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Biological activity and chemical composition of the essential oil of *Nepeta cataria* L.

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ABSTRACT: In this study, chemical composition, antioxidant and antimicrobial activity of the essential oil of *Nepeta cataria* L., a native plant that grows in Lorestan region of Iran, were studied. Chemical constituents of the essential oil were identified by means of gas chromatography/mass spectrometry (GC/MS) and the relative content of each constituent was determined by area normalization. Disc diffusion and broth microdilution assays were used for in vitro antimicrobial screening. Antioxidant activity was employed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radical scavenging. The predominant bioactive ingredients with high percentage in *N. Cataria* essential oil was 4a-α, 7-α, 7a-β-Nepetalactone (53.87%). The essential oil's IC_{50} value of 80.62 µg / mL in the DPPH assay, could be regarded as its strong antioxidant potential. The antibacterial activities of the essential oil were screened and compared against dozen important human pathogens. The essential oil showed good antibacterial activity, especially on *Salmonella spp.*. The biggest diameters of inhibition zones (29 mm) was recorded for *Salmonella typhi* and *Salmonella paratyphi A* showed the lowest MIC (160 µg / mL).

KEYWORDS: Antimicrobial; antioxidant, *Nepeta cataria* L.; essential oil.

1. INTRODUCTION

The genus *Nepeta* with the common Persian name "puneh" belongs to the Lamiaceae family and of about 280 species that widespread in Europe, Asia and Africa. Sixty seven species of this genus are grown endemically in Iran [1]. *Nepeta cataria* (Catnip), a tropical aromatic plant belonging to this family, is native to Asia and Southeast Europe. Its leaves resemble mint in appearance and the flowers are white and finely spotted with purple with a strong odor [2]. In Iran and some other countries, fresh or dried leaves and flowers of *N. cataria* are used in making sauce, soup and cheese. These plants have been used in folk medicine as disinfectant and cure against colds [3]. The medicinal properties of Nepeta species are usually attributed to their essential oils and flavonoids [4]. Recently several researchers have reported chemical composition of the essential oil of *Nepeta cataria* from different parts of the world. The composition varies from region to region, variety, analytical and extraction methods, climatic conditions and vegetation period. The main constituents so far identified, include caryophyllene, caryophyllene oxide, 1,8-cineol, citronellol, geraniol, elemol, nerol, nerolidol, spathulenol, elemene, geranyl acetate, citronellyl acetate and geranial [5, 6]. Bacterial infections as well as microorganisms' resistance to antibiotics are well documented in both plants and humans. In the face of emerging health consequences or side effects of the use of synthetic chemicals in the fight of diseases, a safer alternative could be in the use of natural products. Indeed, one of the most important sources of antioxidants are natural bioactive plant products such as phenolics and specifically triterpenoid compounds that suppress the production of free radicals, delay oxidative stress reactions, and have important role against one of the world major human diseases such as cancer. In the search for such natural products and modern drugs, medicinal plants play a unique role. Medicinal plants are sources of > 50% of all drugs and contribute ~70% to the small molecule anti-infective drugs [7]. In this study, we attempt to determine the composition of the essential oil of *Nepeta cataria* native Lorestan, Iran, anti-bacterial and anti-oxidant effects of this essential oil will be evaluated. In studies of certain chemical compounds and medicinal properties of the plant genus

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Nepeta mentioned. Chemical and antimicrobial essential oil of *Nepeta crispa* were studied and oils derived from plant leaves by GC and GC / MS were analyzed and twenty-three combination of essential oil content was detected 99.8 percent. The main components of the oils consisted of 8 and 1 Cineol to the 47.9 percent isomer of Nepetalactone the 4aα, 7a, 7aβ-nepetalactone by 20.3 percent. The antimicrobial activity of essential oil of *N. crispa* was tested against seven gram-negative or gram-positive bacteria and four fungi. The results showed the interesting antimicrobial activity, in which the gram-positive bacteria, *Bacillus subtilis* and *Staphylococcus aureus*, were the most sensitive to the oil. Also, the oil exhibited a remarkable antifungal activity against all the tested fungi [8]. *Nepeta meyeri* showed very high reducing power. In DPPH radical and NO* scavenging assays the IC50 value of extract was 672.2 microg/mL and 165.32 microg/mL, respectively. Furthermore, The extract revealed antibacterial activity against all gram positive bacteria but not was active against gram negative bacteria [9].

The aim of this study is to investigate the chemical composition of essential oils from *N. cataria* by gas-chromatography coupled with mass spectrometry (GC/MS) method, and to evaluate their antioxidant capacity (DPPH radical scavenging effect) as well as antibacterial activity, against twelve bacterial species, using microdilution method in 96-well microplates.

2. RESULTS

2.1. Chemical Composition of the Essential Oils

Hydrodistillation of the aerial parts of *N. cataria* yielded 0.16% (w/w) yellowish fragrant oil distillates. The chemical composition of the oils can be seen in Table 1. The components were listed in order from their elution on the HP-5MS column. From the leaf essential oil of *N. cataria*, forty components were identified, representing 99.94% of the total oil. The 4a-α, 7-α, 7a-β-Nepetalactone (53.87%) was the major oil constituents of *N. cataria*.

2.2. Antimicrobial activity

The antimicrobial activity of *N. cataria* essential oils was examined by agar disk diffusion and broth microdilution susceptibility assays against a panel of twelve bacteria strains selected for their relevance as food contaminants. Their potency was qualitatively and quantitatively determine from the diameters of the inhibition zones (DDs), minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs). The results are shown in Table 2.

The results from the antimicrobial activity tested by the broth microdilution method are summarized in Table 2. *N. cataria* essential oils exhibited antimicrobial activity against all of the tested strains. The inhibition values ranged from 160.0 to 460.0 µg/mL for MIC and 160.0 to 1280.0 µg/mL for MBC in bacteria.

2.3. Antioxidant activity

In this study, the free radical scavenging activity of the essential oils was studied *in vitro.* As shown in Table 3, the essential oils had the highest ability to reduce the stable radical DPPH to yellow-colored diphenylpicrylhydrazine with an IC_{50} of 80.62 µg/mL. BHT and ascorbic acid were two positive controls that exhibited antioxidant activity, with IC_{50} values of 28.7 µg/mL and 12.8 µg/mL, respectively.

3. DISCUSSION

3.1. Chemical Composition of the Essential Oils

Different studies have been conducted in other regions on the chemical composition of essential oils from different samples of *N. cataria* [5, 6]. Although the comparison between compounds obtained from this study and other reports show several similarities, there are considerable quantitative and qualitative differences between the samples from different locations in Iran. These differences might derive from climatic, seasonal and geographic conditions; harvest periods; and distillation techniques [10].

Table 1. Chemical composition of the essential oils from the aerial parts of *N. cataria*.

RRI-Relative retention indices	Compound name	%	RRI-Relative retention indices	Compound name	%
4.422	2-Hexenal	0.05	13.43	Myrtenol	1.39
5.817	Tricyclene	0.18	14.076	TRANS-CARVEOL	0.59
6.103	α-Pinene	3.32	14.15	Isobornyl acetate	1.70
6.463	Camphene	1.60	14.67	Carvone	1.25
6.6	verbenene	0.19	14.83	Linalyl acetate	0.48
7.074	Sabinene	0.82	14.956	PIPERITON	0.64
7.16	β-pinene	1.86	15.699	Bornyl acetate	0.48
7.406	1-Octen-3-ol	0.20	15.767	α-TERPINOLENE	0.70
7.503	β-Myrcene	1.02	16.407	Thymol	0.11
7.886	l-Phellandrene	0.29	16.86	4a-α,7-α,7a-β-Nepetalactone	53.87
8.017	3-Carene	1.36	17.091	4a-α,7-β,7a-α-Nepetalactone	8.67
8.212	α-Terpinene	0.36	18.528	β-elemene	0.02
8.772	1,8-Cineole	1.86	18.956	α-Gurjunene	0.18
9.36	ɤ-Terpinene	0.75	19.248	trans-Caryophyllene	0.43
10.166	Terpinolen	0.38	19.928	δ-Cadinene	0.10
10.641	Linalool	1.13	20.808	Germacrene D	0.08
11.029	β-THUJONE	0.24	20.911	β- IONONE	0.03
12.881	4-Terpineol	2.73	21.601	alpha.-Amorphene	1.50
13.15	Crypton	4.31	23.357	Caryophyllene oxide	2.51
13.31	isolimonene	2.53	24.46	Aromadendrene	0.09

Table 2. Antimicrobial activity of *N. cataria* essential oils.

Microorganisms	Essential oil			Gentamycin			Vancomycin		
	DD	MIC	MBC	DD	MIC	MBC	DD	MIC	MBC
S. aureus ATCC 12600	17	320	1280				30	16	128
S. epidermidis PTCC 1435	20	160	640				30	16	2
S. agalactiae PTCC 1768	25	160	1280				35	2	64
S. mutans PTCC 1683	13	160	320				29	4	128
E. faecalis ATCC 29219	18	320	1280				23	32	32
L. monocytogenes ATCC 13932	18	160	640				30	160	640
E. coli ATCC 11775	25	640	1280	25	8	64			
S. typhi PTCC 1609	29	160	320	34	5	5			
S. paratyphi A PTCC 1230	27	160	160	32	1	16			
S. enterica PTCC 1709	16	320	640	30	2	2			
P. aeruginosa ATCC 27853	15	320	1280	31	5	5			
K. pneumoniae ATCC 700603	17	320	320	23	8	128			

DD: diameter of inhibition zone (mm) including disk diameter of 6 mm. MIC, MBC: values given as µg/mL (for the essential oils and antibiotics).

Table 3. Antioxidant activity of the essential oils from *N. cataria* and positive controls (BHT and ascorbic acid) in the DPPH assay.

Sample	IC_{50} (µg/mL)
Essential oil	80.62
BHT	28.7
Ascorbic acid	12.8

3.2. Antimicrobial activity

A broad variation in the antibacterial properties of the essential oils against various bacteria was observed. The essential oils showed strong antibacterial activity (inhibition zone > 20 mm), moderate activity (inhibition zone < 12–20 mm) and no inhibition (zone < 12 mm) [11]. According to the width of the inhibition zone diameter, essential oils had the highest antibacterial activity against some *Gram*- negative organisms including *S. typhi*, *S. paratyphi and E. coli* and a few Gram- positive organisms, including *S. agalactiae and S. epidermidis* ($p \leq 0.01$). The lowest antibacterial activity was observed against *S. mutans*, with a 13 mm zone of inhibition ($p \leq 0.01$).

From Table 2 it can be observed that the oils in this study are potential antimicrobial agents because the Gram-positive organisms corresponding to each MIC were low, ranging from 160 to 320 µg/mL. Although the essential oils presented a higher MIC for *E. coli* at 640 µg/mL, the analyzed Gram-negative bacteria showed strong activity for the essential oils.

The antimicrobial activity of the essential oils could be due, to a considerable degree, to the existence of mostly active compounds, such as 4a-α,7-α,7a-β-Nepetalactone and 4a-α,7-β,7a-α-Nepetalactone [12-14]. In addition, the components found in lower amounts, such as crypton, α-Pinene, isolimonene and caryophyllene oxide, could also contribute to the antimicrobial activity of the oils [13, 15]. In fact, the synergistic effects of the diversity between the major and minor constituents present in the essential oils should be taken into consideration to account for their biological activity.

Overall the *N. cataria* essential oils showed stronger antimicrobial effects against Gram-positive bacteria compared with Gram-negative bacteria. In general, the antimicrobial activity of the essential oils tested was more pronounced against Gram-positive compared with Gram-negative bacteria, a general observation derived from studies with essential oils from many other plant species [11, 16]. This generally higher resistance among Gram-negative bacteria could be ascribed to the presence of their outer phospholipidic membrane, which is almost impermeable to lipophilic compounds [17]. The absence of this barrier in Gram-positive bacteria allows for the direct contact of the hydrophobic constituents of the essential oils with the phospholipid bilayer of the cell membrane, where they take effect, causing either an increase of ion permeability and leakage of vital intracellular constituents or an impairment of bacterial enzyme systems [18].

Although tests on food are necessary, the present study indicates that *N. cataria* essential oils can be considered to be an alternative to traditional food preservatives, eliminating or reducing the growth of important food borne pathogens and spoilage bacteria and contributing to enhance food safety and shelf life.

3.3. Antioxidant activity

The DPPH free radical is stable, which has been widely accepted as a tool for estimating the free radical-scavenging activity of antioxidants [19]. In the DPPH test, the antioxidants were able to reduce the stable DPPH radical to the yellow-colored diphenylpricryhydrazine. The effects of antioxidants on DPPH radical scavenging were related to their hydrogen-donating ability [20]. The high antioxidant activity of *N. cataria* essential oils can be attributed to the richness of the oil in nepetalactones (62.5%). Previous studies also revealed that essential oils that have a high percentage of nepetalactones [14]. possess antioxidant activities, which was confirmed by our results. The current results further show that the antioxidant activity of essential oils can be attributed to the synergistic activities of multiform unsaturated compounds. Results should be clear and concise. Text, tables and figures must show minimal overlap, and must be internally consistent. Tables and figures should be designed to maximize the presentation and comprehension of the experimental data. Attention should be paid to the matter of significant figures (usually, no more than three). The same data should not be presented in more than one figure or in both a figure and a table. As a rule, interpretation of the results should be reserved for the discussion section of a Research Article, but under some circumstances it may be desirable to combine results and discussion in a single section.

4. CONCLUSION

The essential oil of *N. cataria* showed strong antimicrobial activity against all 12 microorganisms tested. Current consumers have greater concerns regarding high quality, natural and safe food products to maintain a healthy lifestyle [21, 22]. This study showed very positive results that indicated the good potential of *N. cataria* essential oils for applications in food products; however, further experiments, including elucidation of the mechanism of action and *in vivo* tests are also important to support the benefits and safety of *N. cataria* essential oils.

5. MATERIALS AND METHODS

5.1. Plant material

Flowering aerial parts of wild growing *N. cataria* were collected in July 2014 from Garin Mountain in the Lorestan province, west of Iran. The altitude was *ca.* 2700 m above sea level. The plant material was identified by Dr. Mehrnia, and a voucher specimen numbered LUR 7203 was deposited in the herbarium of the Lorestan Agricultural Research and Natural Resources Center, Khorramabad, Iran.

5.2. Isolation and analysis of the essential oil

A portion (500 g) of the dried and finally ground aerial parts of *N. cataria* was submitted, for 3 h, to water distillation using a Clevenger-type apparatus (British type). The mean yield of the extraction was 0.6% w /w based on the dry weight of the sample. The yellow oil of the plant was dried over dry sodium sulfate and, after filtration, was stored at +4 ° C until tested and analyzed.

The essential oil was analyzed by using gas chromatography with flame ionization detection (GC–FID) and gas chromatography–mass spectrometry (GC–MS). The GC or GC–MS conditions were optimized based on the property of the essential oil extract. GC–FID analysis was performed on a HP 6890 gas chromatograph equipped with a FID detector and an HP-5 fused silica capillary column (30 m×0.25 mm, film thickness 0.25 µm).

Helium (purity 99.999%) was used as the carrier gas, with a flow rate of 0.9 mL/min. The column oven temperature was programmed from 50 ◦C (hold 1 min) to 240 ◦C (hold 10 min) at a 5 ◦C/min rate. The injector and FID detector temperatures were 230 and 300 ◦C, respectively. The injector was operated in the splitless mode and programmed to return to the split mode after 1 min from the beginning of the run. Diluted samples (1/10 in ether, v/v) of 0.3 µL were injected manually, and the split ratio was adjusted to 50:1.

GC–MS analysis was carried out in an Agilent 6890 gas chromatograph interfaced with an Agilent 5973 MSD using helium as the carrier gas (0.9 mL/min, and the same capillary column previously mentioned. The column temperature was programmed from 50 (hold 3 min) to 180 ◦C at 5 ◦C/min and to 240 ◦C at 10 ◦C/min and then remained stable for an additional 20 min at 280 ◦C. The injector and detector temperatures were at 200 and 280 ◦C, respectively. A 0.3-µl sample was injected using the split mode (split ratio 50:1).

Components of the oil were analyzed and identified by comparing the MS fragmentation pattern with pure compounds, corresponding data in the literature [23] and/or computer mass spectra libraries (Wiley 138K and NIST 98). A standard solution of *n*-alkanes (C6–C26) was used to obtain the retention indices [24]. Quantitative data were obtained from the electronic integration of the FID peak areas without using any correction factors.

5.3. Antimicrobial activity

5.3.1. Microbial strains

The antibacterial activity of the essential oils from *N. cataria* was evaluated against Gram-positive and Gram-negative bacteria species (provided by the National Cell Bank of Iran, Pasteur Institute, Tehran, Iran). Gram-positive bacteria included *Staphylococcus aureus* (ATCC 12600), *Staphylococcus epidermidis* (PTCC 1435), *Streptococcus agalactiae* (PTCC 1768), *Streptococcus mutans* (PTCC 1683), *Enterococcus faecalis* (ATCC 29219) and *Listeria monocytogenes* (ATCC 13932), and the group of Gram-negative bacteria included *Escherichia coli* (ATCC 11775), *Salmonella typhi* (PTCC 1609), *Salmonella paratyphi A (PTCC 1230), Salmonella enterica (PTCC 1709), Pseudomonas aeruginosa* (ATCC 27853) and *Klebsiella pneumoniae* (ATCC 700603), which were obtained from Persian Type Culture Collection (PTCC), Iran and America Type Culture Collection (ATCC).

5.3.2. Disk diffusion assay

The protocols used in this study were based on the guidelines of CLSI, formerly known as NCCLS (CLSI, 2006) with slight modifications. Briefly, each sterile Petri dish with a diameter of 9 cm was prepared with 20 mL of Mueller–Hinton medium. After solidifying, 100 µL of a bacterial suspension (10^8 CFU/mL) was spread on the plates. After 5 min, a sterile filter paper disc (6 mm) containing 20 µL of *N. cataria* essential oil was placed on the surface of the plate. To accelerate diffusion of the essential oil into the agar, the plates were incubated at 4 °C for 1 h and then incubated at 37 °C, for 24 to 48 h. The diameters of the inhibition zones (mm) were measured, including the diameter of the disks. Gentamycin (30 µg/disk), for Gram-positive bacteria, and vancomycin (30 µg/disk) for Gram-negative served as positive controls. All tests were performed in triplicate.

5.3.3. Minimum inhibition concentration (MIC) and minimal bactericidal concentration (MBC)

N. cataria essential oils dissolved in 5% dimethyl sulfoxide (Merck, Germany) were diluted to the highest concentration (20480 µg/mL), and then, serial twofold dilutions were made in concentrations ranging from 20 to 5120 µg/mL in Mueller-Hinton broth (MHB). The final DMSO concentration was 5% (v/v), and this solution was used as a negative control.

The minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations were determined by the broth microdilution method in 96-well microtiter plates [25-28]. Briefly, bacterial strains were cultured overnight at 37 °C on MHB, adjusted to a final density of 8 log CFU/mL, and used to inoculate (1-10) 96-well microliter plates containing serial dilutions of the essential oils (5120-20 µg/mL) on MHB. A positive control containing the bacterial culture and broth without the plant oils was included in each test. The contents of each well were mixed on a plate shaker at 300 r/min for 30 seconds and then incubated at 37 C for 24 h, while *Streptococcus agalactiae* and *Streptococcus mutans* were incubated for 48 h. Then, 2, 3, 5-triphenyltetrazolium chloride (Sigma, T8877) was used for the visual indicator of bacterial growth. The MIC of the essential oils was taken as the lowest concentration that showed no growth.

All samples showing no turbidity were sub-cultured; however, the lowest concentration from which the microorganisms did not recover was the minimal bactericidal concentration (MBC). Each experiment was performed in triplicate.

5.4. Antioxidant activity: Free radical scavenging capacity

The free radical scavenging capacity of the essential oils and two positive controls, butylated hydroxytoluene (BHT; Sigma, W218405) and ascorbic acid (Sigma A4403), was measured from bleaching the purple-colored methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to the method of Brand-Williams et al. [29]. Briefly, various methanolic oil concentrations (5, 10, 20, 30, 40, 50, 60, 80 and 100 µg/mL) were mixed with the same volume of a 0.2 mM methanolic solution of DPPH (Sigma, D9132). After a 30 min incubation at room temperature, the absorbance was recorded at 517 nm using a UV/VIS spectrophotometer. The free radical scavenging capacity was calculated as follows: % scavenging = 100 - (Abs sample - Abs control)/Abs DPPH × 100 %, where the Abs sample is the absorbance of the sample without DPPH. The oil concentration providing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentages against the oil concentrations. The assay was carried out in triplicate.

5.5. Statistical analysis

All experiments were repeated three times. The data were analyzed by SPSS computer software version 18 using ANOVA with the least significant difference (LSD) at the 0.05 probability level.

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