for cytotoxicity against H1299 (human non-small cell lung cancer cell line), MCF-7 (breast cancer cell line) and HUVEC (human umbilical vein endothelial cell line) using cytotoxicity assays and cell viability analyses. The analysis results are presented in Figure 2. For this purpose, eight different concentrations of *H. perforatum* extracts were applied to the cells in the range of 15.6-2000 µg mL^{-1}. The extract concentrations that reduced cell viability by 50 % (IC_{50} values) were calculated using the data obtained from the cell viability assays.

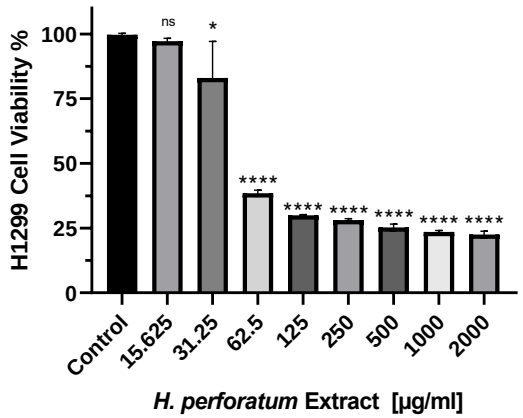

Figure 2. Cell viability inhibition graph of *H. perforatum* extracts against H1299 cell line.

*Data were expressed as the mean ± SD of % Viability as the number of treatments per group n = 3.
*Statistically significant difference was found when compared with the control group
(p > 0.5, *p > 0.01, *p > 0.001, ***p > 0.0001).

When applied to the H1299 (human non-small cell lung cancer cell line) cell line, the methanol extract of *H. perforatum* caused a significant decrease at a concentration of 31.25 µg mL^{-1}, while concentrations of 62.5 µg mL^{-1} and above reduced H1299 cell viability to below 50 % (Figure 3). The concentration of *H. perforatum* methanol extract that reduced H1299 cell viability by 50 % (IC_{50}) was calculated to be 44.07 ± 2.38 µg mL^{-1}.

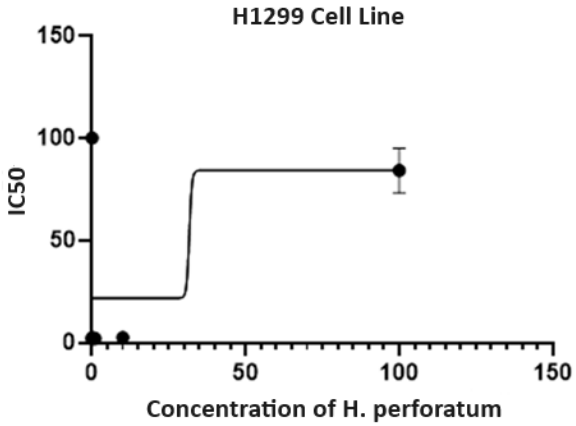

Figure 3. Cell viability inhibition graph of methanol extract of *H. perforatum* extracts against H1299 cell line.

When applied to the MCF-7 (breast cancer cell line) cell line, the methanol extract of *H. perforatum* at a concentration of 31.25 µg mL^{-1} was found to reduce the viability of MCF-7 cells to less than 50 % compared to the control group (Figure 4). The IC_{50} value of the methanol extract of *H. perforatum*, which reduced the viability of MCF-7 cells by 50 %, was calculated as 15.95 ± 2.19 µg mL^{-1}.

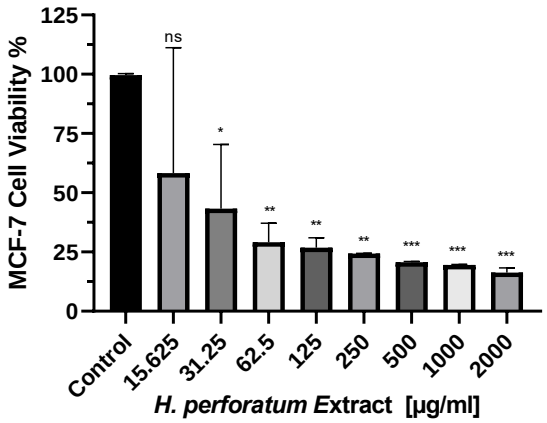

Figure 4. Cell viability inhibition graph of *H. perforatum* methanol extract against MCF-7 cell line.

*Data were expressed as the mean ± SD of % Viability as the number of treatments per group n = 3.
*Statistically significant difference was found when compared with the control group
(p > 0.5, *p > 0.01, *p > 0.001, ***p > 0.0001).

DOI: http://dx.doi.org/10.32571/ijct.1445857 E-ISSN: 2602-277X

When the methanol extract of *H. perforatum* was applied to the HUVEC (human umbilical vein endothelial cell) cell line, it was observed that all concentrations of the methanol extract caused a significant decrease in HUVEC cell viability compared to the control group. However, at a concentration of 2000 µg mL^{-1}, it reduced the viability of MCF-7 cells to less than 50 % (Figure 5). The IC_{50} value of the methanol extract of *H. perforatum*, which reduced the viability of HUVEC cells by 50 %, was calculated as 54.70 ± 0.42 µg mL^{-1}.

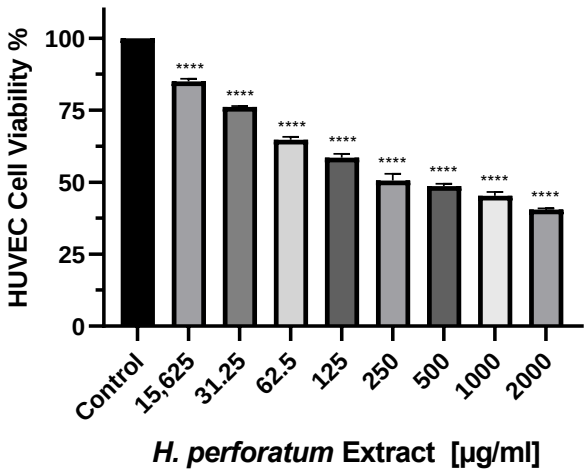

Figure 5. Cell viability inhibition graph of methanol extract of *H. perforatum* against HUVEC cell line.

*Data were expressed as the mean ± SD of % Viability as the number of treatments per group n = 3.
*Statistically significant difference was found when compared with the control group
(p > 0.5, *p > 0.01, *p > 0.001, ***p > 0 .0001, ****p > 0.00001).

Cancer causes more deaths than coronary heart failure or accidents in all less developed, developing and developed countries. Due to the adaptation to living conditions that increase the risk of cancer, a rapid increase in the number of cancer cases and deaths is expected.[39] Therefore, the use of traditional and alternative therapeutic approaches against cancer is being investigated in medical Practice.[40] In recent years, the use of traditional and alternative therapeutic approaches against cancer has become an important research topic. Current studies include in vitro studies examining the anticancer, antioxidant and cytotoxic activities of various plant extracts. Nair[41] reported that flavonoids such as hyperforin, hypericin and quercetin inhibit the growth of cancer cells. In this study, the methanol extract of *H. perforatum* was used to investigate its effects on lung and breast cancer cells. The methanol extract of *H. perforatum* reduces the viability of both H1299 and MCF-7 cancer cells starting from low concentrations of 31.25 µg mL^{-1}, with IC_{50} values of 44.07 ± 2.38 µg mL^{-1} and 15.95 ± 2.19 µg mL^{-1}, respectively, against H1299 and MCF-7 cancer cells, indicating a reduction in cell viability. In a study conducted by Matic[42], the effects of *H. perforatum* extracts prepared using different organic solvents from the leaves and stem-body parts of the plant on the viability of cervical cancer (HeLa), lung cancer (A549), leukemia (K562) and healthy lung (MRC-5) cells were investigated.

The extracts derived from the stem/trunk part of the plant, when prepared using methanol, ethyl acetate, and hexane, demonstrated a notable reduction in cell viability, with IC_{50} values recorded at 100 µg mL-1. Conversely, extracts from the leaf part of the plant, also prepared using the same solvents, exhibited a similar decrease in cell viability across all cell types, again with IC_{50} values of 100 µg mL-1. Particularly noteworthy was their pronounced antiproliferative effect on the HeLa cell line.

It is important to highlight that this study, in line with existing literature, stands out as the first original research endeavor to investigate the effects of these extracts specifically on lung and breast cancer cells. This distinction underscores the novelty and significance of the findings, providing valuable insights into the potential therapeutic applications of these extracts in combating these types of cancer.

Although it is clear that the beneficial effects of H. perforatum arise from numerous molecules, there is currently no study that simultaneously investigates the inhibitory power of flavonoids, melatonin, and various enzymes involved in numerous pathologies for this plant. The collective effect of all compounds identified in H. perforatum and their connection to beneficial effects have been somewhat overlooked. This study's characteristics allow us to understand the link between the plant and its positive effects. While it is evident that the beneficial effects of H. perforatum stem from numerous molecules, there is currently no study that simultaneously investigates the inhibitory power of flavonoids, melatonin, and various enzymes involved in numerous pathologies for this plant.

4. CONCLUSIONS

In this study, antimicrobial, antioxidant and anticancer properties of extracts of *H. perforatum* growing naturally in Kahramanmaraş were evaluated. The findings show that the plant extract of *H. perforatum* contains a total of 18 fatty acids, including 8 saturated and 10 unsaturated fatty acids. The high phenolic and flavonoid content of the extracts indicates strong antioxidant properties. The methanol extract of the plant has been observed to exhibit antimicrobial activity against all tested bacteria and fungi. The samples showed similar levels of activity against both gram-negative and gram-positive bacteria. The highest inhibition zone was observed against *E. coli* among gram-negative bacteria and against *B. subtilis* among

DOI: http://dx.doi.org/10.32571/ijct.1445857 **E-ISSN: 2602-277X**

gram-positive bacteria. According to cell viability analysis results, a significant decrease in cell viability was observed in all tested cell lines (H1299, MCF-7, HUVEC). Notably, the pronounced antiproliferative effects on the HeLa cell line are particularly striking.

Strategies aimed at identifying phytochemicals responsible for antioxidant, antimicrobial, and anticarcinogenic activity can lead to innovative or novel molecular approaches in agricultural or pharmaceutical applications. These strategies can help us better understand the positive effects of bioactive compounds derived from plants on health and develop new treatment methods using plant-derived compounds for the prevention or treatment of various diseases.

Conflict of interest

Authors declare that there is no a conflict of interest with any person, institute, company, etc.

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