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Screening of medicinal plant extracts as novel DNA gyrase inhibitors

O Oyardi[a,*], B Ozcelik[a], U Koca Caliskan[b,c], Y D Simay Demir[d] & M Ark[d]

[a]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Gazi University, 06330, Ankara, Turkey

[b]Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, 06330, Ankara, Turkey

[c]Department of Pharmacognosy, Faculty of Pharmacy, Duzce University, 81620, Duzce, Turkey

[d]Department of Pharmacology, Faculty of Pharmacy, Gazi University, 06330, Ankara, Turkey

*E-mail: ozlemyrd@gmail.com

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Bioactivities of a number of medicinal plants; *Alkanna tinctoria* (L.) Tausch, *Alnus glutinosa* (L.) Gaertn., *Calamintha nepeta* Willk. and *C. nepeta*, *Centaurea iberica* Trevir. ex Spreng., *Citrus paradisi* Macfad., *C. paradisi*, *Citrus sinensis* (L.) *Osbeck*, *Colutea cilicica* Boiss. & Balansa, *Cotinus coggygria* Scop., *Cuscuta arvensis* Beyr. ex Engelm., *Equisetum palustre* L., *Lapsana communis* L., *Laurus nobilis* L., *Olea europea* L., *Plantago major* L., *Rhus coriaria* L, *Salvia verticillata* L., *Sambucus ebulus* L., *Sedum acre* L, *Thymus capitatus* (L.) Hoffmanns. & Link, *T. capitatus*, *Thymbra spicata* L., *T. spicata* (n: 20), which are used for the prevention and treatment of diverse diseases, were investigated. The antimicrobial activities of extracts were evaluated using broth microdilution assay. The cytotoxicities of extracts were investigated on HeLa cell line by MTT assay. Statistical analysis was performed using GraphPad Prism (5.0). The effects of the extracts, which have the highest antimicrobial activity, on the *Escherichia coli* and *Staphylococcus aureus* DNA gyrase gene expression were determined by using quantitative reverse transcription polymerase chain reaction (qRT-PCR). The MICs (μg/ml) of extracts were determined as 32-64, 2-128, 8-128,1-128, 4-128 against Gram-positive, Gram-negative bacteria, yeasts, dermatophytes, and *Mycobacterium spp.,* respectively. No cytotoxicity has been observed in plant extracts tested. DNA gyrase activity was determined for *T. capitatus*-SFE (128 μg/mL) and *L. nobilis*-H*x* (128 μg/mL) extracts according to the inhibition of DNA gyrase gene expression. Overall, *T. capitatus*-SFE and *L. nobilis*-H*x* are good candidates for further antimicrobial studies.

Keywords: Antimicrobial activity, Anti-dermatophytic activity, Anti-*Mycobacterium* activity, Aromatic medicinal plants, Cytotoxicity, DNA gyrase inhibition

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Antibiotic resistance is still a growing problem worldwide in the treatment of infectious diseases[1]. Multi-drug resistant (MDR) microorganisms threaten public health due to the misuse of antibiotics. As MDR microorganisms have rendered antimicrobial drugs ineffective, thus complicating the treatment of infections. Today, most human pathogens, including bacteria, fungi can present as MDRs. Therefore, it is of primary importance to introduce new alternatives in the treatment of these pathogens[2]. Human being has been searching and utilizing remedies from nature to combat diseases for many years. Plants are very rich source for different kinds of phytochemicals that are released to different solvents. Various plant extracts containing different phytochemicals are known to be useful to prevent or treatment of diseases. Several studies have been conducted to determine the antimicrobial activity of medicinal plants in order to overcome the antibiotic resistance problem[2].

Antimicrobial agents act through various mechanisms such as inhibition of bacterial cell wall synthesis, protein metabolism or nucleic acid synthesis. Studies have shown that some active compounds such as flavonoids found in plant extracts can be effective by inhibiting DNA gyrase enzyme, which is involved in DNA synthesis in bacteria[3]. DNA gyrase is an enzyme with an important role in DNA topology. It comprises two subunits named A and B encoded by gyrA and gyrB gene. DNA gyrase B subunit is responsible for the ATPase activity and DNA gyrase A subunit is responsible for the separation of DNA strands. The inhibition of DNA gyrase enzyme is the target of many antibiotics such as quinolone antibiotics, causes death of the bacteria by affecting bacterial replication[4]. Therefore, targeting the DNA gyrase enzyme and identifying

*Corresponding author

plant extracts and active compounds effective on DNA gyrase may be an important research area for the treatment of infections caused by MDR bacteria.

The beneficial effects of the plants in treating various diseases have been known for centuries. Their various parts and extracts are used for various purposes thanks to the active compounds in their structures. Plants are good sources for drug preparation and traditional medicine[5]. Medicinal plants may have antimicrobial and antiseptic effects as they contain secondary metabolites such as phenols, flavonoids, and alkaloids[6,7]. Some of the plant extracts in this study have been shown to have anti-inflammatory, antiseptic, antispasmodic, anticancer, antiviral and antioxidant activities[8-10]. Traditional medicinal use of these plant extracts includes expectorant, diuretic, and gastroprotective, and they are used to treat wounds, diarrhoea, peptic ulcer, haemorrhoids and hepatitis[11-14].

In this study, 32 plant extracts obtained from 20 plants were extensively analysed to determine their effectiveness against Gram-positive and Gram-negative bacteria, fungi, dermatophytes, *Mycobacterium* species that cause infections in humans. In addition, the effects on the viability of the HeLa cell line were determined by the MTT method. The plant extracts with the highest antimicrobial activities were determined and their effects on DNA gyrase expression of *S. aureus* and *E. coli* which are one of the most important pathogens were investigated.

Methodology

Collection of plant materials

The plant samples were collected from various parts of Turkey and the voucher specimens were deposited in the herbarium belongs to Gazi University with the following recorded numbers. *Alkanna tinctoria* (L.) Tausch-(GUE96), *Alnus glutinosa* (L.) Gaertn. (GUE98), *Calamintha nepeta* Willk.-(GUE99) *and C. nepeta, Centaurea iberica* Trevir. ex Spreng. (GUE2603), *Citrus paradisi* Macfad.-(GUE100), *C. paradisi* (GUE101), *C. sinensis* (L.) Osbeck (GUE102), *Colutea cilicica* Boiss. & Balansa (GUE2620), *Cotinus coggygria* Scop. (GUE2932), *Cuscuta arvensis* Beyr. ex Engelm. (GUE25875), *Equisetum palustre* L. (GUE2937), *Lapsana communis* L. (GUE2934), *Laurus nobilis* L. (GUE3093), *Olea europea* L. (GUE3120), *Plantago major* L. (GUE2943), *Rhus coriaria* L. (GUE2940), *Salvia verticillata* L. (GUE440), *Sambucus ebulus* L. (GUE3588), *Sedum acre* L. (GUE2941), *Thymus capitatus* L. Hoffmanns. & Link (GUE138), *Thymbra spicata* L. (GUE188).

Plant extracts

Collected medicinal plant samples from various parts of Turkey were dried in shade and powdered. Solid-liquid extractions were carried out starting with nonpolar to polar solvent system. Due to ethnobotanical usage, different solvent extracts were applied for different medicinal plants. Selected solvents were added to the carefully weighed dry-plant materials and the macerations were conducted at room temperature on a shaker for a day. The combined organic extracts first filtered, then they were concentrated to dryness under reduced pressure in a rotary evaporator, to give the crude extracts which follows; n-hexane-H*x*: *C. arvensis*-H*x, L. nobilis*-H*x S. ebulus*-H*x*, *O. europea*-H*x;* dichloromethane-DCM: *C. arvensis*-DCM, acteone-ACE: *C. paradisi*-ACE, *Citrus sinensis* (L.) Osbeck-ACE, *Laurus nobilis* L.-ACE, ethyl acetate-EtOAc; *C. arvensis*-EtOAc, methanol-MeOH; *Citrus paradisi* Macfad.-MeOH, *C. arvensis*-MeOH, distilled water-H_2O: *Alkanna tinctoria* (L.) Tausch-H_2O, *Alnus glutinosa* (L.) Gaertn.-H_2O, *Centaurea iberica Trevir. ex Spreng.*-H_2O, *Colutea cilicica* Boiss. & Balansa fruit-H_2O, *C. cilicica* herba-H_2O, *Cotinus coggygria* Scop.-H_2O, *Cuscuta arvensis* Beyr. ex Engelm.-H_2O, *Equisetum palustre* L.-H_2O, *Lapsana communis* L.-H_2O, *Olea europea* L.-H_2O, *Plantago major* L.-H_2O, *Rhus coriaria* L.-H_2O, *Salvia verticillata* L.-H_2O, *Sambucus ebulus* L. -H_2O, *Sedum acre* L-H_2O. Only the aqueous extracts were filtered and freeze dried by using lyophilization and kept in a fridge. Moreover, hydrodistillation-HD and super fluid extraction-SFE samples were prepared. Following plant samples have HD and SFE sequentially; *Calamintha nepeta* Willk.-HD, *Thymus capitatus* (L.) Hoffmanns. & Link -HD and *C. nepeta*-SFE, *T. capitatus*-SFE.

Materials and Methods

Antimicrobial activity

Plant extracts were dissolved in dimethylsulphoxide (30%) and H_2O (70%) at a final concentration of 512 μg/mL and sterilized by filtration using 0.22 μm Millipore (MA 01730, USA) and used as the stock solutions. Ampicillin-clavulanate (AMC), meropenem (MPM), gentamicin (GEN), ketoconazole (KET), fluconazole (FLU),

terbinafine (TER), griseofulvin (GRI), itraconazole (ITRA), isoniazid (INH), ethambutol (EMB) and streptomycin (STM) were used as the standard agents. Reference antibacterial agents were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and dissolved in phosphate buffer solution (ampicillin, pH 8.0; 0.1 mol/mL), dimethylsulphoxide (ketoconazole), or in water (gentamicin, levofloxacin, fluconazole). Antibacterial activity tests were carried out against standards; Gram-negative standard strains of *Escherichia coli* ATCC 25922*, Pseudomonas aeruginosa* ATCC 27853*, Klebsiella pneumoniae* RSKK 574*, Acinetobacter baumannii* RSKK 02026, and Gram-positive standard strains of *Staphylococcus aureus* ATCC 25923*, Bacillus subtilis* ATCC 6633*, Enterococcus faecalis* ATCC 07005*, Staphylococcus epidermidis* ATCC 12228. *Candida albicans* ATCC 10231, *C. parapsilosis* ATCC 22019, *C. tropicalis* ATCC 13803 and *C. krusei* ATCC 6258 were used for the determination of antifungal activity using broth microdilution assay[15,16].

Antidermatophytic activity tests were carried out against *T. rubrum* RSKK 486, *E. floccosum* RSKK 3027 and *M. gypseum* NCPF 580 (NCPF; National Collection of Pathogenic Fungi, RSKK; Culture collection of RefikSaydam Central Hygiene Institute). Dermatophytes were subcultured onto potato dextrose agar (PDA) plates at 28°C, during 7–14 days. The fungal colonies were covered with 1 mL of sterile 0.85% saline, and suspensions were made by gently probing the surface with the tip of a Pasteur pipette. The resulting mixture of conidial and hyphal fragments was withdrawn and transferred to a sterile tube. Heavy particles were allowed to settle for 15-20 min at room temperature; the upper suspension was mixed with a vortex for 15s. The turbidity of supernatants was measured spectrophotometrically at a wavelength of 530 nm, and transmission was adjusted to 65-75%. These stock suspensions were diluted 1:50 in RPMI medium to obtain the final inoculum sizes ranging from 0.4×10^4 to 5×10^4 CFU mL^{-1}. The turbidity of the mixed suspension was measured at 530 nm to obtain 75-77% transmission and adjusted to $1\times10^{6-5}\times10^6$ CFU mL^{-1} by a spectrophotometric method. These stock suspensions were diluted 1:50 in RPMI medium, and further diluted 1:20 with medium to obtain the two-fold test inoculum ($1x10^3$-$5x10^3$ CFU mL^{-1}). The (twofold) inoculum was diluted 1:1 when wells were inoculated, and the desired final inoculum size was achieved ($0.5x10^3$-$2.5x10^3$ CFU mL^{-1}). The minimal inhibition concentration (MIC) of each extract was determined by using broth microdilution techniques[15,17].

The strains *Mycobacterium tuberculosis* H37Rv ATCC 27294; American Type Culture Collection reference strain and *M. avium* ATCC 15769 were maintained on Lowenstein–Jensen medium and subcultured on Middlebrook 7H11 agar (Becton Dickinson) resuspended in 7H9-S broth medium supplemented with 10% [OADC; 0.1% casitone, 0.5% glycerol, supplemented oleic acid, albumin, dextrose, and catalase], 0.2% glycerol and 0.1% Bactocasitone. Suspensions were prepared in 0.04% (vol/vol) Tween 80-0.2%+bovine serum albumin so that adjusted to McFarland tube number 1. This was diluted to 1:20 and 100 µL aliquot was used as inoculum. As for screening of the compounds as MICs against *M. tuberculosis* and *M. avium,* the resazurinmicroplate assay procedure (REMA) was carried out as described previously[18].

Cytotoxicity

The cytotoxic effects of plant extracts on the viability of HeLa cells were evaluated by MTT assay. HeLa cells were cultured in phenol red-free DMEM (Dulbecco's Modified Eagle's medium; Gibco) enriched with 10% fetal bovine serum (Gibco), 100 mg mL^{-1} of streptomycin and 100 IU mL^{-1} of penicillin under a humidified 5% CO_2 atmosphere[18].

The cells were seeded into 96-well microplates at 5000 cells/well and cultured for 24 h, and then treated with MICs of extracts. After the incubation for 48h, fresh media containing 0.5 g/L MTT solution was replaced to each well, and the cells were cultured for another 4 h at 37°C. Media was carefully removed and 160 µLDMSO was added to dissolve the insoluble formazan crystals to each well. The absorbance was measured at 570 nm by a microplate reader (VERSAmax, Molecular Devices). The percentage cell viability was calculated as follows:

Cell viability (%) = (absorbance of the plant extract group/absorbance of the control group) ×100

Statistical analysis was performed by using GraphPad Prism 5.0 software programme. The data were analyzed with one-way ANOVA and post-hoc Tukey test using GraphPad Prism 5 software. Values of $p<0.05$ were considered statistically significant.

Analysis of DNA Gyrase Gene expressions. Quantitative Reverse-transcription Polymerase Chain Reaction (RT-qPCR)

RT-qPCR analysis of DNA gyrase gene region was performed with *C. coggygria*-H_2O, *L. nobilis*-ACE,

L. nobilis-Hx, *R. coriaria*-H_2O, *C. arvensis*-EtOAc, *Cuscuta arvensis*-DCM and *T. capitatus*-SFE which have high antimicrobial activities and the effect of the extracts on *S. aureus* and *E. coli* DNA gyrase gene region was tested. The effects of the active samples on *E. coli* (DNA gyrA) and *S. aureus* (DNA gyrA) DNA gyrase (Topoisomerase II) expressions were determined by RNA isolation, following RT-qPCR. 1.7% agarose gel electrophoresis was performed, and images were obtained by using an imaging device (UVP Ltd., Cambridge, United Kingdom). Band densities were calculated by using ImageJ densitometric analysis programme in order to obtain quantitative results.

Results

The MICs (µg/mL) of the extracts tested against Gram-negative and Gram-positive bacteria are shown in Table 1 and the MICs (µg/mL) of the

Table 1 — The MICs of the extracts tested against Gram-negative and Gram-positive bacteria (µg/mL)

Extract	***Gram-negative microorganisms***			***Gram-positive microorganisms***				
	***K. pneumonia* RSKK 574**	***E. coli* ATCC 49766**	***P. aeruginosa* ATCC 10145**	***A. baumannii* RSKK 02026**	***E. faecalis* ATCC07005**	***B. subtilis* ATCC6633**	***S. aureus* ATCC 25923**	***S. epidermidis* ATCC 12228**
A. tinctoria-H_2O	64	64	64	64	64	64	64	64
A. glutinosa-H_2O	64	64	64	64	32	64	64	64
C. nepeta-HD	64	32	32	64	8	64	32	64
C. nepeta-SFE	64	64	64	64	64	64	64	64
C. iberica-H_2O	64	64	64	64	64	64	64	64
C. paradise-MeOH	64	64	64	64	128	64	64	32
C. paradise-ACE	32	64	64	64	128	64	64	64
C. sinensis-ACE	64	64	64	64	64	64	64	64
C. arvensis-H_2O	32	64	64	64	32	64	64	64
C. arvensis-MeOH	32	64	32	64	32	32	64	64
C. arvensis-EtOAc	32	64	32	64	16	32	64	64
C. arvensis-DCM	32	64	32	64	16	32	32	64
C. arvensis-Hx	64	64	32	64	32	64	32	64
C. cilicica fruit-H_2O	64	64	64	64	32	64	64	64
C. cilicica herb-H_2O	64	32	64	64	32	64	64	32
C. coggygria-H_2O	64	32	64	32	16	64	64	4
E. pallustre-H_2O	64	64	64	64	64	64	64	64
L. communis-H_2O	64	64	64	64	64	64	64	64
L. nobilis-ACE	32	32	64	64	32	64	64	16
L. nobilis-Hx	64	64	64	64	128	64	64	2
O. europea-H_2O	64	64	64	64	64	128	64	64
O. europea-Hx	64	64	64	64	128	64	64	32
P. major-H_2O	64	64	64	64	64	128	64	64
R. coriaria-H_2O	64	32	32	32	128	64	64	8
S. vertisillata-H_2O	64	64	64	64	128	64	64	64
S. ebulus-H_2O	64	64	64	64	64	64	64	32
S. ebulus-Hx	64	64	64	64	64	64	64	32
S. acre-H_2O	64	64	64	64	64	64	64	32
T. capitatus-HD	64	64	64	64	16	64	64	64
T. capitatus-SFE	32	32	32	32	16	32	32	32
T. spicata-HD	64	64	32	64	16	64	64	64
T. spicata-SFE	32	32	32	64	16	32	32	64
Ampicillin clavunate	<0.12	-	-	<0.12	<0.12	<0.12	<0.12	<0.12
Meropenem	-	0.12	-	-	-	0.12	0.25	0.25
Gentamicin	-	-	0.5	-				

H_2O: water, ACE: Acetone, Hx: Hexane, DCM: Dichloromethane, MeOH: Methanol, EtOAc: Ethyl acetate, HD: Water distillation, SFE: Supercritical fluid extraction

extracts tested against yeasts, dermatophytes and mycobacteria are shown in Table 2.

All the extracts displayed 32-64 µg/mL MICs against Gram-negative microorganisms. *L. nobilis*-H*x* (MIC; 2 µg/mL against *S. epidermidis*), *C. coggyria*-H_2O (MIC; 4 µg/mL against *S. epidermidis*) showed the highest antibacterial activity against Gram-positive microorganisms. The other extracts were

Table 2 — The MICs of the extracts tested against yeasts, dermatophytes and mycobacteria (µg/mL)

Extract	*Yeasts*				*Dermatophytes*			*Mycobacterium*	
	***C. albicans* ATCC 10231**	***C. tropicalis* ATCC 13803**	***C. parapsilosis* ATCC 90028**	***C. krusei* ATCC 6258**	***T. rubrum* NCPF 375**	***E. floccosum* RSKK 3027**	***M. gypseum* NCPF 580**	***M. tuberculosis* ATCC 15769**	***M. avium* ATCC 27294**
A. tinctoria-H_2O	64	64	32	64	32	1	32	32	32
A. glutinosa-H_2O	64	64	32	64	32	1	32	32	32
C. nepeta-HD	32	16	32	32	64	64	32	128	64
C. nepeta-SFE	128	16	32	32	64	32	32	16	128
C. iberica-H_2O	64	64	32	64	32	64	32	16	32
C. paradise-MeOH	64	16	64	64	64	128	32	16	32
C. paradise-ACE	64	16	32	32	64	128	32	128	128
C. sinensis-ACE	64	16	64	32	4	16	4	8	32
*C.cilicica*fruit-H_2O	64	64	32	64	32	4	64	64	32
*C.cilicica*her-H_2O	64	16	32	64	32	4	32	64	32
C. arvensis-H_2O	64	128	32	64	16	8	32	64	32
C. arvensis-MeOH	64	64	16	64	4	8	32	32	32
C. arvensis-EtOAc	64	64	16	64	4	8	32	32	32
C. arvensis-DCM	32	64	16	32	4	8	32	32	16
C. arvensis-Hx	32	16	64	32	4	8	32	32	16
C. coggygria-H_2O	64	16	64	64	64	64	32	16	32
E. pallustre-H_2O	64	64	32	64	32	1	32	32	32
L. communis-H_2O	64	64	32	64	32	1	32	32	32
L. nobilis-ACE	32	16	64	64	64	128	32	128	64
L. nobilis-H*x*	128	32	64	128	64	64	4	32	64
O. europea-H_2O	64	32	32	64	4	32	16	4	64
O. europea-H*x*	64	16	64	64	32	8	16	4	64
P. major-H_2O	64	64	32	64	4	16	4	4	64
R. coriaria-H_2O	32	8	64	32	64	128	16	8	64
S. vertisillata-H_2O	64	64	64	64	128	128	32	64	32
S. ebulus-H_2O	64	128	64	64	4	32	16	4	32
S. ebulus-Hx	64	32	32	32	4	32	16	4	64
S. acre-H_2O	64	64	64	64	32	64	32	128	64
T. capitatus-HD	64	16	32	32	32	64	64	32	16
T. capitatus-SFE	32	16	32	16	8	64	32	32	16
T. spicata-HD	64	16	32	32	16	64	64	128	16
T. spicata-SFE	32	16	32	16	8	64	32	128	64
Ketokonazole	1	1	1	4	-	-	-	-	-
Flukonazole	2	2	4	64	-	-	-	-	-
Terbinafine	-	-	-	-	-	-	-	-	-
Griseofulvin	-	-	-	-	0,125	0,25	0,25	-	-
Itraconazole	-	-	-	-	0,5	0,5	0,5	-	-
Izoniasid	-	-	-	-	0,25	0,125	0,125	0,125	0,125
Ethambutol	-	-	-	-	-	-	-	2	2
Streptomycin	-	-	-	-	-	-	-	1	2

H_2O: water, ACE: Acetone, Hx: Hexane, DCM: Dichloromethane, MeOH: Methanol, EtOAc: Ethyl acetate, HD: Water distillation, SFE: Supercritical fluid extraction

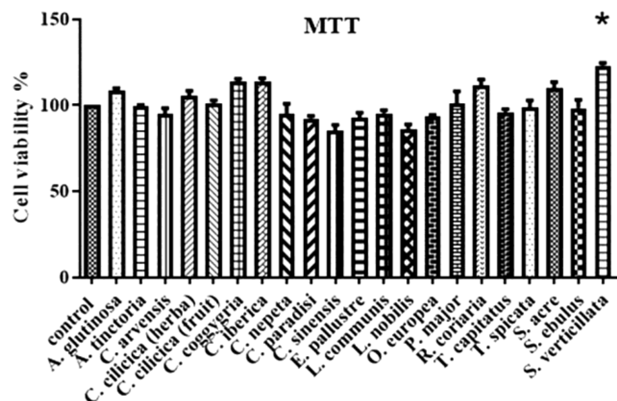

Fig. 1 — Cytotoxicity of the extracts against HeLa cells. Cell viability was measured using MTT assay. Data are representative of three independent experiments. *Statistically significant differences compared with the control ($p<0.05$). MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

determined to be active at MIC values of 16-128 µg/mL (Table 1). The MIC ranges of all extracts against yeasts were determined as 8–128 µg/mL. The highest activity was observed with *R. coriaria* against *C. tropicalis* at 8 µg/mL. The anti-dermatophytic activity was determined between 1–128 µg/mL for all extracts tested. The highest activity was shown against *E. floccosum*. Moreover, anti-mycobacterial activity was observed between 4–128 µg/mL (Table 2).

According to the MTT cytotoxicity assay, among all the plant samples, *S. verticillata* aqueous extract has been found to increase proliferation of cells (Fig. 1). For the rest of the studied plant extracts, there was no statistically significant cytotoxicity observed.

When the effects of plant extracts on *E. coli* DNA gyrase enzyme gene region were compared, *T. capitatus*-SFE was seen as having the most active extract (Fig. 2). For the impact of the extracts on *S. aureus* DNA gyrase enzyme gene region, *T. capitatus*-SFE and *L. nobilis*-ACE were found bioactive (Fig. 3).

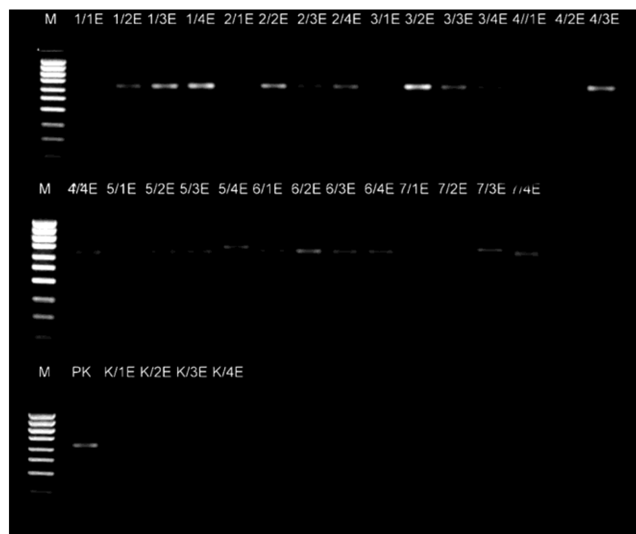

Fig. 2 — DNA gyrA electrophoresis results for *E. coli*. 1= *C. coggygria*-H_2O (1/1; 1/2; 1/3; 1/4: 128 µg/mL-16 µg/mL), 2= *L. nobilis*-ACE (2/1; 2/2; 2/3; 2/4: 128 µg/mL-16 µg/mL), 3= *L. nobilis*-Hx (3/1; 3/2; 3/3; 3/4: 128 µg/mL-16 µg/mL), 4= *R. coriaria*-H_2O (4/1; 4/2; 4/3; 4/4: 128 µg/mL-16 µg/mL), 5= *C. arvensis*-EtOAc (5/1; 5/2; 5/3; 5/4: 128 µg/mL-16 µg/mL), 6= *C. arvensis*-DCM (6/1; 6/2; 6/3; 6/4: 128 µg/mL-16 µg/mL), 7= *T. capitatus*-SFE (7/1; 7/2; 7/3; 7/4: 128 µg/mL-16 µg/mL), E: *E. coli*, PK: *E. coli*, K: ciprofloxacin (K/1; K/2; K/3; K/4: 128 µg/mL-16 µg/mL)

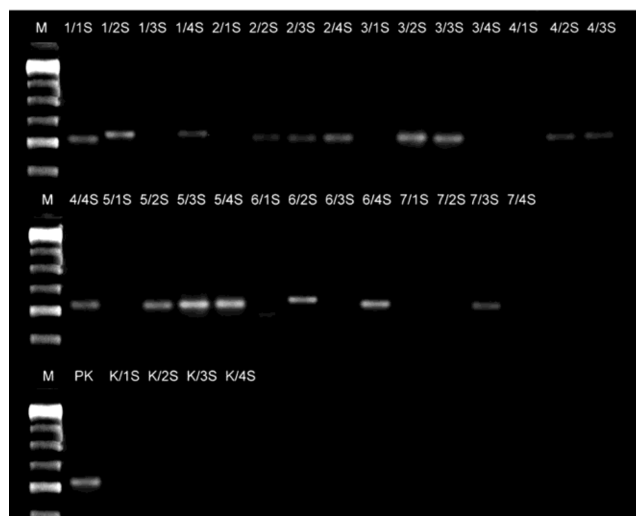

Fig. 3 — DNA gyrA electrophoresis results for *S. aureus*. 1= *C. coggygria*-H_2O (1/1; 1/2; 1/3; 1/4: 128 µg/mL-16 µg/mL), 2= *L. nobilis*-ACE (2/1; 2/2; 2/3; 2/4: 128 µg/mL-16 µg/mL), 3= *L. nobilis*-Hx (3/1; 3/2; 3/3; 3/4: 128 µg/mL-16 µg/mL), 4= *R. coriaria*-H_2O (4/1; 4/2; 4/3; 4/4: 128 µg/mL-16 µg/mL), 5= *C. arvensis*-EtOAc (5/1; 5/2; 5/3; 5/4: 128 µg/mL-16 µg/mL), 6= *C. arvensis*-DCM (6/1; 6/2; 6/3; 6/4: 128 µg/mL-16 µg/mL), 7= *T. capitatus*-SFE (7/1; 7/2; 7/3; 7/4: 128 µg/mL-16 µg/mL), S: *S. aureus*, PK: *S. aureus*, K: ciprofloxacin (K/1; K/2; K/3; K/4: 128 µg/mL-16 µg/mL)

Discussion

The need to search for novel and effective antimicrobials has increased in order to reduce the burden of antimicrobial drug resistance and to prevent and treat microbial infections. Therefore, nature/plants since that have been utilized for centuries have become an option for the discovery of new agents against health-threatening pathogens. In this study, results revealed that plant extracts may

have antimicrobial activities and thus justifying and explaining the uses of medicinal plants in traditional medicine to treat infection diseases.

According to the results of the study, in a broad spectrum, all the plant extracts had MICs between 32 and 64 µg/mL against Gram-negative bacteria (Table 1). While all the plant extracts presented 32-128 µg/mL MICs against *B. subtilis* and *S. aureus*, some of them were more effective against *E. faecalis* and *S. epidermis* standard strains. The most active plant extract against *E. faecalis* was determined as *C. nepeta*-HD. Furthermore, *L. nobilis*-Hx was determined as the most effective (MIC: 2 µg/mL) against *S. epidermis* followed by *C. coggygria*-H_2O (MIC: 4 µg/mL) and *R. coriaria*-H_2O (8 µg/mL) extracts. Hexane extract of *L. nobilis* displayed stronger antimicrobial effect than acetone extract against *S. epidermidis*, but lower activity against *E. faecalis*. Most of the studies about *L. nobilis* are related to the essential oil obtained from different parts of the plant. Studies have shown that essential oil contains many active compounds and has powerful antimicrobial effects against several microorganisms including fungi as well as possible effects on central nervous system[19]. Studies about the leaf extracts of *L. nobilis* are very limited. The results of the study conducted by Santoyo *et al.*[20] suggested that the fractions obtained by supercritical CO_2 extraction from *L. nobilis* leaves showed moderate antioxidant and high antimicrobial activity.

Although the most common cause of Candida infections is *C. albicans*, the incidence and drug resistance of non-albicans Candida (NAC) species are also increasing[21]. Among all the extracts tested, *R. coriaria*-H_2O showed the highest anti-yeast activity against *C. tropicalis*. *R. coriaria* is commonly used as spice in traditional dishes. Numerous active compounds such as phenol, flavonoids, terpenoids have been isolated and the antimicrobial activities have also been demonstrated[22]. Additionally, it has been reported that *R. coriaria* water extract could be an important natural preservative for ground sheep meat[23]. Therefore, its benefits should be evaluated in terms of inhibiting fungal growth in foods as well as *Candida* infections.

Dermatophytes cause superficial infections of mostly keratinized tissues called dermatophytosis. Sometimes these recurrent persistent infections can be difficult to treat[24]. In our study, the anti-dermatophyte effects of plant extracts were found to be more promising than their other antimicrobial effects. Especially, *A. tinctoria*-H_2O, *A. glutinosa*-H_2O, *E. pallustre*-H_2O, *L. communis*-H_2O displayed 1 µg/mL MIC results against *E. floccosum*. In addition, all tested extracts of *C. cilicica* (MIC: 4 µg/mL) and *C. arvensis* (8 µg/mL) led to high antimicrobial effects against *E. floccosum*. The extracts were determined to be the least active on *M. gypseum*. According to comparative evaluation, *C. sinensis*-ACE, *L. nobilis*-Hx and *P. major*-H_2O have the strongest activity among all the extracts. Numerous studies concerning the effects of plant extracts against dermatophytes have been published. Phenolic derivatives, alkaloids and saponins have been identified as the most promising anti-dermatophytic compounds, recently[25]. In a study conducted on *Calendula* species, it was determined that they contain phenolic acids, flavonoids and saponins and have a strong effect against dermatophytes[26].

The most effective extracts were determined as *O. europea*-H_2O, *O. europea*-Hx, *P. major*-H_2O, *S. ebulus*-H_2O and *S. ebulus*-Hx against *M. tuberculosis* and *M. avium*. Camacho-Corona *et al.*[27] reported that *O. europea* hexane extract was effective against drug-resistant *M. tuberculosis*, which is in agreement with our results. In a previous study by Tosun *et al.*[28], etanol extract of *S. ebulus* aerial parts and its chloroform fraction displayed good anti-mycobacterial activity (MIC: 50 µg/mL).

By considering that plant extracts are natural, it is dangerous to believe that they are always safe for use. Similarly, some plants have been known for their anti-cancer properties, therefore, it is important to conduct cytotoxicity studies. In literature, there are studies about the cytotoxixites of *A. glutinosa*[29], *C. coggygria*[30], *L. nobilis*[31], *O. europea*[32], *P. major*[30], *S. ebulus*[33], *T. spicata*[34] against HeLa cells. In our study, toxic effects of all the plant extracts against HeLa cells were not observed. It has been shown that only *S. verticillata* increased the cell viability statistically and supported proliferation.

In our study, it was determined that *T. capitatus*-SFE was the most active extract for the inhibition of both *E. coli* DNA-GyrA and *S. aureus* DNA-GyrA (Fig 2). Furthermore, MIC results of *T. capitatus*-SFE was promising, and no cytotoxicity was observed. According to our knowledge, the effects of the extracts used in our study on the DNA gyrase enzyme do not exist in the literature. Chedia *et al.*[35] demonstrated the antimicrobial activity of *T. capitatus*

essential oil against various bacteria (*E. coli, S. typhimurium, S. aureus, P. aeruginosa, Aeromonas hydrophila, L. monocytogenes* and *B. cereus*) and fungi (*A. niger, A. flavus*ve, *C. albicans*) by agar dilution method and disk diffusion method. Qaralleh *et al.*[36] investigated the antimicrobial activity of aqueous, ethanol, dichloromethane and hexane extracts of leaves and root of *T. capitatus* against *E. coli, S. aureus* and *P. aeruginosa*. Qaralleh *et al.*[36] revealed that leaves extracts were most active than root extracts. In our study, the antimicrobial activities and supercritical fluid extraction of essential oils of *T. capitatus* obtained by hydrodistillation were investigated and the results showed that essential oil obtained by supercritical fluid extraction was more active than essential oil obtained by hydrodistillation against *E. coli, S. aureus, P. aeruginosa* and *C. albicans* (Table 1, Table 2). The differences in the antimicrobial activity of *T. capitatus* essential oils can be explained by differences in the content and amount of active compounds due to different extraction methods.

Conclusions

Plant extracts are significant resources for fighting serious diseases due to their active compounds. The plant extracts used in the study not only showed promising antimicrobial properties, but also not caused cytotoxicity. In addition, the effect of *T. capitatus*-SFE on DNA gyrase gene expression has been demonstrated. These results show that plant extracts can be useful alternatives for pharmaceutical therapy. Further studies are needed to better understand antimicrobial mechanisms of the individual plant extracts.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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Author's Contributions

OO and BO designed the study. OO and YDSD conducted the experiments. BO, UKC and MA analyzed the data. OO and BO wrote the manuscript. All authors read and approved the final version of the manuscript.

References

1 World Health Organization, Antimicrobial resistance: global report on surveillance, Geneva, Switzerland. https://apps.who.int/iris/bitstream/handle/10665/112642/9789241564748_eng.pdf;jsessionid=8A0D718D3B64285A936C33703CF6C505?sequence=1(2014).

2 Subramani R & Narayanasamy M Feussner K D, Plant-derived antimicrobials to fight against multi-drug-resistant human pathogens, 3 *Biotech*, 7 (3) (2017) 1-15.

3 Wu T, Zang X, He M, Pan S & Xu X, Structure–activity relationship of flavonoids on their anti-*Escherichia coli* activity and inhibition of DNA gyrase, *J Agric Food Chem*, 61 (34) (2013) 8185-8190.

4 Collin F, Karkare S & Maxwell A, Exploiting bacterial DNA gyrase as a drug target: current state and perspectives, *Appl Microbiol Biotechnol*, 92 (3) (2011) 479-497, doi: 10.1007/s00253-011-3557-z.

5 Meenu C & Manokari L, Analysis of phytochemical constituents and antibacterial activity of *Wrightia tinctoria*: traditional medicinal plant of India for application on wound dressing materials, *Indian J Tradit Know*, 21 (1) (2022) 48-54.

6 Mishra R C, Kumari R & Yadav J P, Screening of antimicrobial efficacy of traditionally used Indian plants against microorganisms associated with dandruff, *Indian J Tradit Know*, 20(4) (2021) 934-939.

7 Erdoğan Eliuz E A, Antimicrobial performance of methanol extract of *Foeniculum vulgare* Mill. as a sanitizer agent, *Indian J Exp Biol*, 61 (02) (2023) 124-130.

8 Arantes S M, Piçarra A, Guerreiro M, Salvador C, Candeias F, *et al*., Toxicological and pharmacological properties of essential oils of *Calamintha nepeta*, *Origanum virens* and *Thymus mastichina* of Alentejo (Portugal), *Food Chem Toxicol*, 133 (2019) 110747, https://doi.org/10.1016/j.fct.2019.110747.

9 Marčetić M, Božić D, Milenković M, Malešević N, Radulović S, *et al*., Antimicrobial, antioxidant and anti-inflammatory activity of young shoots of the smoke tree, *Cotinus coggygria* Scop, *Phytother Res*, 27 (11) (2013) 1658-1663.

10 Goudjil M B, Zighmi S, Hamada D, Mahcene Z, Bencheikh S E, *et al*., Biological activities of essential oils extracted from *Thymus capitatus* (Lamiaceae), *S Afr J Bot,* 128 (2020) 274-282, doi: 10.1016/j.sajb.2019.11.020.

11 Sicari V, Pellicanò T M, Giuffrè A M, Zappia C, Capocasale M, *et al*., Physical chemical properties and antioxidant capacities of grapefruit juice (*Citrus paradisi*) extracted from two different varieties, *Int Food Res J*, 25(5) (2018) 1978-1984.

12 Qi X L, Peng X, Huang Y Y, Li L, Wei Z F, *et al.,* Green and efficient extraction of bioactive flavonoids from *Equisetum palustre* L. by deep eutectic solvents-based negative pressure cavitation method combined with macroporous resin

enrichment, *Ind Crops Prod,* 70 (2015) 142-148, https://doi.org/10.1016/j.indcrop.2015.03.026.

13 Younessi-Hamzekhanlu M, Ozturk M, Altay V, Nojadeh M S & Alakbarli F, Ethnopharmacological study of medicinal plants from Khoy city of West Azerbaijan-Iran, *Indian J Tradit Know,* 19 (2) (2020) 251-267.

14 Stanković J S K, Srećković N, Mišić D, Gašić U, Imbimbo P, *et al*., Bioactivity, biocompatibility and phytochemical assessment of lilac sage, *Salvia verticillata* L. (Lamiaceae)-A plant rich in rosmarinic acid, *Ind Crops Prod*, 143 (2020) 111932.

15 Pirasa A, Rosa A, Marongiu B, Porcedda S, Falconieri D, *et al.*, Chemical composition and *in vitro* bioactivity of the volatile and fixed oils of *Nigella sativa* L. extracted by super critical carbondioxide, *Ind Crop Prod*, 46 (2013) 317-323, Doi:10.1016/j.indcrop.2013.02.013.

16 Ustun O, Özçelik B & Baykal T, Bioactivities of ethanolic extract and its fractions of *Cistus laurifolius* L. (Cistaceae) and *Salvia wiedemannii* Boiss. (Lamiaceae) species. *Pharmacogn Mag*, 12 (1) (2016) 582-585, doi: 10.4103/0973-1296.176125.

17 Özçelik B, Gürbüz I, Karaoglu T & Yesilada E, Antiviral and antimicrobial activities of three sesquiterpene lactones from *Centaurea solstitialis* L. ssp. solstitialis. *Microbiol Res*, 164 (2009) 545-552, doi: 10.1016/j.micres.2007.05.006.

18 Karakaya G, Aytemir M D, Özçelik B & Çalış Ü, Design, synthesis and *in vivo*/*in vitro* screening of novel chlorokojic acid derivatives, *J Enzyme Inhib Med Chem,* 28 (3) (2013) 627-638, https://doi.org/10.3109/14756366.2012.666538.

19 Caputo L, Nazzaro F, Souza L F, Aliberti L, De Martino L, *et al., Laurus nobilis*: Composition of essential oil and its biological activities, *Molecules,* 22 (6) (2017) 930, doi: 10.3390/molecules22060930.

20 Santoyo S, Lloria R, Jaime L, Ibanez E, Senorans F J, *et al*., Supercritical fluid extraction of antioxidant and antimicrobial compounds from *Laurus nobilis* L. Chemical and functional characterization, *Eur Food Res Technol*, 222 (5-6) (2006) 565, doi: 10.1007/s00217-005-0027-9.

21 Whaley S G, Berkow E L, Rybak J M, Nishimoto A T, Barker K S, *et al*., Azole antifungal resistance in *Candida albicans* and emerging non-albicans *Candida* species, *Front Microbiol*, 7 (2017) 2173, doi: 10.3389/fmicb.2016.02173.

22 Alsamri H, Athamneh K, Pintus G, Eid A H & Iratni R, Pharmacological and antioxidant activities of *Rhus coriaria* L. (Sumac), *Antioxidants*, 10 (1) (2021) 73, doi: 10.3390/antiox10010073.

23 Aliakbarlu J & Mohammadi S, Effect of sumac (*Rhus coriaria* L.) and barberry (*Berberis vulgaris* L.) water extracts on microbial growth and chemical changes in ground sheep meat, *J Food Process Preserv,* 39 (6) (2015) 1859-1866, doi.org/10.1111/jfpp.12423.

24 Khurana A, Sardana K & Chowdhary A, Antifungal resistance in dermatophytes: Recent trends and therapeutic implications, *Fungal Genet Biol,* 132 (2019) 103255, doi: 10.1016/j.fgb.2019.103255.

25 Lopes G, Pinto E & Salgueiro L, Natural products: an alternative to conventional therapy for dermatophytosis?, *Mycopathologia*, 182 (1-2) (2017) 143-167, doi: 10.1007/s11046-016-0081-9.

26 Faustino M V, Pinto D C, Gonçalves M J, Salgueiro L, Silveira P, *et al*., *Calendula L.* species polyphenolic profile and *in vitro* antifungal activity, *J Funct Food,* 45 (2018) 254-267, https://doi.org/10.1016/j.jff.2018.04.013.

27 Camacho-Corona M D R, Ramírez-Cabrera M A, Santiago O G, Garza-González E, Palacios I D P, *et al.,* Activity against drug resistant-tuberculosis strains of plants used in Mexican traditional medicine to treat tuberculosis and other respiratory diseases, *Phytother Res*, 22 (1) (2008) 82-85, doi: 10.1002/ptr.2269.

28 Tosun F, Kızılay Ç, Şener B, Vural M & Palittapongarnpim P, Antimycobacterial activity of some Turkish plants, *Pharm Biol,* 42 (1) (2004) 39-43, https://doi.org/ 10.1080/13880200490505465.

29 Sahin Yaglıoglu A, Eser F, Tekin S & Onal A, Antiproliferative activities of several plant extracts from Turkey on rat brain tumor and human cervix carcinoma cell lines, *Front Life Sci*, 9 (1) (2016) 69-74, https://doi.org/10.1080/21553769.2015.1089949.

30 Bozkurt-Guzel C, Serbetci T & Kultur S, Cytotoxic activities of some Turkish medicinal plants against HeLa cells in vitro, *Indian J Tradit Know*, 17 (2018) 43-49.

31 Berrington D & Lall N, Anticancer activity of certain herbs and spices on the cervical epithelial carcinoma (HeLa) cell line, *Evid Based Complement Alternat Med*, 2012 (2012) 564927, doi: 10.1155/2012/564927.

32 Kouka P, Chatzieffraimidi G A, Raftis G, Stagos D, Angelis A, *et al.,* Antioxidant effects of an olive oil total polyphenolic fraction from a Greek *Olea europaea* variety in different cell cultures, *Phytomedicine*, 47 (2018) 135-142, doi: 10.1016/j.phymed.2018.04.054.

33 Meriç Z İ, Antioxidant, antimicrobial and anticarcinogenic activities of *Sambucus ebulus* L. flowers, fruits and leaves, *J Res Pharm,* 18 (1) (2014) 22-25, doi: 10.12991/mpj.201414122.

34 Khalil M, Khalifeh H, Baldini F, Serale N, Parodi A, *et al*., Antitumor activity of ethanolic extract from *Thymbra spicata* L. aerial parts: effects on cell viability and proliferation, apoptosis Induction, STAT3, and NF-kB signaling, *Nutr Cancer,* 2020 (2020) 1-14, doi:10.1080/01635581.2020.1792517.

35 Chedia A, Ghazghazi H, Dallali S, Houssine S, Brahim H, *et al*., Comparison of chemical composition, antioxidant and antimicrobial activities of *Thymus capitatus* L. essential oils from two Tunisian localities (Sousse and Bizerte), *Int J Agro Plant Prod*, 4 (8) (2013) 1772-1781.

36 Qaralleh H N, Abboud M M, Khleifat K M, Tarawneh K A & Althunibat O Y, Antibacterial activity in vitro of *Thymus capitatus* from Jordan, *Pak J Pharm Sci*, 22 (3) (2009) 247-251.