Acta Pharm. Sci. Vol 63:(3), 2025
Original article
DOI: 10.23893/1307-2080.APS6334

Antibacterial activity and UPLC analysis of *Hypericum perforatum* L. extracts

Elif GÜZEL[1,2*], Mukhlesur RAHMAN[3]

1 Istanbul Medipol University, Institute of Health Sciences, Istanbul, Türkiye

2 Istanbul Medipol University, School of Medicine, Department of Medical Pharmacology, Istanbul, Türkiye

3 University of East London, School of Health, Sport and Bioscience, Department of Bioscience, Medicines Research Group, London, UK, United Kingdom

ABSTRACT

Hypericum perforatum L. has been used for centuries as a herbal remedy against variety of diseases for its biological activities. It has been also used as an antibacterial agent against different bacteria. The aim of this study is to investigate the antibacterial properties of the *H. perforatum* L. extracts against several clinical isolates and explore their chemical composition. In terms of *S. aureus* strains, *n*-hexane extract demonstrated the best activity against XU212 strain, with the MIC of 256 μL/mL. The MIC was 512 μL/mL for other *S. aureus* strains for *n*-hexane and dichloromethane extracts. *n*-hexane extract demonstrated activity against *E. coli* ATCC 25922 with the MIC of 1 μL/mL. UPLC was performed for dichloromethane and methanol extracts. The main compounds were identified as catechin, hyperforin, and rutin from methanol extract, and hypericin and luteoskyrin from dichloromethane extract. *S. aureus* demonstrated good antibacterial activity against tested MRSA and *Bacillus subtilis* strains.

Keywords: *Hypericum perforatum* L., extract, antibacterial activity, MRSA, UPLC

* Corresponding author: Elif GÜZEL
E-mail: elif.guzel@medipol.edu.tr
ORCIDs:
Elif GÜZEL: 0000-0002-2737-0568
Mukhlesur RAHMAN: 0000-0001-8180-7970
(Received 9 Jan 2025, Accepted 12 Mar 2025)

INTRODUCTION

Staphylococcus aureus commonly presents in the mucosal surfaces and the skin of the human body, and it is a Gram-positive bacterium[1]. When there is a breach on the mucosal surfaces or the skin, *S. aureus* enters the body, and the infection is initiated. For centuries, *S. aureus* has been one of the widespread and life-threatening causes of infections in health-care settings, because the bacteria mostly spread to the adjacent organs and cause severe invasive infections such as bacteremia or pneumonia. *S. aureus* has been identified as one of the six bacteria that are the most dangerous nosocomial infections in many countries, including the USA, the UK, and Canada, by The Infectious Diseases Society of America[2]. According to a report published by WHO in 2014, which is about antibiotic resistance surveillance, *S. aureus* spread is increasing in all continents. Some countries reported the number of *S. aureus* infections up to 80% which results in longer hospital stays or the use of a second-line antibiotic treatment[3]. Significant number of the bacteria that have been isolated from the patients mostly in ventilators or from the surgical site of the patient, approximately 43-58%, was identified as Methicillin Resistant *Staphylococcus aureus* (MRSA)[4]. According to a survey which was carried out by the European Centre for Disease Control and Prevention (ECDC), which includes 33 different countries in Europe, it has been recorded that *E. coli* is the first and *S. aureus* is the second most common cause of nosocomial infections[5].

Bacteria can acquire resistance against antibacterial therapeutics via different routes such as efflux pump activation, enzymatic destruction of the bacteria, modification of antibacterial agent's enzymes, and target site alteration of the antibacterial agents. Efflux pumps have a crucial role in bacterial resistance mechanisms as they work as an export system for antibacterial agents. Throughout the efflux pumps, an antibacterial agent is pushed out of the bacteria faster than it gets in, as a result, antibiotic resistance is seen in bacteria. Because of this mechanism, efflux pumps are one of the most important target sites for potential antibacterial therapeutics against multidrug-resistant bacteria. Therefore, developing novel antibacterial therapeutics that can prevent bacteria to efflux the antibacterial agent is an emerging issue today.

Several synthetic antibacterial agents are widely used around the world; however, plant-based antibacterial agents still attract many of the researchers[6]. Antibacterial compounds derived from natural sources have shown significant results against several multidrug-resistant bacteria[7]. Depending on the chemical structures of these naturally derived antibacterial compounds, they can be mainly classified as alkaloids, terpenoids, polyphenols, and sulphur containing compounds[8].

Hypericum perforatum L. is a perennial shrub and has yellow flowers. It has five sepals and petals in its flowers. The plant has opposite leaves, and, in its stamen, it has five bundles[9]. *H. perforatum* L. is in the family *Clusiaceae,* which includes around 400 different species worldwide[10]. *Hypericum perforatum* L. is mainly native to Western Asia, North Africa, and around Europe, however, it is also distributed among Australia and North America[11]. For centuries, *Hypericum perforatum* L. has been used as a herbal remedy for several diseases such as skin lesions, gastrointestinal tract diseases, anxiety and depression, mucosal lesions, and superficial injuries, and as a nursing remedy[11]. Today, different preparations of dried and fresh *Hypericum perforatum* L. are used for various purposes. Fresh plant species are used as a mother tincture in homeopathy as drops. Also, oil of the plant species is used for ointments and capsules. Dried extract of *Hypericum perforatum* L. is used for tablets and capsules, fluid extract is used for ointments and tinctures, and dried raw preparations are used as tea[10]. The aim of this study is to investigate the antibacterial properties of *H. perforatum* L. extracts against clinical isolates of MRSA and determine its chemical composition by Ultra Performance Liquid Chromatography (UPLC) analysis.

METHODOLOGY

Plant material

Hypericum perforatum L. plant's dried aerial parts were obtained from Herbal Apothecary, UK, with batch number 15522, in October 2012.

Preparation of plant extracts

14 grams of plant material was grinded and used for extraction, and the Soxhlet extraction method was used. Increasing polarity of 150 mL of 3 different solvents were used to obtain extracts, including *n*-hexane, dichloromethane, and methanol, respectively. After the extraction, the residual solvent was evaporated with a rotary evaporator (Heidolph, Germany).

Bacterial strains

The standard Gram-positive bacteria (*S. aureus* ATCC 25923 and *B. subtilis* ATCC 6633) and Gram-negative bacteria (*E. coli* ATCC 25922) were used in this study. Additionally, a series of MRSA strains such as *S. aureus* SA 1199B and *S. aureus* XU212 were used. *S. aureus* XU212 is a tetracycline resistant strain as it overexpresses *tetK* efflux pump[12], and *S. aureus* SA 1199B is an Muti Drug Resistant (MDR) strain that overexpresses *norA* MDR efflux pump[13].

Antibacterial assay

Recommended protocol by the British Society for Antimicrobial Chemotherapy (BSAC) was followed to determine the Minimum Inhibitory Concentration (MIC) of the plant extracts and the antibiotic, against all the tested bacteria[14]. The broth microdilution technique was performed in duplicate.

The bacteria were sub-cultured on nutrient agar (Oxoid) prior to the antibacterial assay and incubated at 37°C for 18 hours. Cation levels of Mueller-Hinton Broth (Oxoid) were adjusted to include 20 mg/L of Ca^{2+} (Acros Organics) and 10 mg/L of Mg^{2+} (Acros Organics). Norfloxacin (Sigma Chemical Co.) was used as an antibiotic for positive control. To prepare the stock solution, Norfloxacin was dissolved in DMSO (Dimethyl Sulfoxide) (Sigma-Aldrich), and then further dilution was done with Mueller-Hinton Broth to obtain a final concentration of 128 µL/mL. Extracts were dissolved in DMSO to prepare the stock solutions for the extracts. Then, further dilution was done with Mueller-Hinton Broth to obtain a final concentration of 512 µL/mL. Test organisms were prepared in saline water (0.9% NaCl) with 5 x 10^5 cfu inoculum density and compared with 0.5 MacFarland turbidity standard.

96 well plate was used for the determination of MIC against each bacterium. 100 µL of Mueller-Hinton Broth was dispensed to the wells from columns 1 to 11. 100 µL of samples were dispensed to the first column as follows; wells A and B for *n*-hexane extract, wells C and D for dichloromethane extract, wells E and F for methanol extract, and wells G and H for Norfloxacin. Serial dilution was done starting from the first column up to column 12 by skipping column 11 which was used as growth control. 100 µL of bacterial suspension was dispensed to all wells except column 12 which was used as sterility control. All the plates were incubated at 37°C for 18 hours. After that, 5 mg/mL methanolic MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Alfa Aesar) solution was prepared, and 20 µL was dispensed to all wells. Then incubated for 20 minutes at 37°C to observe the color change. The blue color indicated bacterial growth, and the MIC of all the extracts and antibiotics was recorded. The antibacterial activity of extracts was evaluated in comparison with the positive control, Norfloxacin. The MIC of the extracts and the antibiotic were determined by looking at the lowest concentration where no bacterial growth was seen. The results of the antibacterial assay are provided in the "Table 1".

UPLC analysis

Chromatographic separation was done with normal phase Ultra Performance Liquid Chromatography technique for dichloromethane and methanol extracts. Sample concentrations were prepared as 1 mg/mL initially with HPLC grade methanol, then further diluted with the ratio of 1:4 with the same solvent. A 0.22 µm pore size filter was used to filter samples before the analysis. Agilent Technologies 1260 Infinity Series was used with Agilent Technologies Poroshell 120 EC-C18 column with the column size of 3 x 50 mm, and 2.7 µm particle size. Two elution binary gradients were used. As a mobile phase, HPLC-grade water was used as an aqueous, and HPLC-grade acetonitrile was used as an organic phase. They were acidified with Trifluoroacetic acid (TFA) with 0.01% ratio, and then further filtered with a 0.22 µm pore size filter. The flow rate was 0.750 mL/min, and the pressure was 242.43 bars. Column temperature was between 18.61°C to 18.85°C. The injection volume was 5.00 µL, and full loop injection was used. From 0 to 2 min 100% A (0.01% TFA in water), from 2 to 3.5 min 100% A, from 3.5 to 5.5 min 100% B (0.01% TFA in acetonitrile), from 5.5 to 6 min 100% B and at the min 6 100% A was used. A Photodiode Array (PDA) detector was used to detect chemical compounds. Separation was performed under 3 different wavelengths which were 210 nm, 260 nm, and 350 nm, and all the chromatograms were recorded. The run time was 6 minutes for each sample.

RESULTS and DISCUSSION

Antibacterial assay

All the plant extracts were tested for *in vitro* antibacterial activity with broth micro-dilution assay to determine the MIC. All the antibacterial activity was evaluated in comparison with the positive control, Norfloxacin. Among all extracts, *n*-hexane extract demonstrated the highest antibacterial property against all the tested bacteria, whereas methanol extract did not have activity against any of the tested bacteria.

n-hexane extracts showed the best activity against the *S. aureus* XU212 strain which is the tetracycline resistant strain. MIC against *S. aureus* XU212 was 256 µL/mL for *n*-hexane extract. MIC of *n*-hexane extract against *S. aureus* ATCC 25923 standard strain and *S. aureus* SA 1199B strain, which overexpress *norA* MDR efflux pump, was same and 512 µL/mL. MIC of dichloromethane extract against all the tested *S. aureus* strains was the same, which was 512 µL/mL. The MIC of the Norfloxacin was recorded as 16 µL/mL for *S. aureus* XU212 strain, and 64 µL/mL for *S. aureus* ATCC 25923 standard strain and *S. aureus* SA 1199B. Methanol extract was not effective against any of the tested *S. aureus* strains.

n-hexane extract demonstrated the best antibacterial activity against tested *B. subtilis* ATCC 6633 strain. MIC was between 64 – 128 µL/mL. And the MIC of dichloromethane extract was between 256 – 512 µL/mL. The MIC of Norfloxacin was recorded as 8 µL/mL, and methanol extract was not effective against this bacterium. One bacterium was used as a Gram-negative bacterium which was *E. coli* ATCC 25922. *n*-hexane extract had the best activity with the MIC of 1 µL/mL. The MIC of Norfloxacin was recorded as 0.25 µL/mL. Dichloromethane and methanol extracts did not show any activity against this bacterium.

The summary of the results of the antibacterial assay is provided in "Table 1" below.

Table 1. MIC of the plant extracts and the antibiotic

Bacteria	Description	MIC (µL/mL)		
		n-hexane	Dichloromethane	Norfloxacin
S. aureus ATCC 25923	Standard strain	512	512	64
S. aureus XU212	*tetK* efflux pump, tetracycline-resistant	256	512	16
S. aureus SA 1199B	*norA* efflux pump, MDR strain	512	512	64
B. subtilis ATCC 6622	Commonly used strain	64 – 128	256 – 512	8
E. coli ATCC 25922	Commonly used strain	1	–	0.25

UPLC analysis

The chemical composition of dichloromethane and methanol extract of *Hypericum perforatum* L. was determined with normal phase UPLC analysis using a gradient mobile phase consisting of HPLC grade water as an aqueous phase and HPLC grade acetonitrile as an organic phase. The analysis was conducted with 3 different wavelengths including 210 nm, 260 nm, and 350 nm. The run time was 6 minutes for each extract and all wavelengths. UPLC analysis was not carried out for *n*-hexane extract because of its highly non-polar nature.

Different compounds were observed with different wavelengths for methanol extract. 4 peaks and 6 peaks were observed under 210 nm and 350 nm, respectively. However, the best separation for methanol extract was seen under 260 nm. There were 8 different compounds with different peaks. The highest peak was observed at the retention time (RT) of 3.659 min, and determined as the major compound, as Hyperforin, which is the fourth compound in the chromatogram.

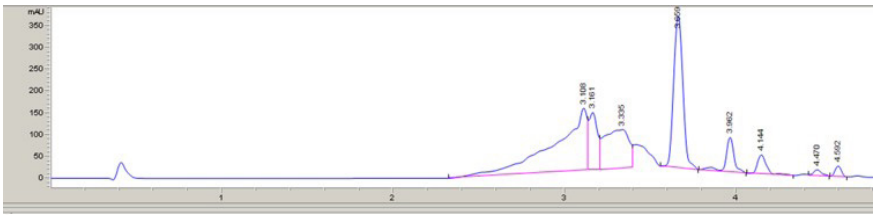

Figure 1. UPLC chromatogram of the methanol extract under 260 nm

Previous studies have been performed to investigate the chemical composition of the methanolic extracts of *H. perforatum*. Depending on those studies, compounds found with UPLC were determined by comparing the retention time. Previous studies have shown that the methanolic extract of the plant species includes catechin which was seen at 3.216 minutes under 275 nm. Compound number 2 in our analysis with the retention time of 3.108 min was determined as Catechin as the retention time and the observation wavelength is very close. The same study also determined Procyanidin B1 at the time of 3.383 min under 275 nm, and compound number 3 in our analysis is also believed to be Procyanidin B1 as the retention time is very close to 3.161 min, and our wavelength was 260 nm[15]. Another previous study was conducted to identify Hyperforin and its metabolites which are present in *Hypericum perforatum* L. by using UPLC, and it was reported that the standard Hyperforin was seen at the minute of 3.64. In our results, compound 4 has retention time of 3.659 min, which was determined as Hyperforin[16]. Similarly, Rutin was identified in the previous studies at 3.95 min under 255 nm. Our compound 5 with the retention time of 3.962 is identified as Rutin depending on the literature[15]. The same study also identified 2 derivatives of Quercetin which are Quercetin-3-O-galactoside (Hyperoside) and Quercetin-3-O-rhamnoside (Isoquercetrin) with the retention time of 4.083 min and 4.383 min, respectively. In our results, Compounds 6 and 7 have retention times of 4.144 min and 4.470, respectively. By comparing the literature, they were identified as Hyperoside and Isoquercetrin, respectively[15]. To determine the chemical composition of *Hypericum perforatum*, former studies were performed with HPLC for methanolic extract of the plant species as well. The study has reported that Quercetin was seen at 34.6 min for a 40 min run time HPLC analysis. By comparing the total run time and the time Quercetin was seen, our compound 8 was identified as Quercetin[17]. Compound 1 was unidentified as there is no proof of compound in the literature for the specific retention time and wavelength of this compound. The list of the compounds in the methanolic extract is provided below, in “Table 2”.

Table 2. Possible compounds found in the methanolic extract of *H. perforatum* L. via UPLC under 260 nm

#	RT (min)	Area %	Possible Compound	Reference
1	3.108	37.810	Unknown	–
2	3.161	8.886	Catechin	(15)
3	3.335	17.438	Procyanidin B1	(15)
4	3.659	25.519	Hyperforin	(16)
5	3.962	5.196	Rutin	(15)
6	4.144	3.081	Quercetin-3-O-galactoside (Hyperoside)	(15)
7	4.470	0.898	Quercetin-3-O-rhamnoside (Isoquercetrin)	(15)
8	4.592	1.172	Quercetin	(17)

The dichloromethane extract was observed under 3 different wavelengths as well. No peak was observed under 350 nm, and 14 peaks were observed under 210 nm. However, the best separation was observed under 260 nm again, with 13 peaks. The highest peak was seen at the retention time of 3.667 min.

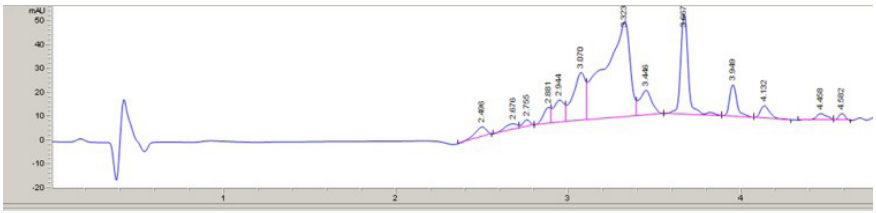

Figure 2. UPLC chromatogram of the dichloromethane extract under 260 nm

Because of the limited number of previous studies of HPLC and UPLC analysis of dichloromethane extract of *H. perforatum*, the majority of the compounds could not be determined by comparing the literature. Therefore, the main compounds at the retention time of 3.323 min and 3.667 min could not identified. A recent UPLC-MS study was carried out to determine the naphthodianthrones, emodin, skyrin, and bisanthrones in the *H. perforatum* L. extracts. The extraction was performed with several solvents including methanol, ethanol, ethyl acetate, acetone, and dichloromethane with changing ratios. This UPLC-MS analysis suggested that the extracts of the plant species are rich in the naphthodianthrones and bisanthrones. UPLC run time was 13 minutes in the mentioned study and

depending on the ratio of the UPLC run time and the time where compounds are observed, some possible compounds are determined in our analysis. Depending on the ratio, Luteoskyrin was seen at 2.673 min, and our compound 2 was determined as Luteoskyrin as it has retention time of 2.676 min. Similarly, depending on the ratio, the study identified Protohypericin at 2.876, and compound 4 was determined as Protohypericin as it has very close retention time of 2.881 min. And lastly, the previous study identified Hypericin at 3.165 min, and our compound 6 has a close retention time of 3.07 min, as a result, it was identified as Hypericin[18]. Due to the lack of previous studies, the rest of the compounds could not be identified. The list of the identified compounds and the retention times of the unknown compounds are provided below, in "Table 3".

Table 3. Possible compounds found in the dichloromethane extract of *H. perforatum* L. via UPLC under 260 nm

#	RT (min)	Area %	Possible Compound	Reference
1	2.496	2.321	Unknown	–
2	2.676	1.432	Luteoskyrin	(18)
3	2.755	0.940	Unknown	–
4	2.881	2.428	Protohypericin	(18)
5	2.944	4.602	Unknown	–
6	3.07	11.879	Hypericin	(18)
7	3.323	46.102	Unknown	–
8	3.446	5.688	Unknown	–
9	3.667	12.225	Unknown	–
10	3.949	5.173	Unknown	–
11	4.132	4.013	Unknown	–
12	4.458	2.176	Unknown	–
13	4.582	1.021	Unknown	–

The summary of the identified compounds by UPLC analysis has been shown below in "Table 4".

Table 4. Summary of the possible compounds found in the dichloromethane and methanolic extracts of *H. perforatum* L. via UPLC under 260 nm

Extract	#	RT (min)	Area %	Possible Compound	Reference
Methanolic Extract	**1**	3.161	8.886	Catechin	(15)
	2	3.335	17.438	Procyanidin B1	(15)
	3	3.659	25.519	Hyperforin	(16)
	4	3.962	5.196	Rutin	(15)
	5	4.144	3.081	Quercetin-3-O-galactoside (Hyperoside)	(15)
	6	4.470	0.898	Quercetin-3-O-rhamnoside (Isoquercetrin)	(15)
	7	4.592	1.172	Quercetin	(17)
Dichloromethane Extract	**1**	2.676	1.432	Luteoskyrin	(18)
	2	2.881	2.428	Protohypericin	(18)
	3	3.07	11.879	Hypericin	(18)

The findings from both the antibacterial assays and UPLC analyses provide a comprehensive overview of the bioactive potential of the tested extracts, particularly highlighting the remarkable activity of the n-hexane extract. These results are now explored in detail to assess their implications and alignments with existing research as they provide new insights into the antibacterial properties of less-polar extracts, such as n-hexane, a relatively underexplored area in the literature. The discussion contextualizes these findings within the broader framework of antimicrobial research.

In the current study, *n*-hexane extract is the one that demonstrated the best inhibitory effect against all the tested bacteria. The majority of the previous studies were carried out with methanolic, ethanolic, or aqueous extracts of the plant species. Therefore, the knowledge of the bacterial inhibitory property of the extracts obtained with less polar solvents, such as *n*-hexane is limited[19]. The *n*-hexane extract showed the strongest anti-staphylococcal activity against the MRSA strain *S. aureus* XU212, which carries the *tetK* efflux pump, with a MIC value of 256 μL/mL. The rest of the MIC of *n*-hexane and dichloromethane extracts against all the tested clinical isolates of *S. aureus* strains were the same which was 512 μL/mL. Even though there are considerable number of studies in the literature about the bacterial inhibitory effect of methanolic extracts of the plant species, in this study, methanol extract did not show activity against any of the tested *S. aureus* strains[19,20,21]. This can be explained with several reasons including the solvents used to obtain the extract. Many of the studies used methanol as a solvent either directly or with slightly less polar solvents before using methanol, such as acetone or ethanol[19]. However, in this study less polar compounds were first used then, methanol was used to obtain crude extracts. The compounds that exhibit the antibacterial activity in the methanolic extracts in the previous studies might be extracted within the first two solvents, as the activity was the strongest in the *n*-hexane extract. Hence the inhibitory effect of methanolic extract was not observed in the current study. Another reason can be the type of the bacteria used in the MIC assay. Previous studies have demonstrated promising antibacterial activity against several MRSA and PRSA strains with MIC and disc diffusion techniques. It has been proven that *H. perforatum* L. has activity against different bacterial strains that were used in the previous study, including *S. aureus* (PRSA) E12431, *S. aureus* (PRSA) E12398, and *S. aureus* (MRSA) RV5 strains. It was also demonstrated that the plant has activity against *S. aureus* ATCC 25923 strain which is the standard strain used in the current study[22]. Even though the previous study has demonstrated inhibitory activity against the standard strain of *S. aureus* ATCC 25923, no activity was observed in the current study, and this can be explained by the reason mentioned above as the extraction was done with 3 different solvents, as opposed to the study performed previously, as the antibacterial activity was observed for both *n*-hexane and dichloromethane extracts against all the tested *S. aureus* strains with MIC ranging between 256 – 512 μL/mL. Finally, one of the reasons for the lack of antibacterial activity of methanolic extracts can be the extraction type used. In the current study, Soxhlet extraction was used to obtain the crude extracts. However, the extracts were obtained with different techniques including percolation methods, decoction, or supercritical fluid ex-

traction in the previous studies where the antibacterial activity was seen[23,24,25]. The type of extraction could affect the compounds within the extracts, hence the antibacterial activity. In terms of Gram-negative bacteria, *n*-hexane extract has a promising result, as it showed significant MIC of 1 µL/mL, which is the lowest concentration tested against *E. coli* ATCC 25922 strains. Previous studies demonstrated that hyperforin which was isolated from *H. perforatum* L. demonstrated antibacterial activity against the same bacterial strain with the MIC of 0.1 µL/mL[22]. The reason for the strong antibacterial activity of *n*-hexane extract against *E. coli* could be the presence of hyperforin. Thereby, further studies should be focused on this activity and isolating this compound specifically for definitive results. Regarding *B. subtilis*, again *n*-hexane extract had the highest inhibitory activity with the MIC ranging between 64 – 128 µL/mL. Previous studies have shown that extracts of *H. perforatum* L. including methanol extracts have an inhibitory effect against the same bacterial strain of *B. subtilis* ATCC 6633 with MIC ranging between 25 – 50 µL/mL[26]. The reason for the lack of inhibitory activity in the methanol extract could be again the solvents used for extraction. In the previous studies, extraction was done with several solvents with similar polarity index, together with methanol. However, in the current study, methanol was used as the last solvent. Thereby, the activity was the strongest for *n*-hexane extract, followed by dichloromethane extract with the MIC ranging between 256 – 512 µL/mL and no activity for methanol extract. The compounds that showed antibacterial activity within the methanol extract in the previous study might be extracted with the *n*-hexane and dichloromethane extracts. This can be identified by isolation and purification of the compounds in the extracts with further research.

UPLC is a modern technique for liquid chromatography as it provides more precise and reliable results by using a smaller particle size, which is 2 µm, than HPLC which is between 3 – 5 µm with a shorter run time, hence quick results. It was invented in 2004, which can be considered as a fairly new technique, and therefore it is not commonly used in the studies today[27]. Because of this, the reports from previous studies are very limited for UPLC analysis of *H. perforatum*, as the majority of the studies used the

HPLC technique, which is an older commonly used technique. The studies that performed UPLC for the extracts of *H. perforatum* L. have demonstrated some compounds that have antibacterial activity against various bacteria, and some of the compounds include Hypericin, Hyperforin, and Luteoskyrin[15]. In the current study, the compounds were identified depending on the retention times of the compounds in the previous studies. The run times of the samples

were different from some of the former studies. Therefore, the ratio of the retention time and the total run time was used to compare with the peaks of the extracts in this study to determine the compounds. Similarly, some of the compounds were identified by comparing previous HPLC results of the plant extracts in the same way. Further studies should focus on the identification of the compounds by isolation and purification of the compounds and perform UPLC with mass spectrometry for definitive results.

Although the present study evaluated a limited number of bacterial strains, the promising results suggest that future studies should expand the range of clinical isolates tested. Incorporating a broader spectrum of multidrug-resistant (MDR) pathogens could validate the findings and increase the generalizability of the antibacterial activity observed in *n*-hexane and dichloromethane extracts. The strong activity of the *n*-hexane extract against both Gram-positive and Gram-negative bacteria, particularly MDR strains like *S. aureus* XU212 and *E. coli* ATCC 25922, highlights its potential as a natural antibacterial agent. These findings could guide the development of plant-based inhibitors targeting efflux pumps or other resistance mechanisms in pathogenic bacteria. The results underscore the potential application of *H. perforatum*-derived compounds in pharmaceutical sciences. By isolating and characterizing the active components, these extracts could be optimized for therapeutic use, either as standalone agents or in synergy with existing antibiotics. The UPLC analysis revealed the presence of key bioactive compounds such as Hyperforin and Hypericin, which are known for their antimicrobial properties. Further studies employing advanced techniques like UPLC-MS or NMR spectroscopy are necessary to confirm these findings and to identify the unknown compounds detected in both methanolic and dichloromethane extracts. Given the relatively recent introduction of UPLC, its application in studying *H. perforatum* L. extracts remains underutilized. Expanding its use in combination with mass spectrometry could facilitate a deeper understanding of the plant's chemical profile and its link to antibacterial activity. While the MIC values of *n*-hexane extract were higher than those of the synthetic antibiotic Norfloxacin, the results remain significant given that the extract represents a crude mixture. Optimization and purification of the active components could potentially enhance their efficacy and position them as viable alternatives or adjuncts to synthetic antibiotics. The study not only advances our understanding of natural antibacterial agents but also provides a foundation for the integration of less-polar plant extracts into drug discovery pipelines. The demonstrated efficacy against resistant strains suggests a promising avenue for addressing the global challenge of antibiotic resistance.

The majority of the previous studies were focused on methanolic, ethanolic, and aqueous extracts. However, in this study, less polar solvents, such as *n*-hexane, were also used to examine the antibacterial properties of the plant species against variety of bacteria. In addition to the information about the antibacterial activity of methanolic and ethanolic extracts in the literature, it was demonstrated that *n*-hexane extract has a promising inhibitory result for some of the tested bacteria. Especially against *E. coli*, as the *n*-hexane extract inhibited the bacteria for the lowest concentration tested, which was 1 μL/mL. Additionally, as the UPLC technique is a recently discovered liquid chromatography technique, there were a limited number of UPLC analysis of the *H. perforatum* L. extracts. Although there are some UPLC chromatograms for the methanol extract of *H. perforatum* L., this study is the first report on the UPLC chromatograms of the dichloromethane extract. With further investigation, our findings will be helpful for the identification of the chemical composition of *H. perforatum* L.

STATEMENT OF ETHICS

This study does not require any ethical approval.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Concept: E.G., M.R.; Design: E.G., M.R.; Data Collection and Processing: E.G., M.R.; Analysis or Interpretation: E.G., M.R.; Literature Search: E.G.; Writing: E.G.

FUNDING SOURCES

No funding or other financial support was received.

ACKNOWLEDGMENTS

Not applicable.

REFERENCES

1. Wertheim HF, Melles DC, Vos MC, van Leeuwen W, van Belkum A, Verbrugh HA, et al. The role of nasal carriage in *Staphylococcus aureus* infections. Lancet Infect Dis, 2005;5(12):751-762. Doi: 10.1016/S1473-3099(05)70295-4

2. Lee JH, Bae IK, Hee Lee S. New definitions of extended-spectrum β-lactamase conferring worldwide emerging antibiotic resistance. Med Res Rev, 2012;32(1):216-232. Doi: 10.1002/med.20210

3. World Health Organization. Antimicrobial resistance: global report on surveillance 2014 [Internet]. World Health Organization, Geneva; 2014 [Jan 9, 2025]. Available from: https://iris.who.int/bitstream/handle/10665/112642/9789241564748_eng.pdf

4. Sievert DM, Ricks P, Edwards JR, Schneider A, Patel J, Srinivasan A, et al. Antimicrobial-resistant pathogens associated with healthcare-associated infections summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2009–2010. Infect Control Hosp Epidemiol, 2013;34(1):1-14.

5. The European Centre for Disease Prevention and Control. Point prevalence survey of healthcare-associated infections and antimicrobial use in European acute care hospitals: 2011 2012. [Internet]. LU: Publications Office; 2013. Available from: 10.1086/668770

6. Moloney MG. Natural products as a source for novel antibiotics. Trends Pharmacol Sci, 2016;37(8):689-701. Doi: 10.1016/j.tips.2016.05.001

7. Rossiter SE, Fletcher MH, Wuest WM. Natural products as platforms to overcome antibiotic resistance. Chem Rev, 2017;117(19):12415-12474. Doi: 10.1021/acs.chemrev.7b00283

8. Khameneh B, Iranshahy M, Soheili V, Fazly Bazzaz BS. Review on plant antimicrobials: a mechanistic viewpoint. Antimicrob Resist Infect Control, 2019;8(1):118. Doi: 10.1186/s13756-019-0559-6

9. Lee MR. Saint John's wort (Hupericum Perforatum). A balm for hurt minds? Proc R Coll Physicians Edinb, 1999;29(3):253-237.

10. Linde K. St. John's wort - an overview. Forsch Komplementmed, 2009;16(3):146-155. Doi: 10.1159/000209290

11. Saller R, Melzer J, Reichling J. Johanniskraut (Hypericum perforatum): ein plurivalenter rohstoff für traditionelle und moderne therapien. Complement Med Res, 2003;10(1):33-40. Doi: 10.1159/000071690

12. Smith ECJ, Kaatz GW, Seo SM, Wareham N, Williamson EM, Gibbons S. The phenolic diterpene totarol inhibits multidrug efflux pump activity in *Staphylococcus aureus*. Antimicrob Agents Chemother, 2007 Dec;51(12):4480-4483. Doi: 10.1128/AAC.00216-07

13. Abreu AC, Saavedra MJ, Simões LC, Simões M. Combinatorial approaches with selected phytochemicals to increase antibiotic efficacy against *Staphylococcus aureus* biofilms. Biofouling, 2016;32(9):1103-1114. Doi: 10.1080/08927014.2016.1232402

14. Andrews JM. The development of the BSAC standardized method of disc diffusion testing. J Antimicrob Chemother, 2001;48(Suppl.1):29-42. Doi: 10.1093/jac/48.suppl_1.29

15. Farag M, Wessjohann L. Metabolome classification of commercial *Hypericum perforatum* (St. John's wort) preparations via UPLC-qTOF-MS and chemometrics. Planta Med, 2012;78(5):488-496. Doi: 10.1055/s-0031-1298170

16. Hokkanen J, Tolonen A, Mattila S, Turpeinen M. Metabolism of hyperforin, the active constituent of St. John's wort, in human liver microsomes. Eur J Pharm Sci, 2011;42(3):273-284. Doi: 10.1016/j.ejps.2010.12.002

17. Chandrasekera DH, Heinrich M, Ashton D, Welham KJ, Middleton R. Quantitative analysis of the major constituents of St John's wort with HPLC-ESI-MS. J Pharm Pharmacol, 2005;57(12):1645-1652. Doi: 10.1211/jpp.57.12.0015

18. Pradeep M, Kachlicki P, Franklin G. Simultaneous determination of naphtodianthrones, emodin, skyrin and new bisanthrones in *Hypericum perforatum* L. *in vitro* shoot cultures. Ind Crops Prod, 2020;144:112003. Doi: 10.1016/j.indcrop.2019.112003

19. Saddiqe Z, Naeem I, Maimoona A. A review of the antibacterial activity of *Hypericum perforatum* L. J Ethnopharmacol, 2010;131(3):511-521. Doi: 10.1016/j.jep.2010.07.034

20. Gibbons S, Ohlendorf B, Johnsen I. The genus *Hypericum*—a valuable resource of anti-Staphylococcal leads. Fitoterapia, 2002;73(4):300-304. Doi: 10.1016/s0367-326x(02)00082-5

21. Reichling J, Weseler A, Saller R. A current review of the antimicrobial activity of *Hypericum perforatum* L. Pharmacopsychiatry, 2001;34(Suppl.1):116-118. Doi: 10.1055/s-2001-15514

22. Schempp CM, Pelz K, Wittmer A, Schöpf E, Simon JC. Antibacterial activity of hyperforin from St John's wort, against multiresistant *Staphylococcus aureus* and gram-positive bacteria. The Lancet, 1999;353(9170):2129. Doi: 10.1016/S0140-6736(99)00214-7

23. Dadgar T, Asmar M, Saifi A, Mazandarani M, Bayat H, Moradi A, et al. Antibacterial activity of certain Iranian medicinal plants against methicillin-resistant and sensitive *Staphylococcus aureus*. Asian J Plant Sci, 2006;5(5):861-866.

24. Quave CL, Plano LRW, Pantuso T, Bennett BC. Effects of extracts from Italian medicinal plants on planktonic growth, biofilm formation and adherence of methicillin-resistant *Staphylococcus aureus*. J Ethnopharmacol, 2008;118(3):418-428. Doi: 10.1016/j.jep.2008.05.005

25. Voss A, Verweij PE. Antibacterial activity of hyperforin from St John's wort. The Lancet, 354(9180):777. Doi: 10.1016/S0140-6736(05)76018-9

26. Avato P, Raffo F, Guglielmi G, Vitali C, Rosato A. Extracts from St John's wort and their antimicrobial activity. Phytother Res, 2004;18(3):230-232. Doi: 10.1002/ptr.1430

27. Swartz ME. UPLC™: an introduction and review. J Liq Chromatogr Relat Technol, 2005;28(7-8):1253-1263. Doi: 10.1081/JLC-200053046