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Native and endemic Iranian *Nepeta* spp.: powerful antimicrobial agents

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ABSTRACT

Pathogenic microorganisms are more or less successfully treated by synthetic chemical compounds, whose residues often cause serious health problems. Plant specialized metabolites with antimicrobial properties have for a long time been the focus of both medicine and pharmacology. This study was conducted to evaluate the *in vitro* antimicrobial activity of methanol extracts of selected endemic and native Iranian *Nepeta* species against some of the most important pathogenic bacteria and fungi. The results indicated that *N. kotschyi* leaf extract was the most efficient against the tested bacteria, with *Pseudomonas aeruginosa* being the most sensitive and fungal species were more susceptible to the extracts than bacterial strains. *Nepeta* spp. extracts showed a strong antifungal activity against micromycetes, except for quite resistant *Aspergillus niger*. Antibacterial MIC values (mg.mL^{-1}) ranged from 0.01 (*N. kotschyi*) to 0.20 (*N. crassifolia*), while antifungal MIC values ranged from 0.02 (*N. crassifolia*, *N. kotschyi*, *N. menthoides*, and *N. cataria*) to 0.13 (*N. crassifolia* and *N. menthoides*). When compared to positive controls, in most cases the extracts performed much better. The recorded antimicrobial activity candidates the selected 4 endemic and native Iranian *Nepeta* spp. as prospective and promising antimicrobial agents to be used in both pharmacology and biotechnology.

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Introduction

Some fungi and bacteria cause serious problems for living things and numerous synthetic antimicrobials are in use to control such pathogens. The development of resistance against these chemicals represents a serious problem for their effective use. The application of a higher dosage might be a good way to control resistant strains, but this could be hazardous due to the accumulation of high levels of toxins. Thus, researchers are for a very long time focused on the use of natural antimicrobials that have low utility risk. Many studies have investigated the antimicrobial properties of essential oils and plant extracts against pathogens. Natural antimicrobials have two key benefits: 1) they are organic natural substances characterized by both public health safety and environmental friendliness and 2) pathogens are quite unlikely to develop resistance against them due to a mixture of components with apparently different antimicrobial activity mechanisms (1-2).

The *Nepeta* genus is a member of the family Lamiaceae. Its diversity, species richness, and chemical properties have given rise to extensive research on this genus. Nepetalactones, iridoids and their glucosides, diterpenes, triterpenes, and phenolic compounds (phenolic acids and flavonoids) are the major metabolites detected in various *Nepeta* species (3). There is a plethora of scientific reports on the biological activities of *Nepeta* secondary metabolites, which implies the importance of this genus. Effects on the central nervous system, antibacterial, antifungal, antiviral, analgesic (acute pain), anti-inflammatory, antioxidant, cytotoxic, phytotoxic, anti-atherosclerosis, and nerve relaxation effects are some of the reported biological activities (3). Among them, the antimicrobial activities of extracts are principally well-studied in the following species: *N. cataria* (4-6), *N. curviflora* (7), *N. leavigata*, *N. kurramensis* (8), *N. meyeri* (9), and *N. flavida* (10), and they were all reported to be able to effectively control various microbes.

Iran is one of the main biodiversity hotspots of the genus *Nepeta*. There are 79 species described in Iran (11), about 77% out of them being endemic (12). Populations of four *Nepeta* species, including *N. cataria* L., *N. crassifolia* Boiss. & Buhse, *N. menthoides* Boiss. & Buhse, and *N. kotschyi* Boiss. (*N. kotschyi* var. *kotschyi* and *N. kotschyi* var. *persica*) were established in the field (western Tehran, Iran) to form the main germplasm that would be used for various studies. Assessment of variations in their genotypes and chemotypes in terms of the quantity and quality of specialized metabolites were anticipated to provide directions for the introduction of valuable cultivars for field agriculture. Earlier studies proved that Iranian *Nepeta* species contained suitable genetic diversity and richness in essential oils and targeted phenolic compounds (13-16).

As methanol has been proved to be the most efficient

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solvent for the extraction of phenolics compounds (17-18), we opted for this solvent. The present study was aimed at investigating the antibacterial and antifungal properties of leaf methanol extract of Hadi *et al*. (2016, 2017, 2018, 2020) selected Iranian *Nepeta* species.

Materials and Methods

Chemicals and reagents

Methanol (HPLC grade) was purchased from Appli-Chem (Cheshire, USA). Ultrapure water was generated by deionization (Millipore, Billerica, USA).

Plant material

The plant material contained leaves of 4 *Nepeta* spp. (*N. cataria*, *N. crassifolia*, *N. menthoides*, or *N. kotschyi*) that were harvested and stored as described earlier in Hadi *et al*. report (14). In brief, leaves were harvested at the full flowering stage from the top part of plants in the second year of their establishment in the field. The harvested plant material was air-dried in shade and kept in paper bags until use. Three replications per sample were prepared for extraction. Abbreviations used in the text are as follows: KOT – *N. kotschyi*, CAT – *N. cataria*, CRA – *N. crassifolia*, and MEN – *N. menthoides*.

Preparation of extracts

The plant material was weighed and ground in liquid nitrogen. The extraction was performed with 99.8% methanol (w:v = 1:10) in an ultrasonic bath for 15min. After centrifugation for 20 min at 10,000 *g*, the supernatants were filtered through 0.2 µm cellulose filters (Agilent Technologies, Santa Clara, CA) and stored at 4°C until use.

Assessment of antibacterial activity

Antimicrobial activity of the *Nepeta* spp. extracts was tested against Gram-negative bacteria, including *Escherichia coli* ($ATCC_{35210}$), *Pseudomonas aeruginosa* ($ATCC_{27853}$), and *Salmonella typhimurium* ($ATCC_{13311}$) and Gram-positive bacteria, including *Bacillus cereus* (clinical isolate), *Listeria monocytogenes* ($NCTC_{7973}$), *Micrococcus flavus* ($ATCC_{10240}$), *Enterococcus faecalis* (human isolate), and *Staphylococcus aureus* ($ATCC_{6538}$). The organisms were obtained from the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research "Siniša Stanković", University of Belgrade, Serbia. Abbreviations used in the text are as follows: *B.c – Bacillus cereus, E.c – Escherichia coli, E.f – Enterococcus feacalis, L.m – Listeria monocytogenes, M.f – Micrococcus flavus, P.a – Pseudomonas aeruginosa, S.a – Staphylococcus aureus, S.t – Salmonella typhimurium*.

The antibacterial assay was carried out using the microdilution method (19). The minimum inhibitory and bactericidal concentrations (MICs and MBCs) were determined using 96-well microtitre plates. Bacterial suspensions were adjusted with sterile saline to a concentration of 1.0×10^6 CFU.mL^{-1}. The tested extracts were added (10 mg.mL^{-1}) to the tryptic soy broth (TSB) medium (100 µL) with the bacterial inoculum (1.0×10^5 CFU.well^{-1}) to achieve the desired concentrations. The microplates were incubated in a rotary shaker (160 rpm) for 24 h at 37°C. Both MIC and MBC values for bacteria were detected following the addition of 40 µL of *p*-iodonitrotetrazolium violet (INT) (0.2 mg.mL^{-1}, $Sigma_{I8377}$) and incubation at 37°C for 30 min (20). The antibiotics streptomycin and ampicillin, both in the concentration of 1 mg.mL^{-1}, were used as positive controls.

Assessment of antifungal activity

The fungi tested in this study encompassed *Aspergillus fumigatus* ($ATCC_{1022}$), *A. versicolor* ($ATCC_{11730}$), *A. ochraceus* ($ATCC_{12066}$), *A. niger* ($ATCC_{6275}$), *Trichoderma viride* (IAM_{5061}), *Penicillium funiculosum* ($ATCC_{36839}$), *P. ochrochloron* ($ATCC_{9112}$), and *P. aurantiogriseum* that were obtained from the same source as the bacteria strains. Abbreviations used in the text are as follows: *A.f – Aspergillus fumigatus, A.n – Aspergillus niger, A.o – Aspergillus ochraceus, A.v – Aspergillus versicolor, P.au – Penicillium aurantiogriseum, P.f – Penicillium funiculosum, P.o – Penicillium ochrochloron, T.v – Trichoderma viride.*

Micromycetes were maintained on malt agar at 4°C and sub-cultured once a month (21). The antifungal assay was carried out using a modified microdilution technique (19). Briefly, fungal spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% Tween 80 (*v*/*v*). The spore suspension was adjusted with the sterile saline to a concentration of approximately 1.0×10^5 in a final volume of 100 µL.well^{-1}. The inocula were stored at 4°C for further use. Dilutions of the inocula were cultured on the solid malt agar to verify the absence of contamination and to check their validity. Minimum inhibitory concentration (MIC) determinations were performed by a serial dilution technique using 96-well microtiter plates. The tested extracts were diluted in 5% of DMSO (10 mg.mL^{-1}) and added to broth malt medium (MA) that contained the inoculum. The microplates were incubated in a rotary shaker (160 rpm) for 72 h at 28°C. The lowest concentrations without visible growth (at the binocular microscope) were defined as minimal inhibitory concentrations (MICs). The fungicidal concentrations (MFCs) were determined by a serial subcultivation of 10 µL tested extracts dissolved in the medium and inoculated for 72 h, into microtiter plates containing 100 µL broth. well^{-1} and further incubation for 72 h at 28°C. The lowest concentration with no visible growth was defined as MFC, indicating 99.5% killing the original inoculum. The fungicides bifonazole and ketoconazole were used as positive controls.

Results

The results of antimicrobial activity obtained using the microdilution method are summarized in Tables 1 and 2 (See also Supplementary Figures 1 and 2). All 4 extracts of *Nepeta* spp. exhibited considerable antimicrobial activity against the tested microbial strains.

Bacterial MIC values (mg.mL^{-1}) ranged from 0.01_{KOT} to 0.20_{CRA} ($0.05\text{-}0.50_{positive\ controls}$). MBC values (mg.mL^{-1}) ranged between 0.02_{KOT} and $0.25_{CRA,\ KOT,\ MEN,\ and\ CAT}$ ($0.10\text{-}1.00_{positive\ controls}$) (Table 1).

As presented in Table 1, all 4 species showed better activities against *S.a* and *E.c* than positive controls (rank1: KOT, rank2: CRA, and rank3: CAT=MEN). Both KOT and CRA performed better against *B.c* than controls (rank1: KOT and rank2: CRA), while MEN acted better than ampicillin against *B.c* but slightly weaker than streptomycin. In terms of the effect against *M.f*, all 4 species showed better activity than controls (rank1: CRA=KOT

and rank2: MEN≈CAT). All 4 species evenly affected the bacterium *L.m* and, with a small difference, performed better than controls. CRA, KOT, and MEN performed better against *P.a* than controls, among which KOT was the most efficient. All 4 species showed weaker effects against *E.f* than controls. In the case of the bacterium *S.t*, KOT and CRA performed better than controls, with KOT being the most efficient species.

Fungal MIC values (mg.mL^{-1}) ranged from $0.02_{\text{CRA, KOT, MEN, and CAT}}$ to $0.13_{\text{CRA and MEN}}$ ($0.10\text{-}2.50_{\text{controls}}$). MFC values (mg.mL^{-1}) ranged from $0.03_{\text{KOT, MEN, and CAT}}$ to 0.50_{CRA} ($0.20\text{-}3.50_{\text{controls}}$) (Table 2).

As can be seen in Table 2, the plant extracts efficiently diminished fungal growth, among which KOT performed the best. *A.n* was the most resistant fungal species to the *Nepeta* spp. extracts and the KOT extract was again the most efficient.

The microbes were affected differently, depending on the type and plant extract concentration. As can be seen from Tables 1 and 2, the most sensitive bacteria to the plant extracts in terms of MIC were *S.a*, *B.c*, *P.a*, *S.t*, and *M.f*. Among them, *S.a*, *B.c*, *P.a*, and *S.t* were the most sensitive to the KOT extract in the concentration of 0.01 mg.mL^{-1}. Moreover, *M.f* showed the highest sensitivity to the CAT extract in the concentration of 0.06 mg.mL^{-1}. In terms of MBC, the most sensitive bacteria to the plant extracts were *S.a*, *B.c*, *P.a*, *S.t*, *M.f*, and *E.c*. Among them, *S.a*, *B.c*, *P.a*, and *S.t* showed the highest sensitivity to the KOT extract in the concentration of 0.02 mg.mL^{-1}, while *E.c* and *M.f* showed the highest sensitivity to the MEN and CAT extracts in the concentration of 0.13 mg.mL^{-1}. In terms of MIC, the most sensitive fungal species were *A.v* and *T.v*. Although both of them were generally inhibited by the plant extract concentration of 0.02 mg.mL^{-1}, *A.v* preferably reacted on the CRA extract and *T.v* reacted on the remaining species. In terms of MFC, the most sensitive fungi were *P.au* and *T.v*. The successful killing of *P.au* was achieved by the CRA extract in the concentration of 0.06 mg.mL^{-1}, while *T.v* was killed with other extracts in the concentration of 0.03 mg.mL^{-1}.

Discussion

The results of the present study revealed that the extracts of all *Nepeta* species had a powerful and to some extent similar inhibitory effect on bacteria growth, but the KOT extract appeared to be the most efficient. In comparison with bacteria, fungi were even more sensitive and their growth was inhibited by a lower concentration of the extracts. The investigated *Nepeta* spp. extracts exhibited strong antifungal activity against micromycetes than synthetic positive controls (ketoconazole and bifonazole) except in the case of *A. niger* which was considerably more resistant. Similar inhibitory effects of many *Nepeta* species on various pathogenic microorganisms were reported earlier. It was revealed that alcohol extracts, especially

Table 1. Antibacterial activity of *Nepeta* spp. (mg.mL^{-1})*.

	N. crassifolia		*N. kotschyi*		*N. menthoides*		*N. cataria*		Streptomycin		Ampicillin	
Bacteria	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Staphylococcus aureus	0.03**	0.06	0.01	0.02	0.06	0.13	0.06	0.13	0.25	0.50	0.10	0.15
Bacillus cereus	0.03	0.06	0.01	0.02	0.06	0.13	0.13	0.25	0.05	0.10	0.10	0.15
Micrococcus flavus	0.06	0.13	0.06	0.13	0.09	0.13	0.06	0.13	0.13	0.25	0.10	0.15
Listeria monocytogenes	0.13	0.25	0.13	0.25	0.13	0.25	0.13	0.25	0.15	0.30	0.15	0.30
Pseudomonas aeruginosa	0.03	0.06	0.01	0.02	0.03	0.13	0.06	0.13	0.05	0.10	0.10	0.20
Escherichia coli	0.06	0.13	0.03	0.06	0.09	0.13	0.09	0.13	0.50	1.00	0.30	0.50
Enterococcus feacalis	0.20	0.25	0.13	0.25	0.13	0.25	0.13	0.25	0.05	0.10	0.15	0.20
Salmonella typhimurium	0.03	0.06	0.01	0.02	0.13	0.25	0.06	0.13	0.05	0.10	0.15	0.20

*See also Supplementary Fig. 1.

**Each number is mean of three replications.

Table 2. Antifungal activity of *Nepeta* spp. (mg.mL^{-1})*.

	N. crassifolia		*N. kotschyi*		*N. menthoides*		*N. cataria*		Bifonazole		Ketoconazole	
Fungi	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**
Aspergillus versicolor	0.02**	0.13	0.03	0.06	0.03	0.06	0.06	0.13	0.10	0.20	0.20	0.50
A. ochraceus	0.06	0.13	0.03	0.13	0.03	0.06	0.03	0.06	0.15	0.20	0.15	0.20
Trichoderma viride	0.03	0.13	0.02	0.03	0.02	0.03	0.02	0.03	0.10	0.20	0.20	0.30
Penicillium funiculosum	0.03	0.13	0.03	0.13	0.03	0.06	0.03	0.13	0.20	0.25	2.50	3.50
P. ochrochloron	0.06	0.13	0.03	0.13	0.03	0.06	0.03	0.06	0.20	0.25	0.20	0.50
P. aurantiogriseum	0.03	0.06	0.03	0.06	0.03	0.06	0.03	0.06	0.15	0.20	1.00	1.00
A. fumigatus	0.06	0.13	0.03	0.13	0.06	0.13	0.06	0.13	0.15	0.20	0.20	0.50
A. niger	0.13	0.50	0.09	0.13	0.13	0.25	0.09	0.25	0.15	0.20	0.20	0.50

*See also Supplementary Fig. 2.

**Each number is mean of three replications.

methanol extract, which showed the highest extraction rate of phenolics, had strong inhibitory effects on the studied pathogens (8, 22-25).

Considering the antimicrobial activity of *Nepeta* extracts, methanol extract of *N. juncea* leaves was reported to have the strongest effects against *Staphylococcus aureus* and *Bacillus cereus* with the MIC values being in the range of 0.025-0.1 $mg.mL^{-1}$ (25). It was shown that the 50% methanol extract of aerial parts of *N. menthoides* displayed a significant inhibitory effect against *S. aureus*, *B. cereus*, *Pseudomonas aeruginosa*, and *Escherichia coli*, among which the former was the most sensitive to the extract and was affected harder than by standard antibiotics (24). Methanol extract of *N. cataria* leaves had antimicrobial activity against *S. aureus* and *Salmonella typhii* (23). The MIC value of *N. ispahanica* methanol extract against *S. aureus* and *E. coli* was reported 1.9 $mg.mL^{-1}$ and 15.2 $mg.mL^{-1}$, respectively (22).

Phenolic compounds (phenolic acids and flavonoids) are the main group of specialized metabolites in *Nepeta* species (3, 14, 26) and plant biological activities are usually attributed to the presence of these compounds (27). Among numerous studies on biological activities of phenolics, many of them highlight antimicrobial activities (28-34). The mechanisms of action may be assigned into 2 groups: the 1st mechanism includes a modification of cell membrane permeability, the formation of cytoplasmic granules, and the rupture of cytoplasmatic membrane, while the 2nd mechanism is realized through the disturbance of intracellular functions by the formation of hydrogen bonding among phenolics and enzymes (34). Also, to cope with a fungal infection, plants exert toxic compounds, increase reactive oxygen species (ROS) levels, induce apoptosis acting on farnesol, a quorum-sensing molecule that plays an important role in raising the level of ROS and inhibiting hyphal development by targeting the *TUP1* gene, inhibit the morphogenetic switch by impeding biofilm formation due to upregulation of *DPP3*, a gene performing an important role in the farnesol synthesis, disrupt both Ca^{2+} and H^{+} homeostasis in yeast that likely leads to loss of cell viability, and inhibit the synthesis of ergosterol (a component of fungal cell membrane), glucosamine (a growth indicator present only in cells of some fungal genera), and some proteins (28).

The main phenolic compounds found in species belonging to the Lamiaceae family are caffeic acid derivatives (especially rosmarinic acid) and flavonoids, including flavones, flavanones, and their glycosides (35-37). Based on the results of the present study, KOT methanol extract was the most potent agent against the tested bacteria and fungi. According to our previous researches on the *Nepeta* spp. that used UHPLC/-HESI-MS/MS analyses (14), it was shown that methanol extracts of KOT were significantly the richest in targeted phenolics, followed by MEN, CRA, and CAT, respectively.

In addition to the analytics studies, there are numerous reports on antimicrobial activities of specific phenolic compounds. For instance, the flavonoids apigenin and luteolin have been found effective against methicillin-resistant *S. aureus* (38). The flavonoids baicalein, baicalin, and wogonin were reported as growth inhibitors of several bacteria, fungi, and viruses (39). Moreover, many studies showed the antiviral and antibacterial properties of rosmarinic and caffeic acids (29, 40). Rosmarinic acid (an ester of caffeic acid) is one of the most abundant phenolic acids across plant species, particularly in the family Lamiaceae (17, 29). In another study, the presence of nepetoidins A and B, two caffeic acid esters with antifungal activity, was suggested as a chemical trait to distinguish Nepetoideae from other subfamilies within the Lamiaceae family (41).

Based on previous studies on the same plant material as in this study (14), apigenin was the main flavonoid in KOT, MEN, and CRA methanol extracts, while luteolin was the most abundant in CAT. Rosmarinic acid was also reported as the main phenolic acid in CAT, MEN, and CRA, while chlorogenic acids1&2 and an unidentified caffeic acid derivative were reported as the main phenolic acids in KOT. In this research, it was shown that KOT and MEN had unique phenolic profiles and contained the highest levels of phenolic acids and flavonoids, respectively. CAT and MEN had the highest amount of caffeic acid and rosmarinic acid, respectively, but in terms of the amounts of chlorogenic acids1&2 and unidentified caffeic acid derivative, KOT ranked first. MEN was reported to have the highest rutin, luteolin, and apigenin contents, while in terms of the amount of naringenin, CRA and MEN ranked first and second, respectively. The flavonoid quercetin was found only in KOT and CAT populations (14). These phenolic profiles can aid in comprehending the outstanding antimicrobial properties observed for the 4 *Nepeta* species.

Both antibacterial and antifungal activities of plants that contained the mentioned compounds as commercial fungicides and bacteriocides have been previously reported in numerous studies (rutin: 42, luteolin: 34, 42-43, naringenin: 44-45, quercetin: 34, 42, 46-47, apigenin: 34, 48-54, chlorogenic acid: 55-57, rosmarinic acid: 58-59, and caffeic acid: 60-61). In these studies, the antimicrobial effect of each compound ranged from low to high values. It was reported that apigenin inhibited the activity of DNA gyrase and hydroxy acyl-acyl carrier protein dehydratase (34). Apigenin protects adeno-carcinomic human alveolar basal epithelial cells to form α-hemolysin, a pore-forming cytotoxin that is secreted by most *S. aureus* strains, essential for pneumonia pathogenesis (50). When apigenin was applied with LysGH15, a lysin derived from the phage GH15 having high efficiency and a broad lytic spectrum against MRSA, synergism was observed using a mouse *S. aureus* pneumonia model (62). It was also mentioned a reverse antibiotics activity of apigenin against quinolone-resistant *S. aureus* (52). Reverse antibiotics are chemicals ineffective against antibiotic-susceptible bacteria but active against relevant antibiotic-resistant bacteria (49). Apigenin was also found to reverse bacterial resistance to cephalosporin ceftazidime in *Enterobacter cloacae*. The 5,7-OH group of the A ring and one 4'-OH group of the B ring in apigenin were found important in reversing antimicrobial resistance. These significantly enhanced activities of ceftazidime by apigenin may have been the result of the inhibition of peptidoglycan synthesis and certain β-lactamase enzymes as well as alteration of the outer membrane and cytoplasmic membrane permeabilization (51). Also, it has been previously indicated that the main targets of apigenin on bacteria might be nucleic acid-processing enzymes and cell walls/membranes (48, 53). A paper reported that apigenin was able to induce cell shrinkage of the fungus *Candida albicans*, altering the cell membrane potential and causing leakage of intracellular

components (54). Luteolin may also affect bacteria by destroying the cell membrane integrity and well-defined variations in cell morphology. Moreover, luteolin was reported to present robust inhibitory effects on biofilm formation, enhance antibiotics' diffusion within biofilms, and efficiently kill both mono- and dual-species biofilm cells (43). Researchers demonstrated that the naringenin's mechanism of action was dependent on the inhibition of PBP-2a, a penicillin-binding protein. They reported that the combination of different drugs with naringenin suppressed PBP-2a and also found that the combination of naringenin and *β*-lactam antibiotics intensified the antibacterial properties of the latter (45). Quercetin was found to disturb bacterial membrane potential and increase its permeability (34, 46). Regarding the mode of action of chlorogenic acid (CA), It was reported that its binding to the outer membrane disrupted the membrane, exhausted the intracellular potential, and released cytoplasm macromolecules, which leads to cell death (55). It was also reported that CA induced a significant decrease in the intracellular ATP concentration, possibly by affecting either metabolism or cell-signaling transduction. Furthermore, metabolomic results indicated that the CA stress had a bacteriostatic effect by inducing an intracellular metabolic imbalance of the tricarboxylic acid cycle and glycolysis, leading to a metabolic disorder and death of *B. subtilis* (57). It was demonstrated that CA and related compounds had not only bacteriostatic effects but also bactericidal effects (56). Rosmarinic acid strongly affected bacteria by an inhibitory effect on the microbial surface protein components recognizing adhesive matrix molecules (MSCRAMM's) (59). Many additional literature records exist on a wide area of bioactivities of the genus *Nepeta*, witnessing its high potential for use in both pharmacology and medicine.

In addition, based on previous studies on the same plant material as in this study (13, 15), three different nepetalactone isomers mostly 4a*α*,7*α*,7a*α*- and 4a*α*,7*α*,7a*β*-nepetalactones were identified in the essential oil of *Nepeta* species. The highest amount of nepetalactone was obtained in *N. cataria* and *N. kotschyi*. Based on some researches nepetalactone was reported as an important and effective antibacterial component of *Nepeta* essential oil (63-64). Iridoid compounds including non-volatile nepetalactones have also been reported in methanol extract of *Nepeta* species (25, 65-66). So, the antimicrobial effect of the species methanol extracts observed in the present study could be attributed to the presence of nepetalactones in the extracts.

Controlling human, animal, and plant pathogens as well as plant and animal products pathogens that threaten their health and integrity has always been of great human interest. The use of chemicals to control these pathogens has gradually caused their resistance to synthetic antimicrobial agents and has increased the risk of accumulation of residual toxicity in food commodities inducing incurable diseases. Researchers are constantly looking for compounds that could be substituted or combined with these chemicals, which are both less harmful to the environment and do not cause pathogens' resistance. In this way, several commercial fungicides and bactericides have been introduced to stores.

By the achieved results, the present study implies an extraordinary potency of methanol extract of the studied Iranian *Nepeta* species to inhibit the growth and kill the bacteria and fungi tested, and due to a lack of toxicity for human and their surrounding environment, they have the potential to be as commercial fungicides and bactericides. The antimicrobial properties observed in this study can be mainly attributed to the presence of phenolic compounds. Supported by the results of this and previous studies, these 4 selected plant species could be recommended as valuable plant resources for more sophisticated studies targeted toward assessing their bioactivities as well as for the preparation of herbal antimicrobials. However, further research should be conducted to optimise the use method of the extracts and essential oils *in vivo* and to preserve their positive antifungal and antibacterial activities in natural usage.

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Interest conflict

There is no conflict of interest between each of the contributing factors in the production of this article (sponsors, scholars, and writers).

Author contributions

We, the authors (names and orders of appearance are as the below), by awareness of the non-changeability of the names, orders of appearance and information of authors (no authors can be added or removed at all) declare that we all have contributed in producing this article (doing the researches or writing the article) and no names have been added without having an effective role to the article.

Najmeh Hadi: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing—original draft preparation, Writing—review and editing - 35%; Miloš Nikolić: Conceptualization, Methodology, Validation, Formal analysis, Resources, Data curation - 15%; Fatemeh Sefidkon: Resources, Supervision - 11.25%; Abdolali Shojaeiyan; Resources, Supervision - 11.25%; Branislav Šiler: Writing—review and editing, Supervision - 11.25%; Danijela Mišić: Validation, Writing—review and editing, Supervision - 11.25%; Mahdi Yahyazadeh: Writing—original draft preparation and editing - 5%.

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