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Original Article

Phytochemical analyses and antibacterial activities of *Erodium*, *Euphorbia*, *Logoecia* and *Tamarix* species

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Abstract

Introduction: Resistance against commonly used antibacterial agents has become a globally recognized threat to human health. Therefore, the development of new and effective antibacterial agents is necessary to treat infections caused by resistant bacterial strains; plants are a promising source of new agents to be tested.

Methodology: The minimum inhibitory concentrations (MIC) of ethanolic extracts of *Erodium gruinum*, *Euphorbia hierosolymitana*, *Logoecia cuminoides*, and *Tamarix tetragyna* against 10 Gram-negative and 5 Gram-positive bacteria were determined using agar well diffusion and microtiter plate dilution methods, respectively. The phytochemical composition of the crude extracts of the plants was determined using HPLC.

Results: *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae, Proteus mirabilis*, and *Acinetobacter baumannii* were sensitive to *E. gruinum* and *E. hierosolymitana* extracts. *P. aeruginosa* ATCC 27853 and *M. catarrhalis* were sensitive to *L. cuminoides* extract. *P. aeruginosa* ATCC 27853, *P. mirabilis*, and *K. pneumoniae* were sensitive to *T. tetragyna* extracts. For Gram-positive bacteria, *Staphylococcus aureus* ATCC 33591 and ATCC 43300 were sensitive to *E. gruinum* and *E. hierosolymitana* extracts. *S. aureus* ATCC 43300 and ATCC 33591 and Group D *Streptococcus* were sensitive to *T. tetragyna* extract. All Gram-positive bacteria were completely resistant to the extract of *L. cuminoides*. The major phytochemical components of the plant extracts belonged to flavonoids, tannins, terpenes, quinones, phytosterols, phytoestrogens, carbohydrates, fatty acids, and coumarin.

Conclusion: The study showed the potential of the development of antibacterial agents from these plants. Phytochemical analysis revealed compounds that are candidates for new antibacterial drugs.

Key words: antimicrobial activity; medicinal plants; agar well diffusion; MIC.

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Introduction

Antibacterial therapy has been the mainstay treatment of bacterial infections for many decades. However, the misuse of antibiotics has led to the development of multi-drug-resistant (MDR) pathogens [1,2]. In recent years, there has been an alarming and threatening increase in infections caused by MDR pathogens [3], which have become a significant cause of death especially in children, the elderly, and immunocompromised patients [3]. In addition, infections by MDR pathogens have been associated with serious economic impacts due to higher morbidity, increased hospital stays, and increased health care costs [3].

During the last few decades, the rate of the discovery and the development of new antibacterial agents has declined. Therefore, the development of new, effective, and affordable antibacterial agents has become a high priority in the medical community. Many medicinal plants have been used in traditional medicine for centuries in the treatment of a variety of ailments [2]. Medicinal plants contain a vast array of substances that can be used to treat chronic and infectious diseases [4,5]. The antibacterial activity of various plant extracts obtained using a number of organic solvents such as ethanol, methanol, acetone, and water has been reported [5-8].

In folk medicine, *E. gruinum* has been used for its antioxidant, antiviral, bactericidal, antileishmanial, and hemostatic effects [9]. *E. hierosolymitana* has been used in the treatment of skin and hair problems, wounds, eczema, psoriasis, warts, and as a purgative [10]. It was found to act as a protease inhibitor and to have allelopathic, antibacterial, and antifungal effects [11]. Recently, *E. hierosolymitana* has been used in the treatment of ovarian, breast, and prostate cancers [12]. *Logoecia cuminoides* has been used for the treatment of *Leishmaniasis*, bile stones, diarrhea, and abdominal

pain [13]. Studies have shown that *L. cuminoides* has antioxidant and antimicrobial effects [14]. *Tamarix tetragyna* is also believed to have antioxidant, antibacterial, and α-glucosidase inhibitive effects, as well as antitumor, and hepatoprotective activities [15].

To date, many published research papers have focused on listing and classifying medicinal plants using ethnopharmacological or ethnobotanical techniques, while only a few have focused on the pharmacological activities of specific bioactive compounds in these plants. Therefore, the objective of the present study was to determine the phytochemical compositions of four medicinal plants in order to evaluate the antibacterial activities of their ethanolic extracts against 15 bacterial strains.

Methodology

Extraction

Four wild-grown plants were used in the study. Various parts of each plant, including leaves, flowers, and stems, were selected at random to determine their antibacterial activities. The plants were *E. gruinum* (leaves and flowers), *E. hierosolymitana* (leaves and stems), *L. cuminoides* (leaves and stems), and *T. tetragyna* (leaves, flowers, and stems). Extraction was performed according to previously published methods [2]. Briefly, the freshly collected plants were washed and left to air-dry in a dark place at room temperature for five to seven days. Selected plant parts were then ground using a laboratory grinder (Thomas Scientific, Swedesboro, USA). The plant extracts were prepared by adding ultrapure ethanol (99.7%; Merck, Darmstadt, Germany) to the ground plant material at a ratio of 1:3 (w/v). The mixture was incubated under continuous stirring at room temperature (23°C) for 72 hours in the dark. Then the extracts were filtered through Whatman No. 1 filter paper (Merck, Darmstadt, Germany). Ethanol was evaporated at 50°C for 45–60 min under negative pressure (12 mbar) and continuous rotation using a rotary evaporator (Stuart, Staffordshire, UK) until complete dryness.

High Performance Liquid Chromatography (HPLC)

Determination of the plant extracts' phytochemical composition was performed using HPLC [14]. Approximately 50 mg of each of the ground plant materials was placed in a 5 mL glass amber tube (Thomas Scientific, Swedesboro, USA) and 1.5 mL of HPLC-grade methanol was added. The mixture was then vortexed gently for three to five minutes three times (Vortex; Fisher Scientific, Pittsburgh, USA). The tube was then centrifuged at 10,000 g for 5 minutes. The supernatant was filtered using a 0.45 μm PTFE syringe filter (Merck, Darmstadt, Germany). Chromatographic separation of the plant extracts was performed using the Shimadzu HPLC (Shimadzu Scientific Instruments, Addison, USA). The PrimeSIL C18 column (Wesley Technologies, Inc., Suwanee, USA) used an isocratic mobile phase of water (0.1% formic acid): acetonitrile (0.1% formic acid) (10:90, v/v) at a flow rate of 0.2 mL/min for 11 minutes and column temperature of 40°C. The temperature of the auto sampler was maintained at 4°C and the injection volume was 20 μL. For mass spectrometry, an electrospray ionization (ESI) interface was used with positive and negative screening modes at 3 KV capillary voltage, 120°C source block temperature, and 45°C desolation gas temperature.

Preparation of Bacterial Strains

All bacteria used in this study were from the American Type Culture Collection (ATCC) bacterial strains. In total, 10 Gram-negative and 5 Gram-positive bacterial quality control strains were used. Gram-negative bacteria were *Acinetobacter baumannii* ATCC 19606, *Citrobacter koseri* ATCC 27026, *Enterobacter cloacae* ATCC 35549, *Escherichia coli* TG1, *E. coli* ATCC 8739, *Klebsiella pneumoniae* ATCC 13885, *Moraxella catarrhalis* ATCC BAA 1425, *Proteus mirabilis* ATCC 12453, *Pseudomonas aeruginosa* ATCC 27853 and *P. aeruginosa* ATCC BAA 2114.

The Gram-positive bacterial strains were *Staphylococcus aureus* ATCC 33591, *S. aureus* ATCC 43300, Group A *Streptococcus* (GAS) ATCC 12344, Group B *Streptococcus* (GBS) ATCC 13813, and Group D *Streptococcus* (GDS) ATCC 33317.

Bacterial strains were cultured onto Luria–Bertani (LB) agar, except for *Pseudomonas* strains that were cultured onto King's B (KB) agar, and for *Streptococcus* and *Moraxella* that were cultured onto Mueller Hinton agar (Mast Group Ltd., Bootle, UK) supplemented with 5% sheep's blood. The plates were incubated for 24 hours for all bacterial strains except for *Pseudomonas*, *Streptococcus* and *Moraxella,* which were incubated for 48 hours. Carbon dioxide incubator (under 5%) was used for *Streptococcus* and *Moraxella*. Bacterial subcultures were suspended in nutrient broth, supplemented with 15% glycerol, and in case of *Streptococcu*s and *Moraxella*, 5% sheep's blood was added and then stored at -70°C for further use.

Preparation of the Plant Extracts for Antibacterial Activity

To obtain a final stock concentration of 100 mg/mL, 10 g of the powdered plant extract was dissolved in 100

mL of DMSO. The stock solution of the plant extract (100 mg/mL) was further diluted using 0.1% DMSO (prepared with sterile distilled water) to 1 mg/mL for agar well diffusion assay and 0.2 mg/mL for MIC assay.

Antibacterial Activity of the Plant Extracts

The antibacterial activity of the plant extracts was investigated using the agar well diffusion method [6]. Briefly, 100 µL of bacterial suspension containing approximately 10^7 CFU/mL was seeded on Mueller Hinton agar supplemented with 5% sheep's blood for *Streptococcu*s and *Moraxella* (Mast Group Ltd., Bootle, UK) plates. Then, 4.0 mm diameter wells were punched into the agar plate and 10.0 µL of the plant extract (1 mg/mL) were loaded into each well. 10.0 µL of 0.1% DMSO and 10.0 µL chloramphenicol (1 mg/mL) were used in each plate. The plates were then incubated at 37°C for 24 hours for all bacterial strains except for *Pseudomonas*, *Streptococcus* and *Moraxella,* which were incubated for 48 hours. Carbon dioxide incubator (under 5%) was used for *Streptococcus* and *Moraxella.* Each plant extract was investigated in three replicates. The inhibition zones (mm) were measured and the average of the three replicates was calculated.

Determination of Minimum Inhibitory Concentration (MIC)

The MICs of the plant extracts for bacterial strains identified by their sensitivity to that extract by well diffusion were determined in triplicate using microtiter plate dilution [12,16]. Positive and negative controls were used in each plate: chloramphenicol and 0.1% DMSO, respectively. Initially, the first well was filled with 180 µL and the second to the tenth well with 100 µL of MHB supplemented with 5% sheep's blood for *Streptococcus* and *Moraxella*. Twenty microliters (20 µL) of the plant extracts (0.2 mg/mL) were added in the first well to get concentration of (10µg/mL). Then 100µL was transferred from the first well to the second and so on by two-fold dilution to get concentrations of 20 -0.04 µg/mL in the plate. Then, each well was inoculated with 100 µL of approximately 1 McFarland standard of freshly grown bacteria, making the final concentration in the wells in the range 10 - 0.02 µg/mL. Bacterial growth was examined visually after incubation at 37°C for 24 hours for all bacterial strains except for *Pseudomonas*, *Streptococcus* and *Moraxella,* which were incubated for 48 hours. A carbon dioxide incubator (under 5%) was used for *Streptococcus* and *Moraxella.* The MIC was determined visually as the lowest concentration that led to bacterial growth inhibition.

Statistical Analysis

Analysis of variance (ANOVA) was conducted using SAS 9.4 (SAS Institute Inc., USA). Differences among treatment means were determined by Least Significant Difference (LSD) test at 5% confidence interval. Significant values were considered from $p \leq 0.05$.

Results

Antibacterial activity by well diffusion

Antibacterial activities of the ethanolic plant extracts against 10 Gram-negative bacterial strains are presented in Table 1.

Table 1. Antibacterial activity of ethanolic plant extracts against some Gram-negative and Gram-positive pathogenic bacterial strains (inhibition zones in mm).

Bacterial strain	Plant extract				Control
	Erodium gruinum	***Euphorbia hierosolymitana***	***Logoecia cuminoides***	***Tamarix tetragyna***	**Chloramphenicol**
A. baumannii	$16^{b} \pm 0.4$	$15^{bc} \pm 0.4$	$0.0^{d} \pm 0.0$	$13^{c} \pm 0.4$	$24^{a} \pm 0.8$
C. koseri	$0.0^{b} \pm 0.0$	$0.0^{b} \pm 0.0$	$0.0^{b} \pm 0.0$	$13^{a} \pm 0.0$	$11^{a} \pm 0.8$
E. cloacae	$0.0^{c} \pm 0.0$	$0.0^{c} \pm 0.0$	$0.0^{c} \pm 0.0$	$13^{a} \pm 0.4$	$10^{b} \pm 0.8$
E. coli 8739	$0.0^{c} \pm 0.0$	$0.0^{c} \pm 0.0$	$0.0^{c} \pm 0.0$	$15^{b} \pm 0.6$	$23^{c} \pm 0.6$
E. coli TG1	$0.0^{c} \pm 0.0$	$0.0^{c} \pm 0.0$	$0.0^{c} \pm 0.0$	$12^{b} \pm 0.4$	$20^{a} \pm 0.2$
K. pneumonia	$19^{b} \pm 0.3$	$19^{b} \pm 0.3$	$0.0^{c} \pm 0.0$	$18^{b} \pm 0.3$	$23^{a} \pm 0.3$
M. catarrhalis	$10^{c} \pm 0.4$	$10^{c} \pm 0.4$	$7^{d} \pm 0.4$	$16^{b} \pm 0.4$	$30^{a} \pm 1.4$
P. mirabilis	$18^{a} \pm 0.3$	$16^{b} \pm 0.3$	$0.0^{d} \pm 0.0$	$19^{a} \pm 0.3$	$10^{c} \pm 0.0$
P. aeruginosa ATCC 27853	$25^{a} \pm 1.0$	$18^{c} \pm 0.4$	$22^{b} \pm 0.3$	$20^{c} \pm 1.0$	$20^{c} \pm 0.0$
P. aeruginosa ATCC BAA 2114	$0.0^{b} \pm 0.0$	$10^{a} \pm 0.0$	$0.0^{b} \pm 0.0$	$0.0^{b} \pm 0.0$	$12^{a} \pm 0.0$
S. aureus ATCC 33591	$20^{b} \pm 0.3$	$18^{c} \pm 0.6$	$0.0^{d} \pm 0.0$	$17^{c} \pm 0.6$	$35^{a} \pm 0.4$
S. aureus ATCC 43300	$20^{b} \pm 0.7$	$19^{b} \pm 0.7$	$0.0^{c} \pm 0.0$	$18^{b} \pm 0.0$	$23^{a} \pm 0.0$
Group B *Streptococcus* (GBS)	$0.0^{c} \pm 0.0$	$0.0^{c} \pm 0.0$	$0.0^{c} \pm 0.0$	$15^{b} \pm 0.4$	$21^{a} \pm 1.2$
Group D *Streptococcus* (GDS)	$0.0^{b} \pm 0.0$	$0.0^{b} \pm 0.0$	$0.0^{b} \pm 0.0$	$21^{a} \pm 0.3$	$20^{a} \pm 0.0$
Group A *streptococcus* (GAS)	$11^{c} \pm 0.0$	$10^{c} \pm 0.1$	$0.0^{d} \pm 0.0$	$15^{b} \pm 0.3$	$24^{a} \pm 0.4$

Different superscript letters in a raw indicate statistically significant difference at $P \leq 0.05$.

Table 2. MIC (µg/mL) of ethanolic plant extracts against some Gram-negative and Gram-positive pathogenic bacterial strains.

Bacterial Strain	Plant Extract				Control
	Erodium gruinum	***Euphorbia hierosolymitana***	***Logoecia cuminoides***	***Tamarix tetragyna***	**Chloramphenicol**
A. baumannii	2.5	5	NP	5	0.312
C. koseri	NP	NP	NP	10	10
E. cloacae	NP	NP	NP	10	10
E. coli ATCC 8739	NP	NP	NP	5	0.31
E. coli TG1	NP	NP	NP	10	1.25
K. pneumoniae	1.25	1.25	NP	1.25	0.63
M. catarrhalis	10	10	10	2.5	0.078
P. mirabilis	1.25	1.25	NP	1.25	10
P. aeruginosa ATCC 27853	0.156	1.25	0.625	1.25	1.25
P. aeruginosa ATCC BAA 2114	NP	NP	NP	NP	10
S. aureus ATCC 33591	0.625	1.25	NP	1.25	0.078
S. aureus ATCC 43300	1.25	1.25	NP	1.25	0.312
Group B *Streptococcus* (GBS)	NP	NP	NP	5	0.625
Group D *Streptococcus* (GDS)	NP	NP	NP	0.625	0.635
Group A *streptococcus* (GAS)	10	10	NP	5	0.625

NP = Not Performed because the bacteria were found resistant to the plant extract according to well diffusion test (see table 1).

Table 3. Shared phytochemicals of the crude extract of *Erodium gruinum* (leaves and flowers), *Euphorbia hierosolymitana* (leaves and stems), *Logoecia cuminoides* (leaves and stems), and *Tamarix tetragyna* (leaves, flowers, and stems).

Plant	Chemical compound	Molecular weight	Chemical class
Erodium gruinum	Asphodelin A	286.23	Arylcoumarins
Logoecia cuminoides	Hexadecanoic acid	256.43	Saturated fatty acid
	Isorhamnetin-3-O- ß -D-g1ucoside	478.406	Glycosyloxyflavones
	Isorhamnetin-3-O-robinobioside	624.548	Glycosyloxyflavones
Tamarix tetragyna	Gallic acid	170.12	Phenolic acid
Logoecia cuminoides	Isoferulic acid	194.18	Phenolic acid (hydroxycinnamic acid)
	Syringaresinol	418.88	Lignan (polyphenol)
Euphorbia hierosolymitana	Abietadiene	272.4	Diterpene
Erodium gruinum	Luteolin	286.24	Flavonoid
Logoecia cuminoides	Chrysophanol	254.24	Anthraquinones
	Apigenin	270.24	Flavone
Euphorbia hierosolymitana	Quercetin 3,7'-diglucoside	626.52	Flavonol glycoside
Erodium gruinum	ß-sitosterol	414.7	Phytoesterol
	d-3-carene	136.24	Monoterpene
Euphorbia hierosolymitana	Chlorogenic acid	354.3	Phenolic acid
Erodium gruinum	Dillenetin	330.29	Flavonoids
Tamarix tetragyna			
Logoecia cuminoides			
Euphorbia hierosolymitana	Genistein	270.24	Phytoestrogen (isoflavone)
Erodium gruinum			
Tamarix tetragyna			
Erodium gruinum	Quercetin	302.23	Flavonoids
Tamarix tetragyna	Myricetin 3 galactoside	480.37	Glycosyloxyflavone
Logoecia cuminoides			
Tamarix tetragyna	Terpinen-4-ol	154.253	Monoterpenes
Erodium gruinum			

P. aeruginosa ATCC 27853, *K. pneumoniae*, *P. mirabilis*, *A. baumannii*, and *M. catarrhalis* were found to be sensitive to the ethanolic extracts of *E. gruinum* and *E. hierosolymitana,* whereas the other bacteria were completely resistant. The inhibition zone produced by the ethanolic extracts of *E. gruinum* and *E. hierosolymitana* was largest against *P. aeruginosa* ATCC 27853, followed by *K. pneumoniae*, *P. mirabilis*, *A. baumannii*, and *M. catarrhalis*.

Only *P. aeruginosa* ATCC 27853 and *M. catarrhalis* were sensitive to the plant extract of *L. cuminoides,* with the largest inhibition zone observed for *P. aeruginosa* ATCC 27853.

For the *T. tetragyna* plant extract, *P. aeruginosa* ATCC 27853, *P. mirabilis*, and *K. pneumoniae* were found to be sensitive, whereas *P. aeruginosa* ATCC BAA 2114 was completely resistant. However, *C. koseri and E. cloacae* were less sensitive to the *T. tetragyna* plant extract. The largest zone of inhibition was found against *P. aeruginosa* ATCC 27853, followed by *P. mirabilis* and *K. pneumoniae*.

S. aureus strains ATCC 33591 and ATCC 43300 and GAS were sensitive to the extracts of *E. gruinum* and *E. hierosolymitana* plants. The inhibition zones produced by *E. gruinum* and *E. hierosolymitana* extracts were largest against *S. aureus* strains ATCC 33591 and ATCC 43300. GBS and GDS were completely resistant to these two plant extracts (Table 1).

All Gram-positive bacterial strains were completely resistant to the extract of *L. cuminoides*. *S. aureus* strains ATCC 43300 and ATCC 33591, GAS, GBS and GDS were determined to be sensitive to the extract of *T. tetragyna*. The zone of inhibition was greatest against GDS, followed by *S. aureus* ATCC 43300, *S. aureus* ATCC 33591 GAS and GBS.

Antibacterial activity by dilution method

The MICs of ethanolic plant extracts against 10 Gram-negative bacteria are presented in Table 2. *P. aeruginosa* ATCC 27853 was the most sensitive among Gram-negative bacteria to *E. gruinum* and *L. cuminoides* extracts. *K. pneumoni*ae and *P. mirabilis* were equally sensitive to the extracts of *E. gruinum*, *E. hierosolymitana*, and *T. tetragyna. K. pneumoniae, P. mirabilis and P. aeruginosa* ATCC27853 were most sensitive to the extract of *T. tetragyna. P. aeruginosa* ATCC27853 was most sensitive to the extract of *E. gruinum.*

S. aureus ATCC 33591 was the most sensitive to the extract of *E. gruinum*. *S. aureus* ATCC 33591 and *S. aureus* ATCC 43300 were the most sensitive to the extract of *E. hierosolymitana*. GDS was more sensitive to the extract of *T. tetragyna* than GBS and GAS were.

Table 4. Unique phytochemical composition of the crude extract of *Erodium gruinum* (leaves and flowers), *Euphorbia hierosolymitana* (leaves and stems), *Logoecia cuminoides* (leaves and stems), and *Tamarix tetragyna* (leaves, flowers, and stems).

Plant	Chemical compound	Molecular weight	Chemical class
Erodium gruinum	Daidzein 4,7 Dihydroxy Isoflavone	254.23	Phytoestrogen (isoflavone)
Euphorbia hierosolymitana	1-octen-3-ol	128.215	Alkenyl alcohol
	α-pinene	136.23	Monoterpenes
	Kaempferol	286.23	Flavonoids
	Longifolene	204.3	Hydrocarbon
	Verbenone	150.21	Terpene
	Phytol	296.53	Diterpene alcohol
	Verbascose	828.72	Pentasaccharide
	Kaempferol 3-O-(4”-O-P-Coumaroyl)-glucoside	594.5	Flavonoid coumaroyl glycosides
Tamarix tetragyna	1,2,3,6-Tetra-O-galloyl-ß-D-glucose	788.5	Gallotannin
	3-Hydroxy-2-phenyl-2,3-dihydrochromen-4-one	240.25	Flavonone derivative
	Ellagic acid	302.19	Phenolic acid
	Tamarixetin	316.26	Monomethoxyflavone
	Naringenin	272.25	Flavanone
	Dehydrodigallic acid	338.22	Phenolic acid
	Kaempferide	300.26	Flavonol
	Methyl gallate	184.14	Tannin
	Flavone	222.24	Flavone
	Kaempferol-3-O-D-glucoside	447.37	Flavonoid glycoside
	Quercetin-3’-glucuronide	478.32	Flavonol glucuronide
	Isorhamnetin	316.26	Monomethoxyflavone
Logoecia cuminoides	β-Farnesene	204.36	Sesquiterpenes

GAS was more sensitive to the extract of *T. tetragyna* than *E. gruinum* and *E. hierosolymitana* extract (Table 2).

The phytochemical composition of the crude extracts of *E. gruinum* (leaves and flowers), *E. hierosolymitana* (leaves and stems), *L. cuminoides* (leaves and stems), and *T. tetragyna* (leaves, flowers, and stems) are presented in Table 3 and Table 4. HPLC analysis showed that the selected plants contain a wide range of chemicals including flavonoids, tannins, terpenes, quinones, phytosterols, phytoestrogens, carbohydrates, fatty acids, and coumarin compounds. The analysis showed that some plants contain phytochemicals that are only present in one plant but not in others (Table 4).

Discussion

The phytochemical compositions of the crude extracts of leaves and flowers of *E. gruinum*, leaves and stems of *E. hierosolymitana* and *L. cuminoides*, and leaves, flowers, and stems of *T. tetragyna* were determined in the study. The plants were particularly rich in flavonoids, terpenes, tannins, quinones, and phytoestrogens. Many of these components have been found to have distinctive medicinal activity including antibacterial, antifungal, anti-inflammatory, antioxidant and anticancer activities, and as an estrogen agonist [17-21].

In the present study, HPLC analysis revealed that the plants investigated did contain unique phytochemicals. Therefore, the specific antibacterial activities of the extracts of these plants might be attributed to these particular compounds. Daidzein, a metabolite of the isoflavone, was found only in the extract of *E. gruinum*. The compound was reported to be metabolized in gastrointestinal system to equol by the bacteria, which are members of the intestinal flora, and equol is known to have strong estrogenic and antioxidative activities [22,23]. Similar to other flavonoids, daidzein is believed to exert an antibacterial effect against certain Gram-positive and Gram-negative bacteria [24]. In this study, *E. gruinum* extract was shown to have promising antibacterial activity against *P. aeruginosa* ATCC 27853, *K. pneumoniae*, *P. mirabilis*, *A. baumannii*, and *S. aureus*.

The inhibition of *P. aeruginosa* by the ethanolic extract of *E. gruinum* may also be attributed to apigenin, which is another flavonoid known to have antibacterial activity against several pathogenic bacteria including *P. aeruginosa*, *Salmonella Typhimurium*, *P. mirabilis*, *K. pneumoniae*, and *Enterobacter aerogenes* [24].

The extract of *E. hierosolymitana* was found to contain 1-octen-3-ol, which is a mushroom alcohol that has been shown to have strong antifungal and antibacterial activities against Gram-positive bacteria including *S. aureus*, *Staphylococcus epidermidis*, and *Bacillus subtilis* [25]. In the present study, the same metabolite was confirmed to be present in *E. hierosolymitana* and the anti-staphylococcal activity may result from the presence of this metabolite. α-pinene, which was also detected in the extract of *E. hierosolymitana* in the study, is an eugenol of the allylbenzene chemical compounds and has been shown to have a fungicidal effect against *Candida albicans* and a bactericidal effect against methicillin-resistant *S. aureus* [26]. Therefore, the anti-staphylococcal effect of *E. hierosolymitana* extract detected in our study may be attributable to eugenol. Kaempferol (astragalin), which was detected in *E. hierosolymitana*, is a flavonoid that is well known for its wide range of bioactive functions including anti-inflammatory, antioxidant, neuroprotective, cardioprotective, anti-obesity, anti-osteoporotic, anti-cancer, anti-ulcer, and anti-diabetic properties [27,28].

The extract of *T. tetragyna* was found to contain several unique compounds. Ellagic acid (EA) is a herbal polyphenol that is considered a dimer of gallic acid [29] and was found to be bactericidal against *Corynebacterium diphtheriae* and bacteriostatic against *B. subtilis*, *Clostridium sporogenes*, *S. aureus*, *Neisseria meningitidis*, and *M. catarrhalis* [30].

Genistein is an isoflavone with strong estrogenic and antioxidant effects [22]. In this study, genistein was found in *E. hierosolymitana*, *E. gruinum*, and *T. tetragyna* extracts. It could explain the antibacterial activity observed with these plant extracts against *K. pneumoniae*. The finding is in agreement with a previous report that genistein had antibacterial activity against *K. pneumoniae* [31].

ß-sitosterol, a triterpene, was found in *E. gruinum and T. tetragyna* extracts. Previous reports have cited ß-sitosterol as having considerable antibacterial activity against *C. diphtheriae* and, in parallel to our study, against *S. aureus* [32].

Chlorogenic acid, a natural ester, was found in all of the plant extracts included in the study. The metabolite was shown to be effective against *S. aureus*, *Streptococcus uberis*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *E. aerogenes*, and *E. coli* [33].

Quercetin is a flavonoid compound that was found in the extracts of *E. gruinum*, *T. tetragyna*, and *L. cuminoides*. The antibacterial activity of quercetin has

been determined against a wide variety of pathogenic bacteria including *E. coli*, *P. aeruginosa*, *and B. subtilis* [34].

Conclusion

The results of the study showed the promising antibacterial potential of extracts of four medicinal plants. However, further *in vitro* and *in vivo* testing of purified phytochemicals from the crude extracts of these plants is warranted to identify active components that could be used in future formulations of pharmaceuticals and in the development of new and effective antibacterial agents against common pathogenic bacteria.

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