BIODIVERSITAS
Volume 23, Number 9, September 2022
Pages: 4912-4917

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d230961

Biological activity of some phenolic compounds extracted from *Agrimonia eupatoria* against several pathogenic bacteria species

MOHAMMED ARAFAT MOHAMMED[1,♥], JASSIM FATEHI ALI[1,2], YOUNIS SAADI SAEED[1], IBRAHIM HASSAN YASEEN[2], BUSHRA HABEEB AHMAD[2]

[1]Department of Biology, College of Education for Pure Science, University of Mosul. Mosul, Iraq.
Tel./fax.: +96-477-39462008, ♥email: dr.mohamedarafat75@uomosul.edu.iq
[2]Department of Sensing and Nano-Photonics, Laser and Photonics Research Center, University of Al-Hamdaniya. Mosul, Iraq

Manuscript received: 15 July 2022. Revision accepted: 16 September 2022.

Abstract. *Mohammed MA, Ali JF, Saeed YS, Yaseen IH, Ahmad BH. 2022. Biological activity of some phenolic compounds extracted from* Agrimonia eupatoria *against several pathogenic bacteria species. Biodiversitas 23: 4912-4917. Agrimonia eupatoria* L. (Rosaceae) has been used for centuries in traditional medicine to overcome infectious diseases. This study was carried out to isolate, characterize, and determine the antibacterial activity of natural compounds obtained from *A. eupatoria* aerial parts. Three solvents were used in the extraction process. First, hexane was used for defatting plant samples. Then ethyl acetate and ethanol were used to obtain the fraction containing phenolic compounds and their antibacterial activity against several bacteria. The current study identifies several phenolic compounds isolated from *A. eupatoria*, including gallic acid, salicylic acid, cinnamic acids, and resorcinol, using thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC). In addition, the disc diffusion method was used to determine the antimicrobial activity of several phenolic compounds against four bacterial isolates, i.e., *Corynebacterium diphtheria*, *Bacillus cereus*, *Salmonella typhymurium*, and *Enterococcus faecalis*. The results indicated that the plant compounds had an antibacterial activity proportional to the concentration. The *B. cereus* was the most sensitive to the compound isolated from *A. eupatoria*, followed by *C. diphtheria*, whereas *S. typhimurium* exhibited the most insensitive.

Keywords: *Agrimonia eupatoria*, antimicrobial activity, cinnamic acids, gallic acid, HPLC, resorcinol, salicylic acid

INTRODUCTION

Researchers and scientists have studied medicinal plants and their compounds as natural medicines for curing numerous diseases. These compounds are natural products that are not directly on the basic function of plant life but important for plant survival or protection against other living organisms (Mishra and Tiwari 2011; Kumar et al. 2015). There are a lot of studies on plant's natural products (Putra et al. 2020; Hisham et al. 2022; Lakshmanan et al. 2022)

Antibiotics have been essential in treating diseases caused by pathogenic microbes and have increased average life expectancy (Owusu et al. 2021). In addition, antibiotics have a considerable positive influence on society, including a decrease in the number of people exposed to bacterial infections and people dying from infection, especially in underdeveloped nations with weak public health systems (Subramani et al. 2017). However, antibiotics have been misused and inappropriately utilized, leading to increased antibiotic resistance (Shin et al. 2018). It is estimated that there are 500,000 species of plants worldwide, both identified and unidentified. Humans and animals consume only 1% to 10% of them. Since ancient times, plants have been the primary source of medicine and alternative medicine for various ailments, and about 50% of all pharmaceuticals sold in the US originated from plants.

Nevertheless, the use of plant derivatives as antimicrobials has virtually disappeared since the development of antibiotics in the 1950s. Although, due to their accessibility, plant-based drugs are frequently used for self-medication because of their potential and lack of side effects. Natural compounds produced by plants for medical therapies are receiving increased attention. Plants are a great source of beneficial secondary metabolites such as phenols and anther compounds (Srivastava et al. 2014; Cappiello et al. 2020). Excessive and inappropriate use of antibiotics causes bacteria to develop new methods of resistance (Banin et al. 2017; Bassetti et al. 2018) with the help of plasmids, which can be transferred from one bacterium to another; mutations in beta-lactamase enzymes, which convert antibiotics with a beta-lactam ring from active to inactive forms; and the ability of bacterial pathogens to change the antibiotics' receptor sites. The increasing bacterial resistance has increased interest in efforts to develop alternatives sources that can overcome resistance, including the use of probiotics such as Lactobacillus and Lactococcus species to treat a variety of diarrhea-causing bacteria, as well as the use of bacteriophages to treat a variety of infections caused by Staphylococcus aureus, such as intestinal and urinary tract infections (Ahmed et al. 2014).

Among the most important alternatives widely used in treating numerous diseases are medicinal plants, which have been the subject of extensive scientific research due to

their abundance in nature and diversity. It resulted in producing various plant extracts and compounds effective against pathogenic microorganisms. Compared to synthetically synthesized compounds, compounds isolated from plants have lower adverse effects and are efficient against many bacteria due to their structural diversity (Muruzović et al. 2016; Lakshmanan et al. 2022). Plants can produce infinite compounds, including phenolic compounds, fatty acids, alkaloids, volatile oils, glycosides, tannins, and terpenes (Ginovyan et al. 2017; 2020). The goals of this study were to evaluate the antimicrobial activity of three *Agrimonia eupatoria* L. extracts against microbes that were susceptible and resistant to antibiotics and to identify bioactive secondary metabolites.

MATERIALS AND METHODS

Plant collection and extraction

The flowering buds of *A. eupatoria* were collected and thoroughly cleaned, then washed with distilled water and dried in the shade on large filter papers in a suitable location at room temperature, turning them over occasionally to prevent rotting. Then they were sealed in dry paper bags and stored in a moisture-free environment until they were used. The extracts were prepared using a continuous extraction device (Soxhlet) and a series of successive solvents (hexane, ethyl acetate, and ethanol), with the separation process carried out by adding 1000 mL of each solvent to 100 g of extract powder. The extraction process was repeated for a period ranging from 48 to 72 hours for each type of solvent or until the filtrate was colorless. Finally, the extracts were concentrated using a rotary vacuum; the crude extracts were stored in tightly sealed dark-colored bottles to avoid exposure.

Phenolic compounds separation and identification

Thin–layer chromatography (TLC)

Phenolics were identified using TLC by transferring 2 µL of the sample. The solvent and reagent for phenolic identification are presented in Table 1.

High-performance liquid chromatography (HPLC)

Most phenolic compounds isolated from plants are easily ionized in basic conditions and dissolved in polar solvents. It enables their analysis using various separation methods, including HPLC, which depends on capillary and polar properties. The compounds were separated and identified using the Shimadzu LC-2010 high-performance liquid chromatography (Macherey-Nagel). The sample was purified using 0.1 µm membrane filters. The mobile phase was acetonitrile: water (80:20) at a flow rate of 1 ml/minute, using a separation column of type C18 with dimensions (250 x 0.46 mm), at 60°C, equipped with a UV (Mizzi et al. 2020). The detection was carried out at 280 nm.

Rate of flow (RF)

In the case of (TLC) technology, the flow rate values of the standard compounds were matched to the flow rate values of the separated compounds to be diagnosed using the following equation: Sample flow rate (RF) = (distance traveled by sample/distance traveled by the solvent)

Retention time (RT)

The separated compounds are diagnosed by comparing the holding time values of the standard compounds to the separated compounds' retention times (Ferrentino et al. 2021).

Preparation and sterilization of phenolic compounds for antibacterial assay

The stock solution of phenolic compounds was made at 100 mg/mL concentration in DMSO and diluted at the sequential concentrations of 1.562, 3.125, 6.25, 12.5, 25, and 50 mg/mL. Phenolic compounds solution was sterilized using a membrane filter of 0.22 µg (El Jery et al. 2020).

Antibacterial assay of phenolic compounds

Bacteria colonies were transferred to the broth medium and incubated at 37°C for 24 hours. The bacterial suspension was then diluted with physiological saline and compared to the standard McFarland's solution containing 108 cells/mL. Then, using a sterile cotton swab, 0.1 mL of the diluted bacterial suspension was spread homogeneously on the surface of a sterile Mueller-Hinton agar medium. Next, the Petri dishes were incubated for half an hour at 37°C to allow impregnation. Next, 0.1 mL of each sample concentration (1.562, 3.125, 6.25, 12.5, 25, 50, and 100 mg/mL) was transferred to Whatman No. 1 filter paper discs with a diameter of 6 mm. The discs were then put on the Mueller Hinton Agar inoculated with bacteria and followed by incubation at 37°C for 14-16 hours. Finally, the inhibition zone was determined using a graduated ruler and compared to the antibiotics' inhibitory zone (Chen et al. 2019).

Table 1. Solvent systems for phenolics identification and reagents to identify phenolic compounds using the (TLC) technique

Compound	Solvent system	Detector
Gallic acid	Ethyl acetate: benzene (11: 9)	Vanillin – HCl
Salicylic acid	Ethyl acetate: benzene (11: 9)	Vanillin – HCl
Resorcinol	Ethyl acetate: benzene (11: 9)	Vanillin – HCl
Vanillic acid	Ethyl acetate: benzene (11: 9)	Vanillin – HCl
Coumarin	Butanol: acetic acid: water (50:10:40)	UV
Thymol	Petrol	Vanillin – H_2SO_4
Cinnamic acid	Hexane: acetone (20:80)	UV

RESULTS AND DISCUSSION

TLC was used to separate and determine a variety of phenolic compounds from plant extracts, particularly the ethanol extract in this study, by comparing the Rf values of the separated compound with the Rf values of the standard compound (gallic acid, salicylic acid, resorcinol, vanillic acid, coumarin, thymol, and cinnamic acid). The results showed that compounds extracted from *A. eupatoria* exhibited spots with Rf values of 0.58, 0.83, 0.40, and 0.43, respectively, which were identified as gallic acid, salicylic acid, resorcinol compound, and cinnamic acid (Table 2). The isolation and determination of flavonoids and phenolic acids have been accomplished using various analytical techniques, including chromatographic methods. The most popular is TLC. TLC is an important method for pharmacognostic analysis, determination of polyphenols in extracts, and validating and identifying natural compounds of medicinal plants (Jesionek et al. 2015).

HPLC separation and identification of phenolic compounds

This technique was used to separate and determine various phenolic compounds isolated from *A. eupatoria* by comparing the retention time of the isolated phenolic compounds (Rt) to the retention time of the standard phenolic compounds, i.e., gallic acid (Figure 1), salicylic acid (Figure 2), resorcinol (Figure 3), cinnamic acid (Figure 4), resorcinol (Figure 5), gallic acid and salicylic acid (Figure 6), and resorcinol and cinnamic acid (Figure 7). Ten µL of standard phenolic compounds or phenol-containing plant extracts were injected into the HPLC at a rate of 1 mL per second. The chromatogram of each standard phenolic compound was plotted to relate to retention time and concentration. The phenolic compounds were isolated from the plant's ethanol extract. When the area under the curves for the separated compounds was compared, the highest area under the curve (9122) was observed for the resorcinol compound isolated from the ethanol extract, as shown in Figure 5. Gallic acid and salicylic acids were isolated from the ethanol extract and had an area under the curve of (5988 and 3368), respectively, as shown in Figure 6. Resorcinol and cinnamic acid had the area under the standard curve, while the isolated from the extract had an area under the curve (1354) (2903), respectively, as shown in Figure 7.

Antibacterial activity of phenolic compounds

The phenolic compounds in this study showed higher inhibitory activity against several bacteria isolates compared to the standard antibiotics (amikacin, streptomycin, and chloramphenicol), as presented in Table 3. These findings were in comparison to previous studies. For example, a previous study by Emira et al. (2014) showed that Agrimony contains numerous active compounds such as catechin, hydroquinone, and cinnamic acid that have antibacterial activity against several antibiotic-resistant bacterial species, including *S. aureus* and *Salmonella* spp. Hemayet et al. (2013) have separated several active compounds from the Agrimony plant, including rutin, catechin, hyperoside, and quercetin. Islam et al. (2014) evaluated the antibacterial, antioxidative, and antimutagenic properties of several active compounds isolated from the *Agrimony* plant, including catechin and several flavonoids and polyphenols. A study by Monica et al. (2013) demonstrated seed extracts of *Agrimonia* seed extracts have antibacterial activity against various Gram-positive and Gram-negative bacteria. The ethanolic extract had better antibacterial activity than hexane or methanol extracts. The antibacterial activity of phenolic compounds might be due to their high permeability through the bacterial cell wall. Phenolic compounds also inhibit protease enzyme activity, which breaks down the bacterial cell wall and plasma membrane (Pervin et al. 2012).

Table 2. Identification of phenolic compounds in *A. eupatoria* based on the Rf value of standard phenolic compounds

Standard phenolic compounds	Gallic acid	Salicylic acid	Resorcinol	Vanillic acid	Coumarin	Thymol	Cinnamic acid
Rf of standard phenolic compound	0.40	0.82	0.59	0.73	0.92	0.83	0.43
Rfs of *A. eupatoria* compounds	0.40	0.83	0.58	---	---	---	0.43

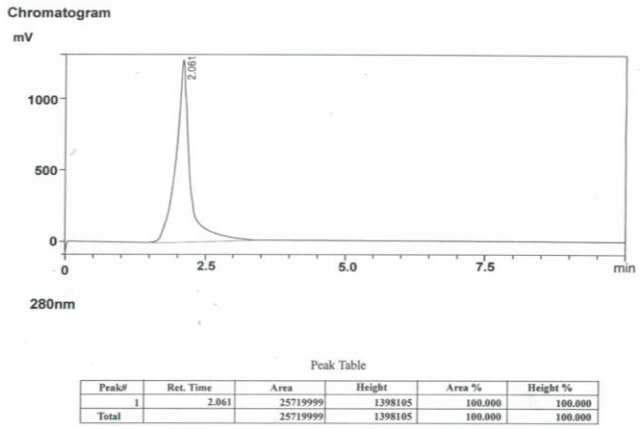


Figure 1. HPLC chromatogram of gallic acid standard using acetonitrile: water (80:20 v/v) solvent system at 280 nm

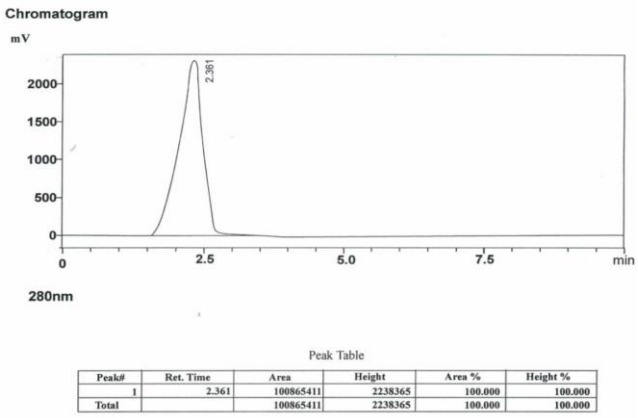
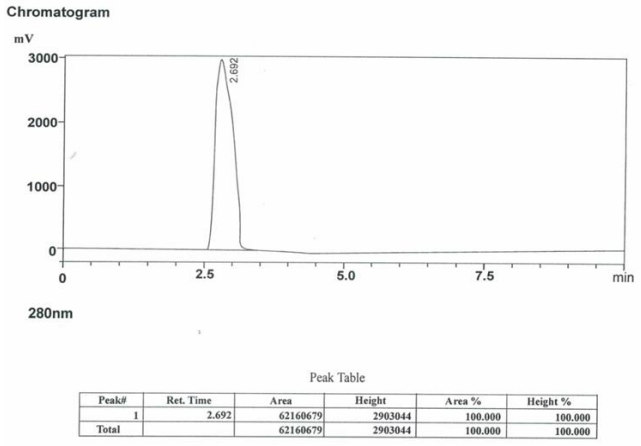


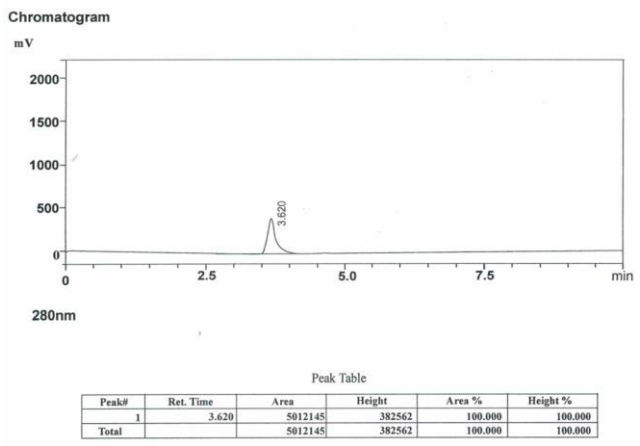
Figure 2. HPLC chromatogram of salicylic acid standard using acetonitrile: water (80:20 v/v) solvent system at 280 nm



Peak Table

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.692	62160679	2903044	100.000	100.000
Total		62160679	2903044	100.000	100.000

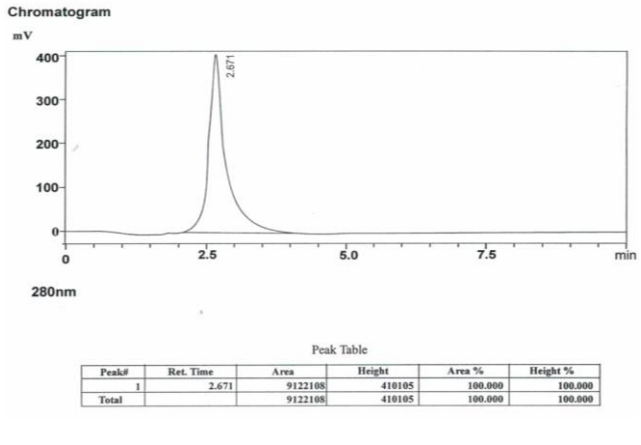
Figure 3. HPLC chromatogram of resorcinol standard using acetonitrile:water (80:20 v/v) solvent at 280



Peak Table

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.620	5012145	382562	100.000	100.000
Total		5012145	382562	100.000	100.000

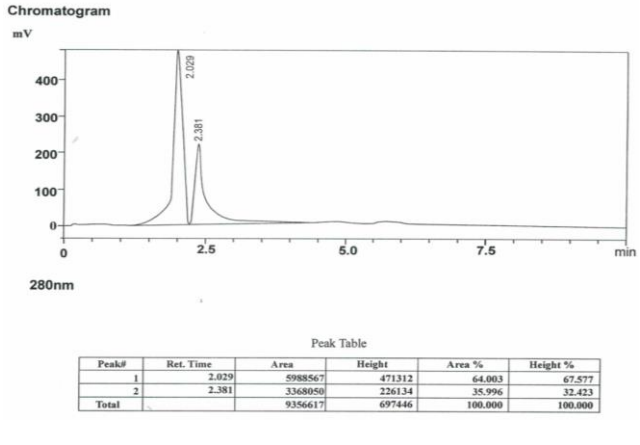
Figure 4. HPLC chromatogram of cinnamic acid standard using acetonitrile:water (80:20 v/v) solvent at 280



Peak Table

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.671	9122108	410105	100.000	100.000
Total		9122108	410105	100.000	100.000

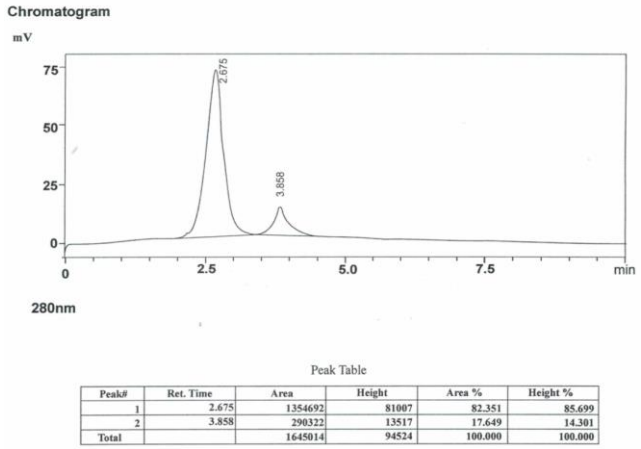
Figure 5. HPLC chromatogram of resorcinol isolated from *A. eupatoria* using acetonitrile:water (80:20 v/v) solvent at 280



Peak Table

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.029	5988567	471312	64.003	67.577
2	2.381	3368050	226134	35.996	32.423
Total		9356617	697446	100.000	100.000

Figure 6. HPLC chromatogram of gallic acid and salicylic acids isolated from *A.eupatoria* using acetonitrile:water (80:20 v/v) solvent at 280



Peak Table

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.675	1354692	81007	82.351	85.699
2	3.858	290322	13517	17.649	14.301
Total		1645014	94524	100.000	100.000

Figure 7. HPLC chromatogram of resorcinol and cinnamic acid isolated from A.eupatoria using acetonitrile:water (80:20 v/v) solvent at 280

The study conducted by Saha et al. (2012) demonstrated that the inhibitory effect of plant extracts and some active compounds isolated from several species of plants, including *Agrimonia*, against several pathogenic Gram-positive and Gram-negative bacteria were significant, particularly quercetrin and catechin isolated from the *Agrimonia*. In addition, the extract of *Agrimonia* and some isolated compounds, such as flavonoids, inhibited the growth of *S. aureus*. However, there is no activity against Gram-negative bacteria.

The effect of varying phenolic compound concentrations on the bacteria under investigation:

The ethanol extract of resorcinol inhibited several bacterial species with the highest diameter inhibition (23 mm) against *Bacillus cereus* at a concentration of 100 mg/mL, while the lowest inhibition diameter (8 mm) was against *Enterococcus faecalis* at a concentration of 6.25 mg/mL, and *Corynebacterium diphtheriae* at a concentration of 3.125 mg/mL. The growth of isolates was not inhibited by resorcinol at the concentration of 1.562 mg/mL (Table 4).

Table 3. Diameter of growth inhibition of phenolic compounds (100 mg/mL) and standard antibiotics against four bacterial species

Plant compound	*B. cereus*	*C. diphtheriae*	*E. faecalis*	*S. typhimurium*
Resorcinol	23	21	20	19
Gallic and salicylic acids	29	26	24	23
Resorcinol and cinnamic acid	26	25	23	20
Compared antibiotics				
Amikacin	18	17	17	16
Chloramphenicol	19	20	19	18
Streptomycin	17	16	15	14

Table 4. The inhibitory effect of fraction of *A. eupatoria* extract containing resorcinol against four bacteria isolates

Resorcinol concentration (mg/mL)	*B. cereus*	*C. diphtheriae*	*E. faecalis*	*S. typhimurium*
100	*23	21	20	19
50	19	17	16	15
25	16	15	13	12
12.5	13	13	11	10
6.25	11	10	8	-
3.125	9	8	-	-
1.562	-	-	-	-

Note: *= mm

Table 5. The inhibitory effect of fraction of *A. eupatoria* ethanol extract containing gallic acid and salicylic acid against bacteria isolates

Gallic and salicylic acid concentration (mg/mL)	*B. cereus*	*C. diphtheriae*	*E. faecalis*	*S. typhimurium*
100	*29	26	24	23
50	25	23	21	19
25	22	19	17	15
12.5	19	17	14	11
6.25	15	14	12	-
3.125	12	10	9	-
1.562	10	-	-	-

Note: *= mm.t

Table 6. The inhibitory effect of fraction of *A. eupatoria* ethanol extract containing resorcinol and cinnamic acid against four bacteria isolates

Resorcinol and cinnamic acid conc. (mg/mL)	*B. cereus*	*C. diphtheriae*	*E. faecalis*	*S. typhimurium*
100	*26	25	23	20
50	21	20	19	17
25	18	17	16	14
12.5	16	14	13	11
6.25	13	12	11	10
3.125	10	10	9	8
1.562	-	-	-	-

Note: *= mm

The inhibitory activity was directly proportional to concentrations. The ethanol extracts of gallic acid and salicylic acid were also able to inhibit bacterial growth, with the highest diameter inhibition (29 mm) against *B. cereus* at a concentration of 100 mg/mL and the lowest diameter of inhibition (9 mm) against *E. faecalis* at a concentration of 3.125 mg/mL. The growth of all species was not inhibited by gallic acid and salicylic acid at a concentration of 1.562 mg/mL (Table 5). The ethyl acetate fraction of *A. eupatoria* containing resorcinol and cinnamic acid also inhibited the growth of bacteria at various concentrations. The lowest diameter of inhibition (8 mm) of the fraction containing resorcinol and cinnamic acid was against *S. typhimurium* at a concentration of 3.125 mg /mL). The 1.562 mg/mL concentration could not inhibit the growth of all bacterial isolates. Table 6 shows that the inhibitory activity is directly proportional to the concentration.

In conclusion, the results of this study revealed that the highest inhibitory activity was obtained at the ethanol extracts of gallic acid and salicylic acid against *B. cereus* (29 mm) at a concentration of 100 mg/mL. The lowest inhibitory activity was obtained at 8 mm against *E. faecalis* at a concentration of 3.125 mg/mL with the smallest diameter of 3.125 mg/mL inhibition. The growth of all bacterial species was not inhibited at a concentration of 1.562 mg/mL. The findings of this study could be used to develop an effective method for extracting phenolic compounds from *A. eupatoria*.

ACKNOWLEDGEMENTS

The authors appreciate the assistance provided by the College of Education for Pure Science, University of Mosul, Iraq. Facilities, which aided in the enhancement of the quality of this work.

REFERENCES

Ahmed EA, Hassan EA, El Tobgy KMK, Ramadan EM. 2014. Evaluation of rhizobacteria of some medicinal plants for plant growth promotion and biological control. Ann Agric Sci 59 (2): 273-280. DOI: 10.1016/j.aoas.2014.11.016.

Banin E, Hughes D, Kuipers OP. 2017. Bacterial pathogens, antibiotics and antibiotic resistance. FEMS Microbiol Rev 41 (3): 450-452. DOI: 10.1093/femsre/fux016.

Bassetti M, Vena A, Russo A, Croxatto A, Calandra T, Guery B. 2018. Rational approach in the management of *Pseudomonas aeruginosa* infections. Curr Opin Infect Dis 31 (6): 578-586. DOI: 10.1097/QCO.0000000000000505.

Cappiello F, Loffredo MR, Del Plato C, Cammarone S, Casciaro B, Quaglio D, Mangoni ML, Botta B, Ghirga F. 2020. The revaluation of plant-derived terpenes to fight antibiotic-resistant infections. Antibiotics 9 (6): 325. DOI: 10.3390/antibiotics9060325.

Chen X, Li S, Yu L, Miller A, Du L. 2019. Systematic optimization for production of the anti-MRSA antibiotics WAP-8294A in an engineered strain of *Lysobacter enzymogenes*. Microbial Biotechnol 12 (6): 1430-1440. DOI: 10.1111/1751-7915.13484.

El Jery A, Hasan M, Rashid MM, Al Mesfer MK, Danish M, Rebah FB. 2020. Phytochemical characterization, and antioxidant and antimicrobial activities of essential oil from leaves of the *common*

sage L. from Abha, Saudi Arabia. Asian Biomed 14 (6): 261-270. DOI: 10.1515/abm-2020-0035.

Ferrentino G, Haman N, Morozova K, Tonon G, Scampicchio M. 2021. Phenolic compounds extracted from spruce (*Picea abies*) by supercritical carbon dioxide as antimicrobial agents against gram-positive bacteria assessed by isothermal calorimetry. J Therm Anal Calorim 145 (6): 3093-3103. DOI: 10.1007/s10973-020-10100-7.

Ginovyan M, Ayvazyan A, Nikoyan A, Tumanyan L, Trchounian A. 2020. Phytochemical screening and detection of antibacterial components from crude extracts of some Armenian herbs using TLC-bioautographic technique. Curr Microbiol 77 (7): 1223-1232. DOI: 10.1007/s00284-020-01929-0.

Ginovyan M, Petrosyan M, Trchounian A. 2017. Antimicrobial activity of some plant materials used in Armenian traditional medicine. BMC Complement Altern Med 17 (1): 1-9. DOI: 10.1186/s12906-017-1573-y.

Hisham SM, Mohammad AM, Mohammed MJ. 2022. The effect of extracts and phenolic compounds isolation from *Rosmarinus officinalis* plant leaves on *Tribolium castaneum* mortality. Technology 12 (2): 814-819.

Islam M, Biswas NN, Saha S, Hossain H, Jahan IA, Khan TA, Awang K, Shilpi JA. 2014. Antinociceptive and antioxidant activity of *Zanthoxylum budrunga* Wall (Rutaceae) seeds. Sci World J 2014: 869537. DOI: 10.1155/2014/869537.

Jesionek W, Majer-Dziedzic B, Choma IM. 2015. Separation, identification, and investigation of antioxidant ability of plant extract components using TLC, LC-MS, and TLC–DPPH. J Liq Chromatogr Relat Technol 38 (11): 1147-1153. DOI: 10.1080/10826076.2015.1028295.

Kumar N, Wani ZA, Dhyani S. 2015. Ethnobotanical study of the plants used by the local people of Gulmarg and its allied areas, Jammu & Kashmir, India. Intl J Curr Res Biosci Plant Biol 2 (9): 16-23.

Lakshmanan G, Selvam R, Altemimi AB, Baldelli A, Bharathi AR, Gopalakrishnan B, Karthik L, Saravanan D, Kavitha M, Mohammed MJ, Cacciola F. 2022. Phytochemical screening of ethanolic extracts of *Cuminum cyminum* L. seeds along with the evaluation of antidiabetic properties by molecular docking approach. Nat Prod Res 1-6. DOI: 10.1080/14786419.2022.2078973.

Mishra BB, Tiwari VK. 2011. Natural products: An evolving role in future drug discovery. Eur J Med Chem 46 (10): 4769-4807. DOI: 10.1016/j.ejmech.2011.07.057.

Mizzi L, Chatzitzika C, Gatt R, Valdramidis V. 2020. HPLC analysis of phenolic compounds and flavonoids with overlapping peaks. Food Technol Biotechnol 58 (1): 12-19. DOI: 10.17113/ftb.58.01.20.6395.

Muruzović MŽ, Mladenović KG, Stefanović OD, Vasić SM, Čomić LR, 2016. Extracts of *Agrimonia eupatoria* L. as sources of biologically active compounds and evaluation of their antioxidant, antimicrobial, and antibiofilm activities. J Food Drug Anal 24 (3): 539-547. DOI: 10.1016/j.jfda.2016.02.007.

Owusu E, Ahorlu MM, Afutu E, Akumwena A, Asare GA. 2021. Antimicrobial activity of selected medicinal plants from a sub-Saharan African country against bacterial pathogens from post-operative wound infections. Med Sci 9 (2): 23. DOI: 10.3390/medsci9020023.

Pervin R, Sabrin F, Billah MM, Islam KD, Elmezughi J, Shilpi JA. 2012. Antibacterial and cytotoxic activity of *Meyna spinosa* Roxb. stem. Turk J Pharm Sci 9 (2): 225-230.

Putra KWE, Pitoyo A, Nugroho GD, Rai M, Setyawan AD. 2020. Review: Phytochemical activities of *Ficus* (Moraceae) in Java Island, Indonesia. Bonorowo Wetlands 10: 98-125. DOI: 10.13057/bonorowo/w100204.

Saha S, Islam MK, Anisuzzman M, Hasan MM, Hossain F, Talukder C. 2012. Evaluation of antioxidant, analgesic and antidiarrheal activity of *Phoenix paludosa* Roxb leaves. Intl J Basic Med Sci Pharm (IJBMSP) 2 (2): 46-52.

Shin J, Prabhakaran VS, Kim KS. 2018. The multi-faceted potential of plant-derived metabolites as antimicrobial agents against multidrug-resistant pathogens. Microb Pathog 116: 209-214. DOI: 10.1016/j.micpath.2018.01.043.

Srivastava J, Chandra H, Nautiyal AR, Kalra SJ. 2014. Antimicrobial resistance (AMR) and plant-derived antimicrobials (PDAms) as an alternative drug line to control infections. 3 Biotech 4 (5): 451-460. DOI: 10.1007/s13205-013-0180-y.

Subramani R, Narayanasamy M, Feussner KD. 2017. Plant-derived antimicrobials to fight against multi-drug-resistant human pathogens. 3 Biotech 7 (3): 1-15. DOI: 10.1007/s13205-017-0848-9.