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Research article

Thymus algeriensis essential oil: Phytochemical investigation, bactericidal activity, synergistic effect with colistin, molecular docking, and dynamics analysis against Gram-negative bacteria resistant to colistin

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ABSTRACT

Due to the increasing resistance prevalence to the last line of antibiotics, such as colistin, and the rising threat of multi-drug resistant bacteria, it is crucial to find alternative therapeutic options. The current study focuses on evaluating antibacterial activities alone and in combination with colistin of *Thymus algeriensis* essential oil (TA-EO) against colistin-resistant *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli* co-harboring *mcr*-1 gene. GC/MS was used to determine the chemical composition of TA-EO. Disc diffusion and microdilution techniques were used to evaluate the antimicrobial activities of TA-EO. Synergism between colistin and TA-EO was evaluated by checkerboard assay. The major compounds of TA-EO were docked with known enzymes involved in resistance to colistin, as well as the biosynthesis of peptidoglycan and amino acids. GC/MS revealed that TA-EO was of carvacrol chemotype (67.94 %). The TA-EO showed remarkable antibacterial activities against all Gram-negative bacterial strains, with the diameter of inhibition zones varied between 30 and 50 mm and a ratio MBC/MIC equal to 1 for the vast majority of bacterial isolates. Interestingly, the checkerboard showed synergism between TA-EO and colistin against colistin-resistant *Escherichia coli* co-harboring *mcr*-1 gene (FICI<1) and reduced the MIC of colistin by 16- to 512-fold and those of TA-EO by 4- to 16-fold. The docking study demonstrated that carvacrol had high binding free energies against MCR-1, a phosphoethanolamine transferase extracellular domain, and its catalytic domain implicated in resistance to colistin, and undecaprenyl pyrophosphate synthase in complex with magnesium which is involved in bacterial peptidoglycan biosynthesis. The molecular dynamics study for 100-ns also revealed the stability of the MCR-1/carvacrol complex with a constant surface area over the

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simulation. These results support using carvacrol or TA-EO as a bactericidal agent, either alone or in combination with colistin, to treat infections caused by colistin-resistant Gram-negative bacteria.

1. Introduction

The World Health Organization (WHO) reported that antimicrobial resistance is one of the greatest challenges for human health worldwide [1]. The overuse of antibiotics in both human and veterinary medicine has been linked to the rise in multidrug-resistant (MDR), extensively resistant (XDR), and pan-drug-resistant (PDR) strains of Gram-negative bacteria. *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli* have multiple inherent or acquired resistance mechanisms [2]. Thus, the rapid and easy emergence of antimicrobial-resistant bacterial strains in the environment, including unit care in hospitals, was demonstrated to be the origin of difficulty in treating infections that caused a longer stay in hospital and mainly improved mortality [1].

Until today, new pharmaceutical drugs have not been developed to resolve and stop the rapid emergence of MDR, XDR, and PDR Gram-negative bacterial strains. Thus, the non-development of new antibiotics against these bacterial strains has oriented the attention to revive older antibiotics that were confirmed to induce toxicity for human use, especially colistin, to be used as a "last resort" antimicrobial. It was well established that antibiotic colistin was infrequently prescribed for patients even in the recent past because of its neurotoxicity and nephrotoxicity, and has now been extensively used as the last therapeutic option, especially in countries characterized by an intensive rate of resistance to antimicrobials for the treatment of infections caused by Gram-negative bacteria resistant to carbapenem [3].

In this setting, WHO has declared that colistin is one of the important antibiotics listed as a "Highest Priority Critically Important Antimicrobial" [4]. Nevertheless, this announcement was confronted by the worldwide dissemination of "mobile colistin resistance 1" (mcr-1) resistant genes to colistin between bacteria from both animals and humans, leading to reduce the clinical efficacy of this antimicrobial agent and to a serious global strategy to use colistin [5].

Resistance to colistin has increased progressively due to the augmented and inappropriate use of this antibiotic [4]. Thus, it was demonstrated that the primary resistance mechanism to colistin was related to mutations occurring in some chromosomal genes, for example, *mgr*B, *pho*PQ, and *pmr*AB [6]. Besides, the easy and rapid spread via horizontal transfer of the plasmid-mediated colistin resistance gene, *mcr*-1, had increased the public and environmental rate risks [1,6].

Accordingly, it was demonstrated that *mcr*-1 can mediate resistance to colistin resistance by a mechanism that does not differ from that found in intrinsically resistant Gram-negative bacteria. Thus, MCR-1 is a phosphoethanolamine lipid A transferase enzyme, belonging to the "YhjW/YjdB/YijP" alkaline phosphatase superfamily [7]. The *mcr-1* gene encodes a PEtN transferase, which plays a key role in the addition of a PEtN moiety to the lipid A of lipopolysaccharide (LPS), leading to an increase of the cationic charges on LPS, and subsequently, reduces the affinity and binding of colistin to LPS [8].

To resolve the worldwide emergence of bacterial resistance to antibiotics, previously discarded antibiotics are once again being introduced for therapeutic use even though it was demonstrated to have important side effects in human organs [9]. Moreover, several collaborations between academic researchers and industry were established to develop new approaches to resolve the worldwide problem of antimicrobial resistance. Numerous potential strategies were proposed such development of alternatives to antibiotics and the research of adjuvants to conventional antibiotics [10]. An attractive and promising strategy was proposed, which was based on the simultaneous use of conventional antibiotics with adjuvants like bioactive compounds of medicinal plants [10].

Various medicinal plants were extensively characterized as a potential natural source to generate a diversity of therapeutic drugs for human use due to their multitude of bioactive compounds. Essential oils produced by medicinal plants are considered natural substances due to their content of multiple volatile secondary metabolites [11,12]. Hence, based on their beneficial therapeutic effects to human and animal health care, some essential oils were classified as safe to use according to the United States Agency for Food and Drug Products [13]. *Thymus algeriensis* belongs to the *Lamiaceae* family, which is native to the Mediterranean region [14]. This plant is considered one of the most popular medicinal plants in the world based on its therapeutic and aromatic properties. Therefore, due to its benefit effects, it is usually used as a therapeutic agent in traditional human medicine to treat several illnesses, such as gastric disorders, heart diseases, respiratory infections, pneumonia, asthma, diabetes, influenza, dermatitis, skin diseases, and infertility in women [15,16].

In the context of the growing imperative to identify novel adjuvants to complement existing antibiotics for combatting multi-resistant pathogenic bacteria, this study has set out the following objectives: (i) to assess the antibacterial efficacy of Tunisian *Thymus algeriensis* essential oil against hospital strains of Gram-negative bacteria that exhibit resistance to colistin; (ii) to examine the potential impact of using *Thymus algeriensis* essential oil as an adjuvant to colistin in reversing the resistant phenotype of Gram-negative bacteria to a susceptible phenotype; and (iii) to conduct molecular docking analysis to evaluate the interaction between the predominant compound in *Thymus algeriensis* essential oil and crucial enzyme MCR-1, a phosphoethanolamine transferase associated with resistance to colistin, and two others enzymes undecaprenyl pyrophosphate synthase, and *S. aureus* ketol-acid reductoisomerase implicated in biosynthesis of peptidoglycan and amino acids, respectively.

2. Materials and methods

2.1. Bacterial strains

Eleven clinical strains of *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli* resistant to colistin, which were previously isolated by microbiological procedures were included in this study [17]. They were collected at Rabta Hospital, Charles Nicolle Hospital of Tunis, National Bone Marrow Transplant Center, and Bechir Hamza Children's Hospital of Tunis Tunisia (Table 1). All bacterial isolates were stored in brain heart infusion broth with 20 % glycerol at −80 °C until use for antimicrobial evaluation. *Escherichia coli* ATCC 25922 was used as an internal quality control strain.

2.2. Antimicrobial susceptibility testing

2.2.1. Disc diffusion assay

Antimicrobial susceptibility tests were performed for all Gram-negative bacteria strains using the standard disk diffusion method on Mueller-Hinton agar plates (Bio-Rad, Marnes-la-Coquette, France) according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [18]. The disk of antibiotics used was as follows: cephems (cefotaxim, ceftazidime), β-lactam (Ceftazidim, Cefoxitin, Cefixime, Cefepime, Aztreonam), aminoglycosides (gentamicin, tobramycin, amikacin), carbapenems (ertapenem, imipenem, eropenem), β-lactam combination agent (Amoxicillin-clavulanic acid, Piperacillin-Tazobactam), quinolones and fluoroquinolones (nalidixic acid, ciprofloxacin), penicillins (ampicillin, piperacillin, ticarcillin).

2.2.2. Determination of minimal inhibitory concentration of colistin

The Minimum Inhibitory Concentrations (MIC) of colistin were determined against all bacterial strains included. It was realized by the standard of broth microdilution assay according to the guidelines of the CLSI [18]. Susceptibility to colistin sulfate (Sigma-Aldrich, St. Louis, MO, USA) was tested over a range of two-fold dilutions by using a broth microdilution assay (512–0.012 mg/L) [18,19]. In a sterile biological safety cabinet (BIOBASE, China), a sterilized Mueller Hinton II cation-adjusted broth was distributed into 96 microtiter plate wells. Then, serial dilution of colistin was done at concentrations ranging from 512 μg/mL to 0.012 μg/mL by a two-fold dilution method. Finally, the wells were inoculated with 100 μL 10^5 CFU/mL of clinical Gram-negative bacteria resistant to colistin as well as *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 as standard strains and incubated overnight at 37 °C (Memmert, Germany).

After incubation, the bacteria that showed visible growth at concentrations >2 μg/mL were considered resistant, as recommended by EUCAST 2024 [19]. The assays were performed in triplicate to confirm the findings.

2.3. Chemical composition analysis of Thymus algeriensis essential oil by gas chromatography-mass spectrometry

The chemical composition of a commercial *Thymus algeriensis* EO purchased from a local company was determined by Gas Chromatography-Mass Spectrometry (GC-MS). The analysis was carried out according to the conditions mentioned in our previous article using a Hewlett Packard (HP) 5890 II GC coupled with an HP-5 MS capillary column (30 m × 0.25 mm, 0.25 μm film thickness, Agilent Technologies) [11].

2.4. Antimicrobial activities of Thymus algeriensis essential oil

2.4.1. Disc diffusion method

The *Thymus algeriensis* essential oil was evaluated by disc diffusion test as we have previously reported [11]. Briefly, in a sterile

Table 1
Antibiotic profile of colistin-resistant Gram-negative bacterial strains.

Bacterial strains[a]	Drug resistance	MIC Colistin (μg/mL)
E. coli-1	NAL, CIP, TCN	16
E. coli-2	AMP, TIC, PIP, CAZ, CXM, FEP, ATM, NAL, CIP, TCN	16
E. coli-3	AMP, AMC, TIC, PIP, CAZ, CXM, FEP, ATM, NAL, CIP, TCN	32
E. coli-4	AMP, TIC, PIP, CAZ, CXM, FEP, ATM, GM, NAL, CIP, TCN	32
K. pneumoniae-1	AMP, AMC, TIC, PIP, TZP, CAZ, FOX, CXM, FEP, ATM, ERT, IMP, MEM, GM, NAL, CIP, TCN	32
K. pneumoniae-2	AMP, AMC, TIC, PIP, TZP, CAZ, FOX, CXM, FEP, ATM, ERT, IMP, MEM, GM, NAL, CIP, TCN	64
K. pneumoniae-3	AMP, TIC, PIP, TZP, TCN	32
K. pneumoniae-4	AMP, AMC, TIC, PIP, TZP, FOX, ERT, IMP, MEM, GM, TCN	128
P. aeruginosa-1	TIC, PIP, TZP, CAZ, FEP, ATM, ERT, IMP, MEM, GM, NAL, CIP, TCN	8
P. aeruginosa-2	TIC, PIP, FOX, ERT, IMP, MEM	4
P. aeruginosa-3	TIC, PIP, TZP, CAZ, GM, NAL, CIP, TCN	128

[a] *Escherichia coli* strain 1: *E. coli*-1; *Klebsiella pneumoniae*: *K. pneumoniae*; *Pseudomonas aeruginosa*: *P. aeruginosa*; Amoxicillin-clavulanic acid: AMC; Ampicillin: Amp; Aztreonam: ATM; Cefepime: FEP; Cefixime: CXM; Cefoxitin: FOX; Ceftazidim: CAZ; Ciprofoloxacin: CIP; Ertapenem: ERT; Gentamicin: GM; Imipenem: IMP; Meropenm: MEM; Nalidixic acid: NAL; Piperacillin/Tazobactam: TZP; Piperacillin: PIP; Tetracycline: TCN; Ticarcillin: TIC.

biological safety cabinet, 5 μL of the essential oil was added on sterile Whatman filter paper discs of a 6 mm diameter which were placed in the center of Petri dishes previously spread with an overnight culture of each bacterial strain. Then, plates were placed at room temperature for 2 h to allow the essential oil to diffuse. Finally, they were incubated upside down at 37 °C overnight.

The antibacterial activity of *Thymus algeriensis* was determined based on the diameter of the transparent zone around the disc (zone of inhibition). The zone of inhibition was interpreted as described previously by Ponce and collaborators [20]: not sensitive = diameter <8 mm; sensitive = diameter of 8–14 mm; very sensitive = diameter of 15–19 mm; extremely sensitive = diameter ≥20 mm.

2.4.2. Determination of minimal inhibitory concentration

The MICs of *Thymus algeriensis* essential oil were determined by microdilution susceptibility as we reported against all Gram-negative bacteria resistant to colistin, and susceptible strains to colistin *Escherichia coli* ATCC 25922 [11]. Initially, *Thymus algeriensis* essential oil was diluted in DMSO. The concentrations of the essential oil were two-fold serial dilutions ranging from 40 to 0.078 mg/mL. Next, the wells were inoculated with 100 μL 10^5 CFU/mL of clinical Gram-negative bacteria resistant to colistin as well as *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 as standard strains and incubated overnight at 37 °C (Memmert, Germany). After incubation, the well with no bacterial growth at the lower concentration of the essential oil was considered the MIC.

2.4.3. Determination of minimal bactericidal concentration

The minimal bactericidal concentration (MBC) of *Thymus algeriensis* essential oil was determined as follows: 10 μL from each well of the Plate without bacterial growth aspired, then they were cultured on Mueller-Hinton agar to verify if the inhibition of bacterial growth was reversible or permanent. Thus, MBC corresponds to the highest dilution (consequently the lowest concentration of the essential oil) at which >99.9 % reduction of initial CFU was obtained. Each test was achieved in triplicate.

2.5. Interactions between Thymus algeriensis essential oil and colistin

To evaluate plausible synergism between colistin and *Thymus algeriensis* essential oil, a checkerboard was applied as we have before described [11]. Briefly, two-fold serial dilutions of the essential oil and colistin were done in Mueller Hinton II cation-adjusted broth and added to 96-well sterile plates. Next, the microplates were placed in the incubator at 37 °C for 24 h under agitation. To analyze the interaction results obtained by checkerboard assays, fractional inhibitory concentration indexes (FICI) were calculated. The FICI was determined as follows:

FICEO + FICantibiotic; with

$$\mathrm{FICEO} = \frac{\text{MICEO used in combination}}{\text{MICEO used alone}}$$

and

$$\mathrm{FICantibiotic} = \frac{\text{MIC antibiotic used in combination}}{\text{MIC antibiotic used alone}}$$

The interpretation of FICI values was done as previously described. Thus, if FICI <0.5 the result indicates a synergistic effect; if 0.5 ≤ FICI ≤4, the result indicates an additive or indifferent effect; and if FICI >4, the result indicates an antagonistic effect [19,21].

2.6. Molecular docking details

The crystal structures of the following enzymes were used as receptors, including a phosphoethanolamine transferase (MCR-1), with its extracellular domain (PDB ID: 5GOV), and its catalytic domain (PDB ID: 5GRR), undecaprenyl pyrophosphate synthase (PDB IDs: 2E98 and 1X06), and *S. aureus* ketol-acid reductoisomerase (PDB ID: 7KH7). The Protein Data Bank (https://www.rcsb.org/, accessed on December 25–26, 2023) provided these structures. The 3d structures of the ligands used were downloaded from PubChem via http://pubchem.ncbi.nlm.nih.gov/ on December 17, 2023. The receptors were prepared by removing water and co-crystallized ligands/ions and were protonated using Pymol software ver. 2.5.1. The 3D structures of the ligands were optimized using Avogadro Software ver. 1.2.0. Blind docking was performed using CB-DOCK2, accessed via http://clab.labshare.cn/cb-dock/php/ on July 24, 2024, as described by Liu and coauthors [22]. The top five cavities were subjected to docking using AutoDock Vina. The best-docked complexes were analyzed using Discovery Studio software described by Farouk and collaborators [23].

2.7. Molecular dynamic simulation

The ligand topology parameters were obtained by the ACPYPE server (https://www.bio2byte.be/acpype/) with the GAFF force field. AMBER99SB force field was used for the protein topology parameters. The complex was solvated in a cubic box with an SPC water model. The system was neutralized by adding 4 Na + ions; then, energy was minimized by the steepest descent algorithm in 50,000 steps and Fmax <500 kJ/mol. After that, the minimized system was equilibrated using an NVT ensemble at 300 K for 200 ps, followed by an NPT ensemble for 500 ps. Finally, the MD simulations were performed for 100 ns under an NPT ensemble with a time step of 2 fs, and long-range electrostatic interactions were calculated using the Particle Mesh Ewald (PME) algorithm. Hydrogen bond lengths were constrained using the Linear Constraint Solver (LINCS) algorithm. After MD simulations, periodic boundary conditions were removed, and the trajectories were analyzed using GROMACS tools like rms for Root mean square deviation (RMSD), rmsf for

Root mean square fluctuation (RMSF), gyrate for Radius of gyration (RG), sasa for Solvent Accessible Surface Area (SASA) and hbond to calculate the number of hydrogen bonds.

3. Results and discussion

3.1. Antibiotic profile resistance

In the current study, the antibiotic resistance obtained by disc diffusion assay and colistin' MICs values achieved by microdilution method against different clinical Gram-negative bacteria strains selected are shown in Table 1. The levels of MIC of colistin ranged from 4 to 128 μg/mL, and they were more than 2 μg/mL, which is the rate defined by EUCAST 2024 to characterize Gram-negative bacterial strains resistant to colistin [19].

3.2. Chemical composition of Thymus algeriensis essential oil

Results obtained using the GC-MS technique revealed the identification of twenty-eight volatile constituents of *Thymus algeriensis* essential oil, where carvacrol is the dominant compound (67.94 %), followed by p-cymene (8.93 %), γ-terpinene (3.25 %), α-terpinyl acetate (2.18 %), linalool (1.43 %), myrcene (1.39 %), and α-terpinene (1.27 %) as shown in Fig. 1 and Table 2. This composition characterizes this essential oil as a carvacrol chemotype.

The chemical of *Thymus algeriensis* essential oil in the current results was not the same as those of other studies previously published from Algeria. Thus, an earlier study from Algeria reported that α-terpinyl acetate (47.4 %) was the major component of EO of *Thymus algeriensis* cultivated in Algerian Saharan atlas [24]. Furthermore, another study indicated that *Thymus algeriensis* EO from El-Guetfaregion, M'sila, Algeria was of camphor chemotype (32.56 %) [25]. The heterogeneity of chemotypes and the chemical compositions of *Thymus algeriensis* EO could be related to one or more of the following parameters: climatic and soil variations, the stage of collection of plants' samples, conditions of storage, techniques used to extract the essential oil, and epigenetic variations in this specie.

3.3. Antibacterial activity of Thymus algeriensis essential oil

3.3.1. Disc diffusion

Results obtained by disc diffusion assay showed very high inhibition zone' diameters of *Thymus algeriensis* essential oil against all tested Gram-negative strains resistant to colistin (Table 3). The interval ranges of inhibition zone diameter varied from 46 mm to 64 mm, with an average value of 54.5 mm in the *E. coli* strains resistant to colistin and co-harboring *mcr*-1 gene; from 35 to 40 mm, with an average value of 37 mm in the *K. pneumoniae*; and from 30 to 40 mm, with an average value 35 mm in the *P. aeruginosa* (Table 3). Thus, to the best of our knowledge, this is the first study to show an advanced inhibition zone of *Thymus algeriensis* essential oil against Gram-negative bacteria resistant to colistin. The *Thymus algeriensis* essential oil gives an inhibitory transparent zone of 60 mm against *E. coli* ATCC 25922 (Table 3).

3.3.2. MIC and MBC determination

MIC assay results confirmed those obtained by the disc diffusion method against all Gram-negative bacteria resistant to colistin and

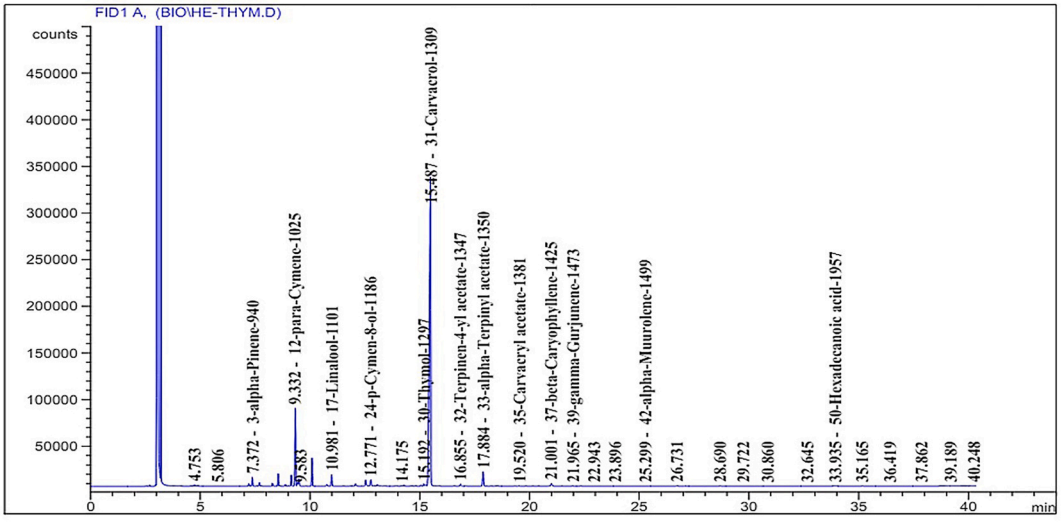

Fig. 1. Chromatogram obtained by GC-MS technique showing the dominant compounds identified in *Thymus algeriensis* essential oil.

Table 2
Percentage of volatile components of *Thymus algeriensis* essential oil identified by GC-MS analysis.

Peak	Compound	RI	Area %
1	Tricyclene	930	0,028
2	α-Thujene	935	0,248
3	α-Pinene	940	0,967
4	Camphene	954	0,066
5	Sabinene	976	0,389
6	1-Octen-3-ol	982	0,350
7	**Myrcene**	991	**1,390**
8	3-OCTANOL	998	0,059
9	α-Phellandrene	1005	0,213
10	δ-3-Carene	1012	0,094
11	**α-Terpinene**	1019	**1,279**
12	**p-Cymene**	1025	**8,938**
13	Limonene	1031	0,527
14	β-Phellandrene	1032	0,909
15	1,8-Cineole	1033	0,045
16	**γ-Terpinene**	1063	**3,255**
17	**Linalool**	1101	**1,432**
18	cis-p-Menth-2-en-1-ol	1121	0,022
19	p-Menth-2-en-1-ol	1140	0,064
20	Pinocarveol	1142	0,039
21	Camphor	1144	0,063
22	Borneol	1168	0,383
23	**Terpinen-4-ol**	1179	**1,067**
24	p-Cymen-8-ol	1186	0,946
25	Dodecene	1190	0,135
26	Nerol	1233	0,271
27	Methyl thymol	1235	0,118
28	Methyl carvacrol	1244	0,148
29	E-Citral	1254	0,162
30	Thymol	1297	0,360
31	**Carvacrol**	1309	**67,941**
32	Terpinen-4-yl acetate	1347	0,305
33	**α-Terpinyl acetate**	1350	**2,181**
34	Thymyl acetate	1355	0,118
35	Carvacryl acetate	1381	0,069
36	Isocaryophyllene	1408	0,050
37	β-Caryophyllene	1425	0,543
38	α-Humulene	1461	0,042
39	γ-Gurjunene	1473	0,086
40	allo-Aromadendrene	1475	0,092
41	ledene	1492	0,072
42	α-Muurolene	1499	0,011
43	β-Bisabolene	1520	0,004
44	δ-Cadinene	1523	0,006
45	Spathulenol	1576	0,009
46	Caryophyllene oxide	1588	0,038
47	α-Bisabolol	1685	0,004
48	(E,E)-Farnesol	1733	0,007
49	Octadecane	1800	0,003
50	Hexadecanoic acid	1957	0,125
51	1-Docosene	2195	0,003
Total			**95,675**

E. coli ATCC 25922 (Table 3). Thus, the MIC values varied from 1.25 to 5, from 0.156 to 0.612, and 0.612–2.5 mg/mL against *E. coli*, *K. pneumoniae*, and *P. aeruginosa*, respectively. To our knowledge, these results are reported for the first time in the current study. Besides, for ten Gram-negative bacteria resistant to colistin, the ratio MBC/MIC was equal to one, and just for *K. pneumoniae* strain number two (*K. pneumoniae*-2) it was equal to two. This finding argues for an advanced *bactericidal* effect of *Thymus algeriensis* essential oil against these bacterial strains, as recommended previously [26].

3.4. Synergism effect between colistin and Thymus algeriensis essential oil

In the current study, we measured the MICs of colistin for all *E. coli* colistin-resistant strains and colistin-sensitive *E. coli* strains (ATCC25922). Interestingly, checkerboard assay revealed that the colistin' MICs were decreased at an advanced rate in the presence of *Thymus algeriensis* essential oil for all tested bacterial strains, and especially for *E. coli* strain number four (*E. coli*-4) from 32 μg/mL to 0.06 μg/mL (Table 4). Thus, simultaneous use of the two antimicrobial agents, i.e., colistin and *Thymus algeriensis* essential oil, intensively reduced the MIC values of colistin by 16 to 512-fold. Furthermore, the MIC value of *Thymus algeriensis* essential oil (when

Table 3
Diameter of inhibition zone (IZ; mm), MICs (mg/mL), and MBCs (mg/mL) of *Thymus algeriensis* essential oil against colistin-resistant Gram-negative bacteria.

Tested strains	IZ±SD (mm)	MIC value (mg/ml)	MBC value (mg/ml)	MBC/MIC ratio
E. coli-1	44 ± 0.2	5	5	1
E. coli-2	48 ± 0.1	5	5	1
E. coli-3	50 ± 0.58	1.25	1.25	1
E. coli-4	46 ± 0.63	1.25	1.25	1
K. pneumoniae-1	35 ± 0.28	0.325	0.325	1
K. pneumoniae-2	37 ± 0.34	0.612	1.25	2
K. pneumoniae-3	40 ± 0.54	0.612	0.612	1
K. pneumoniae-4	36 ± 0.4	0.156	0.156	1
P. aeruginosa-1	30 ± 0.5	2.5	2.5	1
P. aeruginosa-2	35 ± 0.28	2.5	2.5	1
P. aeruginosa-3	40 ± 0.24	0.612	0.612	1
E. coli ATCC 25922	60 ± 0.1	1.25	2.5	2

Escherichia coli strain 1: *E. coli*-1; *Klebsiella pneumoniae*: *K. pneumoniae*; *Pseudomonas aeruginosa*: *P. aeruginosa*; Minimal inhibitory concentration: MIC; minimal bactericidal concentration: MBC.

Table 4
MICs of colistin and *Thymus algeriensis* essential oil used alone or in combination against different *E. coli* strains.

Tested strains	Colistin (μg/mL)	TA-EO (mg/mL)	Colistin $^{A}_{TA\text{-}EO}$ (μg/mL)	TA-EO $^{B}_{colistin}$ (mg/mL)	FICI[a]
E. coli-1	16	5	1	0.312	0.12 (Synergy)
E. coli-2	16	5	0.06	0.312	0.12 (Synergy)
E. coli-3	32	1.25	2	0.156	1.06 (additive)
E. coli-4	32	1.25	0.06	0.312	0.25 (Synergy)
E. coli ATCC 25922	1	1.25	0.06	0.312	0.31 (Synergy)

Thymus algeriensis EO: TA-EO; A: Colistin MICs in the presence of TA-EO; B: TA-EO MICs in the presence of colistin.
[a] Fractional inhibitory concentration index.

used alone) was higher than that of used along with colistin and decreased 4 to 16-fold against colistin-resistant *E. coli* strains and 4-fold against *E. coli* ATCC 25922 (Table 4).

It was established that colistin has dose-dependent side effects such as nephrotoxicity and neurotoxicity, which limits its clinically sufficient dose and long-term treatment options. However, in this study, the simultaneous use of *Thymus algeriensis* essential oil and colistin against *E. coli* co-harboring *mcr*-1 hospital strains diminished importantly the MIC values of colistin under 8 μg/mL which is the limit characterizing *E. coli* NCTC 13846 resistant to colistin co-harboring *mcr*-1 [19]. This finding highlights the important use of *Thymus algeriensis* essential oil as an adjuvant to colistin to combat *E. coli* co-harboring *mcr*-1.

Importantly, we reported that the calculated FICI values ranged between 0.12 and 0.25 for all tested *E. coli* strains resistant to colistin and for the *E. coli* ATCC 25922 sensitive to colistin. These values indicated an advanced synergetic effect between colistin and *Thymus algeriensis* essential oil. However, the FICI was equal to 1.06 only for one strain of *E. coli* resistant to colistin-resistant (*E. coli*-3

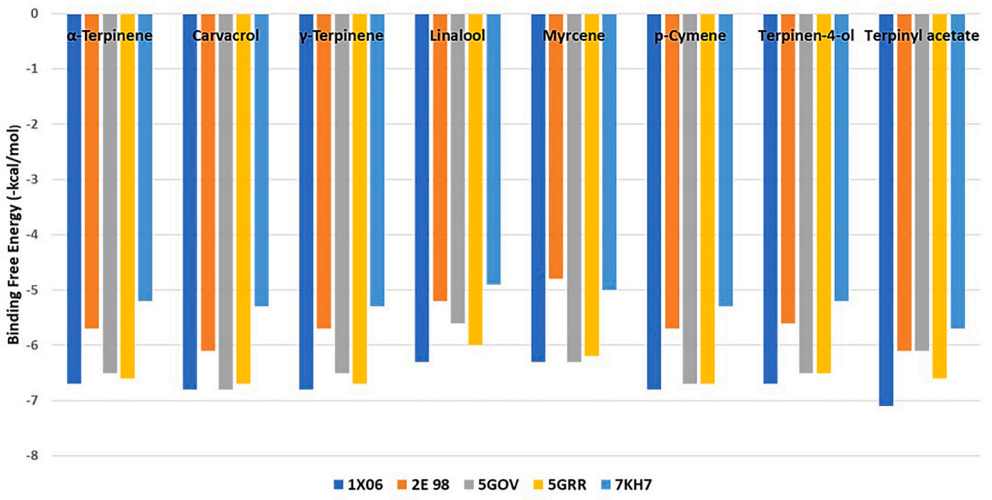

Fig. 2. Molecular docking scores of the major components of *Thymus algeriensis* essential oil and the metabolic enzymes as receptors.

strain), indicating an additive effect of the combination of these two antimicrobial agents.

To avoid the emergence of MDR and XDR Gram-negative superbugs, mainly *E. coli*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, which had different mechanisms of resistance against all available antibiotics, and the lack of development by industrial pharmaceutics of novel antimicrobials, the polymyxins had re-emerged by the mid-1990s as a last-resort to treat infections associated to MDR and XDR Gram-negatives bacteria even of its approved no safety profile [27]. Accordingly, *mcr*-1 has become a main risk of dissemination via horizontal transfer of a plasmid-borne gene coding for a polymyxin resistance mechanism among various Gram-negative bacteria [2,28]. Furthermore, it was previously shown that the *mcr*-1 gene was associated with a 4- to 8-fold increase in the MICs of colistin in *E. coli*. Thus, it was suggested that the *mcr*-1 alone is enough to provide resistance against colistin in *E. coli* and other *Enterobacteriaceae* [29].

3.5. Molecular docking analysis

The molecular docking approach was applied in the current study to understand better how the major *Thymus algeriensis* volatiles under investigation provide antibacterial activities. These volatiles as ligands were docked into vital enzymes involved in the biosynthesis, and resistance toward colistin. MCR-1 modifies lipid A and confers resistance to colistin (PDB ID: 5GOV and 5GRR). Undecaprenyl pyrophosphate synthases (PDB ID: 2E98 and 1X06) catalyze the condensation reactions of farnesyl pyrophosphate with isopentenyl pyrophosphates to generate undecaprenyl pyrophosphate. The branched-chain amino acids (BCAAs) pathway partly relies on the ketol-acid reduction-isomerase (KARI) enzyme for the biosynthesis of leucine, isoleucine, and valine. KARI (PDB ID: 7KH7) is a bifunctional enzyme that converts 2-acetolactate or 2-aceto-2-hydroxybutyrate into precursors of BCAAs via a two-step reaction within a single active site [30–32].

Fig. 2 displays the best poses obtained from molecular docking analyses, revealing binding affinities of each ligand with different bacterial enzymes. Lower ΔG indicates a more vital interaction between receptors and ligands. The major volatile of the investigated oil, carvacrol, has displayed higher binding affinities with high docking scores, ranging from −6.5 to −6.8 kcal/mol, towards the 5GOV, 5GRR, and 1X06 bacterial enzymes, as shown in Fig. 2. Thus, to the best of our knowledge, this study is the first to show high affinities of carvacrol to the enzyme MCR-1 (PDBs: 5GOV, 5GRR) associated with resistance to colistin and to the undecaprenyl pyrophosphate synthase in complex with magnesium which plays a pivotal role in bacterial peptidoglycan biosynthesis (PDB ID: 1X06). The remaining major oil volatiles showed comparable docking scores to carvacrol, especially against 1X06, while similar energies were demonstrated for both carvacrol and terpinyl acetate against 2E98 (−6.1 kcal/mol).

To our knowledge, few studies have yet to be published concerning using essential oils or extracts as inhibitors for the receptors under consideration in the current molecular docking study. For example, Wang and collaborators studied the synergistic effect of eugenol with colistin against clinically isolated colistin-resistant *E. coli* strains with the free energy of binding was −10.087 kcal/mol, which indicates a higher bind compared to the findings of the present study between MCR-1 protein (PDB ID: 5GRR) and eugenol [33]. Meanwhile, comparable results to the present study were shown by Yi and coworkers, where the free energy of binding was −8.13 kcal/mol between MCR-1 protein (PDB ID: 5GRR) and tetrandrine [34]. In the same line, the docking scores of pogostone were −8.5 kcal/mol and −6.1 kcal/mol for MCR-1 and MCR-3, respectively [35].

Fig. 3 depicts how carvacrol interacts with the crystal structure of MCR-1, a phosphoethanolamine transferase, extracellular domain (PDB ID: 5GOV). This interaction exhibits the highest docking score, as illustrated in Fig. 2. The primary reason behind the higher binding affinity of carvacrol with 5GOV (−6.8 kcal/mol) is due to the hydrophobic π-π T-shaped, π-alkyl, and alkyl interactions (Fig. 3). The π-π T-shaped interaction refers to the attractive force between aromatic rings. This is due to π-electron clouds, such as the

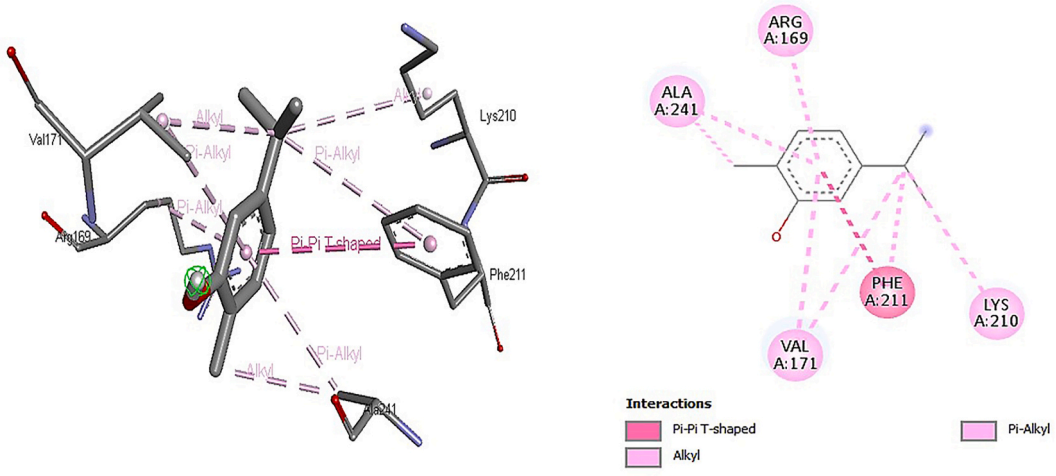

Fig. 3. Interactions of carvacrol with MCR-1,a phosphoethanolamine transferase extracellular domain (PDB ID:5GOV).

π-orbitals of PHE A:211 and carvacrol. When dealing with two π systems, the T-shaped edge-to-face and the parallel-displaced stacking arrangement are the most common forms of interaction. The interaction energies of the parallel, T-shaped, and slipped-parallel benzene dimers are −1.48, −2.46, and −2.48 kcal/mol, respectively [36].

Aromatic ring interactions are prevalent in chemical and biological systems and are considered the third most frequent protein-ligand contact hydrophobic interactions. Almost 50 % of all π-stacking interactions occur between the aromatic ring of phenylalanine and an aromatic ring in the ligand, which supports the present study's findings [37]. Again, hydrophobic interactions between the π-orbitals of PHE A:211 and the alkyl group of carvacrol and between the π-orbitals of carvacrol and alkyl groups of ARG A:169, VAL A:171, and ALA A:241 were observed in Fig. 3-A, which helped in the intercalation of the ligand in the binding pocket of the receptor as reported by Arthur and collaborators [38]. They also reported another type of interaction that also supports the stabilization of ligands inside the receptor pocket was the hydrophobic alkyl one between ALA A:241 and a carvacrol alkyl group, in addition to the binding between the carvacrol alkyl group and that of VAL A:171 and LYS A:210 (Fig. 3-A).

3.6. Molecular dynamic simulation

We conducted all-atom molecular dynamics simulations of receptor-ligand complexes of target proteins, running them for 100 ns, to study how carvacrol binding affects the targeted MCR-1. Various computational analyses were performed, including structural, dynamic, and thermodynamic computations. We used backbone RMSD, RMSF, Rg, SASA, and H-bond analyses to assess the structural behavior of the target proteins in both their bound and unbound states (Fig. 4).

To assess the stability of a protein-ligand complex, analysis was performed for the dynamic movements of atoms and conformational changes of backbone atoms. The complex exhibited low RMSD and minimal variations, indicating high stability, as shown in Fig. 4A [39]. The RMSF analysis showed neutral fluctuation, suggesting that the ligand had no significant effect on the positioning of the residues (Fig. 4B). Additionally, the radius of gyration (Rg) analysis indicated that the compactness of the protein varied based on its coupling with the ligand. The Rg of the MCR-1-carvacrol complex was somewhat lower between 30 and 70 nm compared to the initial and final periods (Fig. 4C).

The study analyzed how the MCR-1-carvacrol complex interacts with solvents using solvent-accessible surface area (SASA) over 100 ns (Fig. 4D). The results showed that the MCR-1 maintained a constant surface area over the simulation and formed two hydrogen bonds with the ligand, indicating a stable structure (Fig. 4E).

4. Conclusions

This study is the first to report the bactericidal effects of Tunisian *Thymus algeriensis* essential oil against Gram-negative bacterial isolates resistant to colistin *in vitro*. Furthermore, the current is the first to demonstrate and approve the synergistic effect of the simultaneous use of *Thymus algeriensis* essential oil and colistin against colistin-resistant *Escherichia coli* co-harboring *mcr*-1 gene. Interestingly, the docking study demonstrated that carvacrol had high binding free energies against MCR-1, a phosphoethanolamine transferase extracellular domain (PDB ID: 5GOV), and its catalytic domain (PDB ID: 5GRR) involved in resistance to colistin, and undecaprenyl pyrophosphate synthase in complex with magnesium which is implicated in bacterial peptidoglycan biosynthesis (PDB IDs: 1X06). MCR-1/carvacrol complex showed high stability through the molecular dynamic study with a constant surface area and two hydrogen bonds during the simulation.

These findings appear as a promising therapeutic strategy to use carvacrol alone or as an adjuvant to colistin against Gram-negative bacteria resistant to colistin.

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Data availability statement

All data generated or analyzed during this study are included in this published article and its supplementary information file.

Ethics declarations

The study was approved by the ethical committee CER-SVS/ of the Higher Institute of Biotechnology of Monastir, with the "003/2022" committee's reference number. The ethical committee CER-SVS waived the need for consent to participate.

CRediT authorship contribution statement

Walid Ben Selma: Writing – review & editing, Writing – original draft, Visualization, Software, Project administration, Methodology, Investigation, Conceptualization. **Amr Farouk:** Writing – review & editing, Writing – original draft, Visualization, Software, Investigation, Conceptualization. **Zhaojun Ban:** Software. **Mohamed Ferjeni:** Software. **Tawfiq Alsulami:** Software, Project administration, Funding acquisition. **Hatem Ali:** Software, Conceptualization. **Jalel Boukadida:** Supervision, Conceptualization.

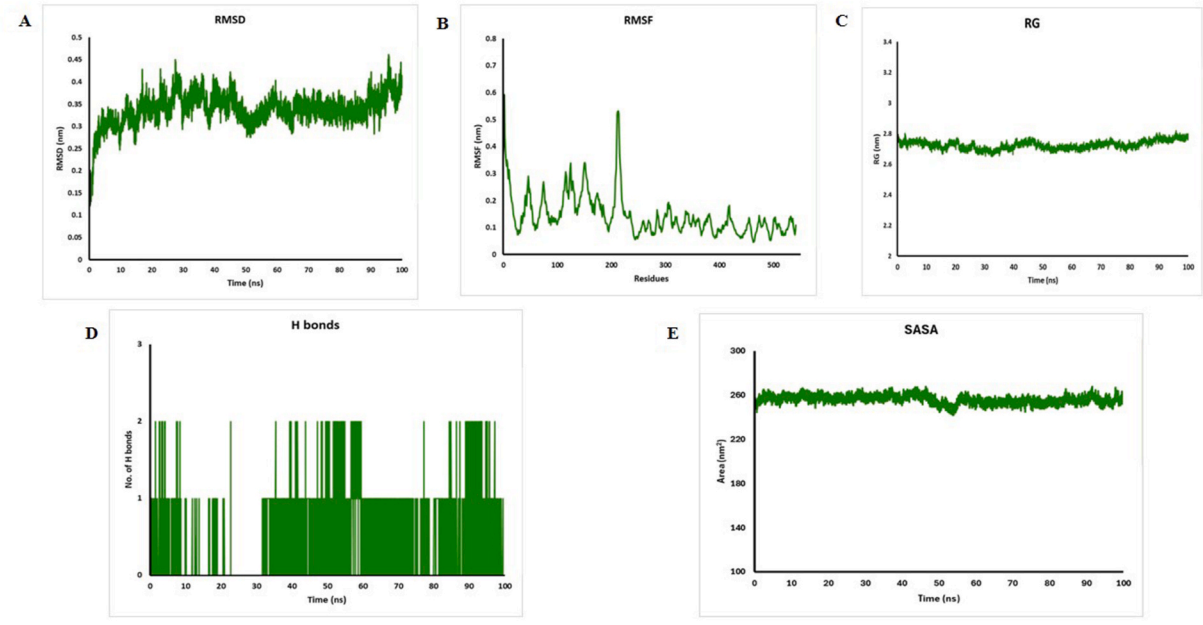

Fig. 4. MD simulations of Carvacrol with MCR-1,a phosphoethanolamine transferase extracellular domain (PDB ID:5GOV).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e38281.

References

[1] WHO, Implementing the global action plan on antimicrobial resistance, 2024, pp. 1–71. https://iris.who.int/bitstream/handle/10665/375008/9789240074668-eng.pdf?sequence=1. Available from:.
[2] P. Srinivas P, K. Rivard, Polymyxin resistance in gram-negative pathogens, Curr. Infect. Dis. Rep. 19 (11) (2017) 38, https://doi.org/10.1007/s11908-017-0596-3.
[3] E. Temkin, A. Adler, A. Lerner, Y. Carmeli, Carbapenem-resistant Enterobacteriaceae: Biology, Epidemiology, and management, 2014, https://doi.org/10.1111/nyas.12537.
[4] WHO, Critically important antimicrobials for human medicine, 6th revision, 2019, pp. 26–41. https://www.who.int/foodsafety/publications/antimicrobials-sixth/en/. Available from:.
[5] L. Poirel, A. Jayol, P. Nordmann, Polymyxins: antibacterial activity, susceptibility testing, and resistance mechanisms encoded by plasmids or chromosomes, Clin. Microbiol. Rev. 30 (2) (2017) 557–596, https://doi.org/10.1128/CMR.00064-16.
[6] A.O. Olaitan, S. Morand, J.M. Rolain, Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria, Front. Microbiol. 5 (2014) 643, https://doi.org/10.3389/fmicb.2014.00643.
[7] R. Gao, Y. Hu, Z. Li, J. Sun, Q. Wang, J. Lin, H. Ye, F. Liu, S. Srinivas, D. Li, B. Zhu, Y.H. Liu, G.B. Tian, Y. Feng, Dissemination and mechanism for the MCR-1 colistin resistance, PLoS Pathog. 12 (11) (2016) e1005957, https://doi.org/10.1371/journal.ppat.1005957.
[8] J. Sun, H. Zhang, Y.H. Liu, Y. Feng, Towards understanding MCR-like colistin resistance, Trends Microbiol. 26 (9) (2018) 794–808, https://doi.org/10.1016/j.tim.2018.02.006.
[9] H.W. Boucher, G.H. Talbot, J.S. Bradley, J.E. Edwards, D. Gilbert, L.B. Rice, M. Scheld, B. Spellberg, J. Bartlett, Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America, Clin. Infect. Dis. 48 (1) (2009) 1–12, https://doi.org/10.1086/595011.
[10] K. Bush, P. Courvalin, G. Dantas, et al., Tackling antibiotic resistance, Nat. Rev. Microbiol. 9 (12) (2011) 894–896, https://doi.org/10.1038/nrmicro2693.
[11] W. Ben Selma, S. Alibi, M. Ferjeni, S. Ghezal, N. Gallala, A. Belghouthi, A. Gargouri, M. Marzouk, J. Boukadida, Synergistic activity of *Thymus capitatus* essential oil and cefotaxime against ESBL-producing *Klebsiella pneumoniae*, Int. J. Environ. Health (2023) 1–11, https://doi.org/10.1080/09603123.2023.2280149.

[12] W. Ben Selma, S. Ferjeni, A. Farouk, M. Marzouk, J. Boukadida, Antimicrobial activity of Cinnamomum zeylanicum essential oil against colistin-resistant gram-negative bacteria, Int. J. Environ. Health Res. (2024) 1–13, https://doi.org/10.1080/09603123.2024.2348094.

[13] F. Fancello, G.L. Petretto, S. Zara, M.L. Sanna, R. Addis, M. Maldini, M. Foddai, J.P. Rourke, M. Chessa, G. Pintore, Chemical characterization, antioxidant capacity and antimicrobial activity against food related micro-organisms of Citrus limon var, LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.) 69 (2016) 579–585.

[14] A.G. Pirbalouti, Z.E. Bistghani, F. Malekpoor, An overview on genus *Thymus*, J Herbal Drugs 6 (2) (2015) 93–100, https://doi.org/10.3390/plants11070954.

[15] B. Mustafa, A. Hajdari, F. Krasniqi, E. Hoxha, H. Ademi, C.L. Quave, A. Pieroni, Medical ethnobotany of the Albanian alps in Kosovo, J. Ethnobiol. Ethnomed. 8 (2012) 6, https://doi.org/10.1186/1746-4269-8-6.

[16] B. Wahida, M. Amor, C. Nabil, M. Amor, C. Nabil, An inventory of ethnomedicinal plants used in Tunisia, Ethnomed. Plants (2011) 28, https://doi.org/10.4314/ajtcam.v11i3.27.

[17] S. Ferjani, E. Maamar, A. Ferjani, K. Meftah, H. Battikh, B. Mnif, M. Hamdoun, Y. Chebbi, L. Kanzari, W. Achour, O. Bahri, A. Hammami, M. Zribi, H. Smaoui, I. B. Boubaker, Tunisian multicenter study on the prevalence of colistin resistance in clinical isolates of gram negative bacilli: emergence of *Escherichia coli* harbouring the *mcr-1* gene, Antibiotics 11 (10) (2022) 1390, https://doi.org/10.3390/antibiotics11101390.

[18] CLSI, Performance Standards for Antimicrobial Susceptibility Testing, 29th ed, CLSI Suppl M100, 2019, https://doi.org/10.1007/978-3-662-48986-4.

[19] EUCAST, Clinical breakpoint (v 14.0) [Updated January 1]. Available from: https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_14.0_Breakpoint_Tables.pdf, 2024.

[20] A.G. Ponce, R. Fritz, C. Del Valle, S.I. Roura, Antimicrobial activity of essential oils on the native microflora of organic Swiss chard, LWT–Food Sci. Technol. 36 (2003) 679–684, https://doi.org/10.3390/antibiotics9010029.

[21] F.C. Odds, Synergy, antagonism, and what the chequerboard puts between them, J. Antimicrob. Chemother. 52 (1) (2003) 1, https://doi.org/10.1093/jac/dkg301.

[22] Y. Liu, X. Yang, J. Gan, S. Chen, Z.X. Xiao, Y. Cao, CB-Dock2: improved protein-ligand blind docking by integrating cavity detection, docking and homologous template fitting, Nucleic Acids Res. 50 (W1) (2022) W159–W164, https://doi.org/10.1093/nar/gkac394.

[23] A. Farouk, T. Alsulami, H.S. Ali, A.N. Badr, In-vitro and in-silico investigation for the spent-coffee bioactive phenolics as a promising aflatoxins production inhibitor, Toxins 15 (3) (2023) 225, https://doi.org/10.3390/toxins15030225.

[24] M. Rezzoug, B. Bakchiche, A. Gherib, A. Roberta, Kilinçarslan Ö. FlaminiGuido, R. Mammadov, S.K. Bardaweel, Chemical composition and bioactivity of essential oils and ethanolic extracts of Ocimum basilicum L. and Thymus algeriensis Boiss. & Reut. from the Algerian Saharan Atlas, BMC Compl. Alternative Med. 19 (1) (2019) 146, https://doi.org/10.1186/s12906-019-2556-y.

[25] H. Ouakouak, A. Benarfa, M. Messaoudi, S. Begaa, B. Sawicka, N. Benchikha, J. Simal-Gandara, Biological properties of essential oils from Thymus algeriensis Boiss, Plants 10 (4) (2021) 786, https://doi.org/10.3390/plants10040786.

[26] G.A. Pankey, L.D. Sabath, Clinical relevance of bacteriostatic versus bactericidal mechanisms of action in the treatment of Gram-positive bacterial infections, Clin. Infect. Dis. 38 (6) (2004) 864–870, https://doi.org/10.1086/381972.

[27] S.J. Son, R. Huang, C.J. Squire, I.K.H. Leung, MCR-1: a promising target for structure-based design of inhibitors to tackle polymyxin resistance, Drug Discov. Today 24 (1) (2019) 206–216, https://doi.org/10.1016/j.drudis.2018.07.004.

[28] K. Jeannot, A. Bolard, P. Plésiat, Resistance to polymyxins in Gram-negative organisms, Int. J. Antimicrob. Agents 49 (5) (2017) 526–535, https://doi.org/10.1016/j.ijantimicag.2016.11.029.

[29] B. Zheng, C. Huang, H. Xu, L. Guo, J. Zhang, X. Wang, X. Jiang, X. Yu, L. Jin, X. Li, Y. Feng, Y. Xiao, L. Li, Occurrence and genomic characterization of ESBL-producing, MCR-1-harboring *Escherichia coli* in farming soil, Front. Microbiol. 8 (2017) 2510, https://doi.org/10.3389/fmicb.2017.02510.

[30] Y. Lv, S. Zheng, A. Goldenzweig, F. Liu, Y. Gao, X. Yang, A. Kandale, R.P. McGeary, S. Williams, B. Kobe, et al., Enhancing the thermal and kinetic stability of ketol-acid reductoisomerase, a central catalyst of a cell-free enzyme cascade for the manufacture of platform chemicals, Appl. Biosci. Appl. Biosci 1 (2) (2022) 163–178, https://doi.org/10.3390/applbiosci1020011.

[31] M. Hu, J. Guo, Q. Cheng, Z. Yang, E.W.C. Chan, S. Chen, Q. Hao, Crystal structure of Escherichia coli originated MCR-1, a phosphoethanolamine transferase for colistin resistance, Sci. Rep. 6 (2016) 38793, https://doi.org/10.1038/srep38793.

[32] R.T. Guo, T.P. Ko, A.P. Chen, C.J. Kuo, A.H. Wang, P.H. Liang, Crystal structures of undecaprenyl pyrophosphate synthase in complex with magnesium, isopentenyl pyrophosphate, and farnesyl thiopyrophosphate: roles of the metal ion and conserved residues in catalysis, J. Biol. Chem. 280 (21) (2005) 20762–20774, https://doi.org/10.1074/jbc.M502121200.

[33] Y.M. Wang, L.C. Kong, J. Liu, H.X. Ma, Synergistic effect of eugenol with Colistin against clinical isolated Colistin-resistant *Escherichia coli* strains, Antimicrob. Resist. Infect. Control 7 (2018) 17, https://doi.org/10.1186/s13756-018-0303-7.

[34] K. Yi, S. Liu, P. Liu, X. Luo, J. Zhao, F. Yan, Y. Pan, J. Liu, Y. Zhai, G. Hu, Synergistic antibacterial activity of tetrandrine combined with colistin against MCR-mediated colistin-resistant Salmonella, Biomed. Pharmacother. 149 (2022) 112873, https://doi.org/10.1016/j.biopha.2022.112873.

[35] S. Xie, L. Li, B. Zhan, X. Shen, X. Deng, W. Tan, T. Fang, Pogostone enhances the antibacterial activity of colistin against MCR-1-positive bacteria by inhibiting the biological function of MCR-1, Molecules 27 (9) (2022) 2819, https://doi.org/10.3390/molecules27092819.

[36] V. Pahal, U. Devi, K.S. Dadhich, Quercetin, a secondary metabolite present in methanolic extract of *Calendula officinalis*, is a potent inhibitor of peptide deformylase, undecaprenyl pyrophosphate synthase and DNA primase enzymes of *Staphylococcus aureus*: an *in vitro* and *in silico* result analysis, MOJ Drug Des Develop Ther 2 (4) (2018) 216–225, https://doi.org/10.15406/mojddt.2018.02.00050.

[37] R. Ferreira de Freitas, M. Schapira, A systematic analysis of atomic protein-ligand interactions in the PDB, Medchemcomm 8 (10) (2017) 1970–1981, https://doi.org/10.1039/c7md00381a.

[38] D.E. Arthur, J.N. Akoji, R. Sahnoun, et al., A theoretical insight in interactions of some chemical compounds as mTOR inhibitors, Bull. Natl. Res. Cent. 45 (2021) 67, https://doi.org/10.1186/s42269-021-00525-x.

[39] A. Farouk, T. Alsulami, H.S. Ali, A.N. Badr, In-vitro and in-silico investigation for the spent-coffee bioactive phenolics as a promising aflatoxins production inhibitor, Toxins 15 (2023) 225, https://doi.org/10.3390/toxins15030225.