ORIGINAL ARTICLE

A C G publications

Rec. Nat. Prod. 9:2 (2015) 224-233

records of natural products

Chemical Composition, Antioxidant and Antimicrobial Activities of the Essential Oil of *Nepeta hindostana* (Roth) Haines from India

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(Received April 07, 2012; Revised August 08, 2014; Accepted October 12, 2014)

Abstract: Aerial parts of *Nepeta hindostana* (Roth) Haines collected from Gorakhpur Division, U. P., India possessed an essential oil in 0.42% yield. GC and GC-MS analysis of the oil revealed recognition of thirty three compounds, representing 91.0% (area percent) of the total oil composition. Oil was rich in sesquiterpene hydrocarbons, exhibited higher percentage of (E) *β*- farnesene (10.4%) followed by ageratochromene (9.7%), spiro [4.5] decan-1-one, 6-hydroxy (9.5%), -caryophyllene (8.6%) and spiro [4.5] decan-6-ol, 6-methyl (8.2%). At 60 µL, oil showed 80.7% antioxidant activity by *β*-carotene bioassay (IC_{50} = 8 µL) and 73.4% by DPPH free radical scavenging bioassay (IC_{50} = 8.5 µL). Furthermore, the oil was tested for its antimicrobial activity in opposition to five plant and human pathogenic bacteria namely *Bacillus subtilis*, *Erwinia herbicola*, *Escherichia coli*, *Pseudomonas putida*, *Salmonella typhi* and six post-harvest fungi such as *Aspergillus flavus* Link., *A. niger* van Tieghem, *A. ochraceus* Wilhelm, *A. terreus* Thom, *Fusarium nivale* (Fries) Cesati and *F. oxysporum* Schlecht. The oil was found to be more efficacious for *E. coli*, *Erw. herbicola* and *A. ochraceus.*

Keywords: *Nepeta hindostana*; essential oil; GC/GC-MS; antioxidant; antimicrobial.

1. Introduction

The genus *Nepeta hindostana* (Roth) Haines (Bilaiyalotan) belongs to family Lamiaceae is an annual herbaceous taxon, wildly distributed along ditches and wetland of Gorakhpur Division [1]. Genus comprises 280 species. *Nepeta* species are known for their utilization in traditional folk medicine and are reported to have diuretic, antitussive, expectorant, antispasmodic, anti-asthmatic and antiseptic activities [2]. Extracts and essential oils of *Nepeta* species exhibited anti-inflammatory, antioxidant activities and many of them are used as bacteriostatic and disinfectants as well as for ailment of skin disorders [3]. Although investigations have been carried out on the chemical composition, antioxidant and antimicrobial activities of several *Nepeta* species [4-6] but, little work has been undertaken on chemical characterization and antioxidant activity of *N. hindostana* oil. In earlier communications, a new triterpene nepehinol [7] and a new triterpenoidal aldehyde nepehinal [8] have been isolated from the alcoholic extract of the whole plant of *N. hindostana.* The present paper reports chemical composition and antioxidant activity of *N. hindostana* oil obtained by hydrodistillation from the aerial parts of the plant. In addition, because of certain constituents of the oil

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The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 01/01/2015 EISSN:1307-6167

have been reported from other plant species to possess antimicrobial activity [9], testing its antimicrobial activity is done.

2. Materials and Methods

2.1. Plant material collection and oil extraction

Flowering aerial parts of plant were collected from the bank of Ramgarh Lake of Gorakhpur Division (latitude of 27º05' to 27º25' North and longitude of 83º20' to 84º10' East at an elevation of about 91 m above sea level), U. P. India in January, 2010. The plant was identified by the aid of literature [1] as well as by matching with its specimen lodged in the Departmental Herbarium of Gorakhpur University. A voucher specimen of the plant (Accession No. 112867) was deposited to the Herbarium of Botanical Survey of India (NRC), Dehradun. 250 g of dried plant parts were cut in small pieces and the essential oil was obtained by hydrodistillation in 300 mL H_2O for 4 h using Clevenger's apparatus [10]. The oil content (v/w %) was estimated on dry weight basis. The essential oil obtained was dehydrated over anhydrous sodium sulphate and was stored at 4 ºC for further study.

2.2. Gas chromatography

Requisite amount (0.1 µL) of pure oil sample of *N. hindostana* was subjected to GC and GC/MS analysis. The GC was composed of an Agilent Technology 6890 N gas chromatograph data handling system equipped with a split-splitless injector (split ratio 50:1) and fitted with a FID using N_2 as the carrier gas at flow rate 1mL/min. The column was HP-5 capillary column (30 m x 0.32 mm, 0.25 µm film thickness) and temperature program was used as follows: initial temperature of 60 ºC (hold: 2 min) programmed at a rate of 3 ℃ /min to a final temperature of 220 ºC (hold: 5 min). Temperatures of the injector and FID were maintained at 210 ºC and 250 ºC, respectively.

2.3. Gas chromatography-mass spectrometry

The GC-MS analysis of oil was carried out using Perkin Elmer Clarus 500 gas chromatograph (Shelton, CT06484, USA) equipped with a split-splitless injector (split ratio 50:1) data handling system. The column was an Rtx®-5 capillary columns (60 m x 0.32 mm, 0.25 µm film thickness). Helium (He) was the carrier gas at a flow rate 1.0 mL/min. The GC was interfaced with (Perkin Elmer Clarus 500) mass detector operating in the EI^+ mode. The mass spectra were generally recorded over 40-500 amu that revealed the total ion current (TIC) chromatograms. Temperature program was used as the same as described above for GC analysis. The temperatures of the injector, transfer line and ion source were maintained at 210 ºC, 210 ºC and 200 ºC, respectively.

2.4. Qualitative and quantitative analysis

Identification of the individual component was made by matching their recorded mass spectra with the library (*NIST/ Pfleger /Wiley*) provided by the instrument software, and by comparing their calculated retention indices with GC alkanes standard solution (C_8-C_{20}) as well as literature value [11]. Relative area percentages of the individual component were obtained from GC-FID analysis.

2.5. DPPH (2, 2'- diphenyl-1-picrylhydrazyl) radical scavenging bioassay

This experiment was conducted following the method of Dordevic et al. [12]. Different concentrations of *N. hindostana* oil (5, 10, 20, 40 & 60 µL) were mixed with 400 µL of 0.5 mM DPPH in ethanol, and final volume adjusted up to 2000 µL with ethanol. All the contents were vigorously shaken and left for 30 min in dark. Similarly, experiment with ascorbic acid as positive control was also run parallel for comparison purpose. Absorbance was measured at 517 nm using ethanol as blank.

1:4 ratios of 0.5 mM DPPH and ethanol respectively was used as control. Inhibition of DPPH radicals was calculated using the equation:

$$I\ (\%) = 100 \times (A_0 - A_s) / A_{0,}$$

where A_0 is the absorbance of control and A_s is the absorbance of the tested sample. The experiments were replicated three times. Probit analysis was used for the estimation of IC_{50} value.

2.6. Antioxidant activity by β-carotene bleaching bioassay [13]

A stock solution of *β*-carotene and linoleic acid was prepared with 0.25 mg of *β*-carotene in 0.5 mL chloroform, 12.5 µL of linoleic, acid and 100 µL Tween 80. The chloroform was evaporated under vacuum and 50 mL of aerated distilled water was then added to the residue. Different concentrations (5, 10, 20, 40 & 60 µL) of *N. hindostana* oil were mixed separately in 2.5 mL of the above mixture in test tubes. The test tubes were incubated in a hot water bath at 50 ºC for 2 h, together with two blanks, one contained the antioxidant ascorbic acid as a positive control, and the other contained the same volume of solution instead of the oil or ascorbic acid. The absorbencies were measured at 470 nm on an ultraviolet spectrometer. Antioxidant activity (inhibition percentage, I%) of the samples were calculated using the following equation:

$$I\% = (A_{\beta\text{-carotene after 2 h assay}} / A_{\text{initial } \beta\text{-carotene}}) \times 100$$

where $A_{\beta\text{-carotene after 2 h assay}}$ is the absorbance of *β*-carotene after 2 h assay remaining in the samples and $A_{\text{initial β-carotene}}$ is the absorbance of β-carotene at the beginning of the experiments. All tests were carried out in triplicates. Probit analysis was used for the estimation of IC_{50} value.

2.7. Reference microbial strains

The bacterial strains (*Bacillus subtilis* MTCC3053, *Erwinia herbicola* MTCC 3609, *Escherichia coli* MTCC443, *Salmonella typhi* MTCC733, *Pseudomonas putida* MTCC 1190) used during experimentation were procured from Institute of Microbial Technology, Chandigarh and fungal strains (*Aspergillus flavus* Link., *A. niger* van Tieghem, *A. ochraceus* Wilhelm, *A. terreus* Thom, *Fusarium nivale* (Fries) Cesati, *F. oxysporum* Schlecht) were isolated from stored pigeon pea seeds. Subcultures were maintained by growing bacteria for 24-48 h on King's B medium for *Ps. putida*, Wilbrink's Agar (WA) for *Erw. herbicola*, Nutrient Agar (NA) for other bacterial species and fungi for 7 days on Czapek-Dox Agar (CDA) medium.

2.8. Antimicrobial bioassay

The antibacterial activity of *N. hindostana* oil was done by disc diffusion method of Andrews [14]. For disc diffusion bioassay, Petri plate filled with 10 mL Nutrient Agar/WA medium depending on the bacterial species, was seeded with the 10^7 CFU/mL target bacterial suspension. The inocula of bacteria were prepared from 24 h broth cultures, and suspensions were adjusted 0.5 McFarland standard turbidity. Disc of Whatmann filter paper No. 1 (5 mm in diam.) was impregnated with 0.07 µL/mL of the oil and placed aseptically on the surface of agar plate. After 24-48 h of incubation at 32 ± 2 °C, the zone of inhibition (ZOI) around the disc was measured in millimeter (mm). For antifungal bioassay Inverted Petri plate method of Bocher [15] was considered and the concentration of oil was 0.36 µL/mL. The results were expressed in terms of per cent mycelial inhibition (PMI) after 7^{th} days at 28 ± 2 °C of incubation. All the experiments were replicated three times. Solution of 1 mg/mL streptomycin (antibiotic) and aluminum phosphide (fungicide) in sterilized distilled water was used as standard for bacteria and fungi respectively. All the data were subjected for standard deviation.

2.9. Agar dilution bioassay

Modified methods of NCCLS [16] and CLSI [17] for agar dilution susceptibility test were considered to determine the minimum inhibitory concentration (MIC) of *N. hindostana* oil and antibiotic. A series of dilutions of the *Nepeta* oil (0.06-16 µL/mL) and antibiotic (0.06-16 µL /mL) were prepared in NA/WA plates depending upon the bacterial species using Tween 80 as an emulsifier for appropriate assimilation of oil with medium. After solidification, the plates were aseptically spotted with 5 µL of overnight grown bacterial cultures approximately containing 1×10^8 CFU/mL inocula. A plane media plate inoculated by bacteria was served as positive control and un-inoculated plate was served as negative control. The plates were incubated aerobically overnight at 32 ± 2 ºC for 24-72 h. The inhibition of the bacterial growth was compared with the growth of control plate. The MIC was defined as the lowest concentration of the oil inhibiting the visible growth of each organism on the agar plate. Further the poisoned plates showing no growth were sub-cultured onto fresh medium (without oil and antibiotic) for determination of minimum bactericidal concentration (MBC) [18]. The least concentration from which the bacteria do not recover growth on fresh medium was considered as MBC. Each test was replicated three times.

2.10. Fungitoxic properties (MIC/MFC) of N. hindostana oil

Inverted Petri plate method of Bocher [15] was adopted for the determination of minimum inhibitory concentration (MIC) of *Nepeta* oil against fungi. 10 mL CDA was poured into 80 mm Petri plates. 6 mm disc of fungal inoculum was deposited on the surface of agar medium in a central position. Whatmann filter papers No. 1 of 6 mm diameter were put in the cover of the Petri plates and soaked with various amounts of pure oil and synthetic fumigant separately. The inoculated Petri plates were turned upside down and observations were made after 7^{th} days of incubation at 28 ± 2 ºC. MICs were determined as the concentration with no visible growth in fungal diameter. The inhibited fungal discs of the oil treated sets were re-inoculated into fresh medium and revival of their growth was observed. The concentration at which the growth of fungus could not revive was read as MFC (minimum fungicidal concentration) [19]. All the experiments were carried out in triplicates and a control set without oil/synthetic fumigant was kept during each experiment.

3. Results and Discussion

The oil sample has characteristic pale yellow colour having 0.42% (v/w) yield on dry weight basis. The identified constituents with their respective percentages and Kovat's indices are summarized in Table 1. GC and GC-MS analysis of the oil revealed recognition of thirty three major and minor compounds, representing 91.0% (area percent) of the total oil sample. Of which, (*E*)-*β*-farnasene (10.4%), spiro [4.5] decan-1-one, 6-hydroxy (9.5%), spiro[4.5] decan-6-ol, 6-methyl (8.2%), -caryophyllene (8.6%) and ageratochromene (9.7%) were reported as major constituents while *α*-pinene (0.1%), camphene (0.1%), *β*-ylangene (0.3%), linalool (0.2%) and *trans*-*α*-bisabolene epoxide (0.2%) were turned out in lower amounts. The oil was characterized by higher content of sesquiterpene hydrocarbons (39.6%) followed by 34.0% of oxygenated monoterpenoids and 16.2% of oxygenated sesquiterpenoids while the studied taxon exhibited only 1.2% of monoterpene hydrocarbons. The other species of *Nepeta* such as *N. nuda* subsp. *nuda* posses' different chemical profile as it consist of mainly 1,8-cineole (16.7%) and caryophyllene oxide (16.3%) [20]. Further 1, 8-cineole was reported as major constituent of some other *Nepeta* species [4, 21]; however, no such compounds were reported in present studied taxon. The present report noted the highest percentage of (*E*) β- farnasene. Earlier nepetalactone was reported as a major constituent of *N. argolica* subsp. *dirphya* [5], on the contrary, in the present study only 0.5 and 0.6% of two isomers of neptalactone were recognized. Additionally, presence of (*E*)-*β*- farnesene, *α*-pinene, *β*-pinene, limonene, linalool and caryophyllene oxide in *N. hindostana* oil corroborate with essential oil composition of *N. camphorate* [5]. These chemical differentiations and similarities might be due to chemotaxonomic

differences among plant species, their intra-specific origin and differences in occurrence of taxon in different geographical regions.

Table 1. Chemical composition of *Nepeta hindostana* (Roth) Haines essential oil.

Components	**Kovat's indices**	**% Content**
α-Pinene	939	0.1
Camphene	953	0.1
β-Pinene	969	0.5
Limonene	1031	0.4
Linalool	1142	0.2
Bornyl acetate	1281	0.4
α-Cubebene	1353	0.5
4aα,7α,7aα-Nepetalactone	1357	0.6
4aα,7β,7aα-Nepetalactone	1391	0.5
β-Ylangene	1419	0.3
trans-α-Bergamotene	1432	3.3
(E)-β- Farnesene	1451	10.4
-Caryophyllene	1452	8.6
(Z)-β-Farnesene	1454	1.1
α-Humulene	1456	1.2
Bicyclo [2.2.2] octane,1-methoxy-4 methyl	1458	1.7
Spiro [4.5] decan-1-one,6-hydroxy	1460	9.5
6-Demethoxyageratochromene	1461	4.6
Spiro [4.5] decan-6-ol, 6-methyl	1472	8.2
Germacrene D	1481	1.2
-Bisabolene	1481	2.4
α-Bisabolene	1538	6.6
Caryophyllene oxide	1584	6.3
1,5,5,8-Tetramethyl-1, 2 oxabicyclo [9.1.0] dodeca-3,7-diene	1590	3.6
δ-Cadinol	1652	0.5
Ageratochromene	1658	9.7
2-Buten-1-one,1-phenyl	1672	1.5
3,5 Diisopropenyl-1,1,2-trimethylcyclohexane	1682	3.8
Germacr-4-en-12-oic acid, 6-alpha-hydroxy-, gamma-lactone (11S)-	1684	0.5
α-Bisabolol	1685	1.1
trans- *α*-Bisabolene epoxide	1704	0.2
cis-*α*-Bisabolene epoxide	1709	0.7
Longipinene epoxide	1710	0.7
Total identified		**91.0**
Class composition of the oil hydrocarbons	Monoterpene	1.2
	Oxygenated monoterpenoids	34.0
	Sesquiterpene hydrocarbons	39.6
	Oxygenated sesquiterpenoids	16.2

It is well-known that the antioxidant activity of plant essential oils containing terpenes is due to their capacity to be donors of hydrogen atoms or electrons and to capture the free radicals. DPPH

analysis is one of the tests, used to prove the ability of the components of the *N. hindostana* oil to act as donors of hydrogen atoms. The obtained results are shown in Figure 1. The *N. hindostana* oil showed a significant effect in inhibiting free radicals produced by DPPH, reaching up to 73.4% at 60 µL and IC_{50} value was found as 8.5 µL. This capability was decreased with the decrease of oil concentration 40 (72.82), 20 (64.09), 10 (52.46) and 5 µL (32.79%). These findings suggested that oil was able to reduce the stable free radical 2, 2-diphenyl-1-picrylhydrazyl to the transparent diphenyl-picryl-hydrazine. In β-carotene bleaching bioassay, linoleic acid produces hydroperoxides as free radicals during incubation at 50 ºC. The presence of antioxidants in the essential oils minimizes the oxidation of β-carotene by hydroperoxides. Thus, the degradation rate of β-carotene depends upon the

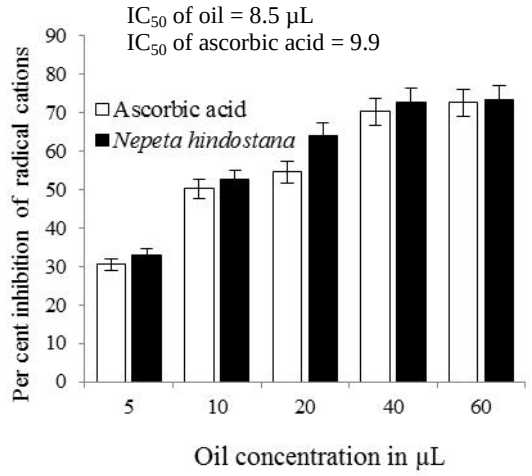

Figure 1. Radical scavenging effect of *N. hindostana* oil and ascorbic acid on DPPH.

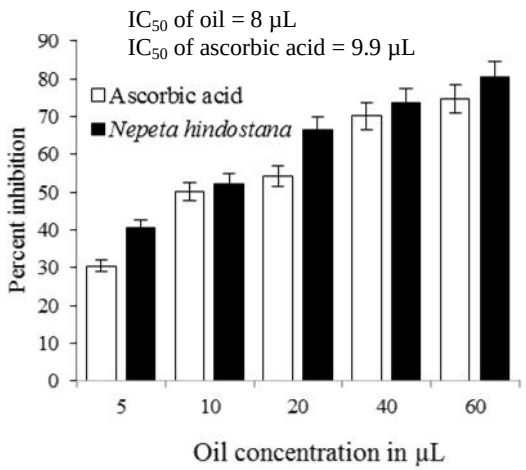

Figure 2. Antioxidative effect of *N. hindostana* oil and ascorbic acid by *β*-carotene bleaching test.

antioxidant activity of the oils which can hinder the extent of β-carotene bleaching by acting on the lipid free radicals form in the system [22]. In present *β*-carotene bioassay, the obtained inhibition activity was enhanced with oil concentration and exhibited 8 µL of IC_{50} value (Figure 2). At 5, 10, 20, 40 and 60 µL, the antioxidant activity increased to 40.6, 52.46, 66.78, 73.9 and 80.7% respectively. During both bioassays, no significant differences between radical scavenging and antioxidant activity of oil and ascorbic acid (synthetic antioxidant) were observed. Similar results were observed by Fraternale et al. [23] with *Monard adidyma* L. oil by comparing its antioxidant activity with BHT (Butylated hydroxy toluene) and ascorbic acid. The antioxidant activity observed in *N. hindostana* oil could be due to the synergistic effect of two or more compounds present in it [24].

The essential oil of *N. hindostana* was highly efficacious to *Erw. herbicola* (28.58 mm, ZOI), *E. coli* (24.50 mm, ZOI) and *A. ochraceus* (84.56%, PMI) than other test pathogens, inhibited the microbial growth significantly (Table 2). From time to time the antimicrobial activities of essential oils isolated from other *Nepeta* species have been investigated. In a laboratory bioassay [6], the essential oils from six Himalayan *Nepeta* species viz., *N. leucophylla* Benth., *N. discolour* Royle ex Benth., *N. govaniana* Benth., *N. clarkei* Hook. f., *N. elliptica* Royle ex Benth. and *N. erecta* Benth. were found to be effective and have variable range of toxicity against several human pathogenic microorganisms (*Pseudomonas aeruginosa*, *Escherichia coli*, *Pasteurella multocida*, *Proteus vulgaris*, *Serratia marcescens*, *Staphylococcus aureus*, *Candida albicans*, *Trichophyton rubrum*).

The oil of *N. rtanjensis* and its constituents were reported to be mycotoxic against several fungal species such as *Alternaria alternata*, *Cladosporium cladosporoides*, *Trichoderma viride* and *Bipolaris spicifera* [25]. Screening of the essential oils of four Morocco *Nepeta* species (*N. atlantica* Ball, *N. tuberosa* L. subsp *reticulata* (Desf.) Maire, *N. cataria* L., *N. granatensis* Boiss) have showed pronounce variable antibacterial activity against *E. coli, S. aureus* and *Ps. aeruginosa* [26].

Similarly, in the present study, investigated oil also showed variable range of toxicity against the tested microbial pathogens. Our results coincided perfectly with the previous studies [27-28]; different microorganisms tested had different susceptivity to the same essential oil, likewise, the sensitivity of same organism changed because of variation of percentage of the constituents. The obvious differences of inhibitory effect of oil on several microorganisms might be due to the variation in amounts of essential oil's constituents.

Table 2. Inhibitory effect of *N. hindostana* oil and antibiotic/fungicide against bacteria and fungi.

Microbial strains	Inhibitory effect(ZOI/PMI) on microbial growth*± SD	
	Oil	Antibiotic(a)/fungicide(f)
Bacillus subtilis	19.50 ± 1.20	10.20 ± 2.50[a]
Erwinia herbicola	28.58 ± 3.95	9.00 ± 1.22[a]
Escherichia coli	24.50 ± 0.80	8.50 ± 0.80[a]
Pseudomonas putida	14.58 ± 0.53	15.60 ± 0.34[a]
Salmonella typhi	17.30 ± 0.90	12.40 ± 0.50[a]
Aspergillus flavus	59.23 ± 4.25	16.01 ± 0.90[f]
A. niger	63.84 ± 1.89	32.42 ± 1.03[f]
A. ochraceus	84.56 ± 3.78	53.90 ± 0.67[f]
A. terreus	47.87 ± 2.01	4.20 ± 0.30[f]
Fusarium nivale	32.80 ± 0.50	19.90 ± 1.20[f]
F. oxysporum	50.30 ± 2.00	14.30 ± 2.00[f]

*Values in zone of inhibition (ZOI) in mm for bacteria and per cent mycelial inhibition (PMI) for fungi

Table 3. Antimicrobial properties (MIC/MBC/MFC) of *N. hindostana* oil and antibiotic/fungicide.

Microbial strains	Oil (µL/mL)			Antibiotic (µL/mL)		Fungicide (µL/mL)	
	MIC	MBC	MFC	MIC	MBC	MIC	MFC
B. subtilis	4.0	8.0	NA	8.0	>16	NA	NA
Erw. herbicola	2.0	8.0	NA	4.0	16	NA	NA
E. coli	2.0	8.0	NA	4.0	16	NA	NA
Ps. putida	4.0	>16	NA	8.0	>16	NA	NA
Sal. typhi	8.0	>16	NA	16.0	>16	NA	NA
A. flavus	1.0	NA	3.5	NA	NA	3.5	>5.0
A. niger	1.5	NA	5.0	NA	NA	3.0	>5.0
A. ochraceus	0.5	NA	1.5	NA	NA	2.0	4.5
A. terreus	1.5	NA	>5.0	NA	NA	3.0	>5.0
F. nivale	2.0	NA	3.5	NA	NA	2.5	>5.0
F. oxysporum	2.0	NA	4.5	NA	NA	3.0	>5.0

NA-Not Applicable; MIC-Minimum Inhibitory Concentration, MBC-Minimum Bactericidal Concentration; MFC-Minimum Fungicidal Concentration

Table 3 summarizes the MIC, MBC and MFC values of oil and synthetics. Amongst the tested bacterial species, the MIC value of *N. hindostana* oil was least (2.0 µL/mL) for *Erw. herbicola* and *E. coli*. Further, oil showed lowest MIC value for *A. ochraceus* (0.5 µL/mL) among the above mentioned test fungi. Oil was cidal in nature at higher dosage, and reported to be more efficacious over antibiotic/fungicide during antimicrobial, agar dilution and fungistatic/fungicidal bioassays. These findings are in accordance with Dorman and Deans [27], they investigated the antimicrobial properties of *Monarda, Myristica, Origanum, Pelargonium* and *Thymus* oils were superior over commercial antibiotics. The MICs of studied *N. hindostana* oil were also found to be lower than *N. granatensis* (MICs ranged from 22.5 to 80 µL/mL), *N. tuberose* (4.37 µL/mL), *N. atlantica* (7.50 µL/mL), and *N. cataria* (5.00 µL/mL) essential oils [26] which concluded that the oil can be use as more effective

antimicrobial agent than other ones. From the current results the observed toxicity of the oil is suspected to be associated with the higher content of (E) *β*-farnasene, spiro [4.5] decan-1-one, 6-hydroxy, spiro [4.5] decan-6-ol, 6-methyl, -caryophyllene and ageratochromene, especially *E. coli*, *Erw. herbicola* and *A. ochraceus* which were responsible for wide variety of infections [29-31], suggested the *N. hindostana* oil could be medicinal resource for antimicrobial agent. In addition, the components in lower amounts such as *α*-pinene, *β*-pinene and linalool could also contribute to the antimicrobial activity of the oil. Linalool had been demonstrated to have strong inhibitory effect against seventeen bacteria and ten fungi [28]. *α*-Pinene and *β*-pinene have also been reported to be antibacterial agents against several pathogenic microorganisms [32]. In fact, it was also possible that the components in lower percentage might be involved in some type of synergism with other active compounds and exhibited inhibitory effect [32]. The antimicrobial potency of the *N. hindostana* oil could also be explained by disturbance of the permeability barrier of the bacterial membrane structure [33]. Earlier findings revealed that tea tree oil damages the cell membrane structure of *E. coli*, *S. aureus* and *Candida albicans* [34]. Such an incident is due to the dissemination of monoterpenes through the cell wall and cell membrane. In fact, monoterpenes are lipophilic, and may persuade the growth of cell membranes, increases fluidity, destroy the membrane structure and inhibit membrane embedded enzymes [34] which further inhibit the respiration and leads to the cell death.

Based on the described experiments and present results, it can be summarized that investigated species (*N. hindostana*) is rich in sesquiterpenes hydrocarbons. The free radical scavenging and antioxidant activity of studied taxon's oil indicate that it has a protective effect against ROS and can therefore be used as a natural preservative ingredient in the food or pharmaceutical industry. Our antimicrobial results confirmed that oil efficaciously inhibits the growth of *Erw. herbicola*, *E. coli* and *A. ochraceus E. coli* which make serious sanitary problems worldwide. Thus, this plant may be useful for developing alternative antimicrobials to treat infections caused by these pathogens.

Acknowledgments

The authors are thankful to the Head, Department of Botany, DDU Gorakhpur University, Gorakhpur for providing necessary Lab. Facilities, to CST UP Lucknow for financial assistance, and to the staff, BSI (NRC) Dehradun for authentication of plant specimens.

References

[1] T.N. Srivastava (1972). Flora Gorakhpurensis. Today & Tomorrow Printers and Publishers, New Delhi.

[2] R.N. Chopra, S.L. Nayar and I.C. Chopra (2006). Glossary of Indian medicinal plants. Publishers National Institute of Science Communication, Dr K. S. Krishnan Marg, New Delhi.

[3] H.L. De Pooter, B. Nikolai, L.F. De Buyck, P. Goetghebeur and N.M. Schamp (1987). The essential oil of *Nepeta nuda*. Identification of a new nepetalactone diastereoisomer, *Phytochem.* **26,** 2311-2314.

[4] J.C. Chalchat, S.D Petrovic and M.S. Gorunovic (1998). Quantity and composition of essential oil of the wild plant *Nepeta nuda* L. from Yugoslavia, *J. Essent. Oil. Res*. **10,** 423-425.

[5] E. Kalpoutzakis, N. Aligiannis, A. Mentis, S. Mitaku and C. Charvala (2001). Composition of the essential oil of two *Nepeta* species and *in vitro* evaluation of their activity against *Helicobacter pylori*, *Planta Med*. **67,** 880-883.

[6] D.S. Bisht, R.C. Padalia, L. Singh, V. Pande, P. Lal and C.S. Mathela (2010). Constituents and antimicrobial activity of the essential oils of six Himalayan *Nepeta* species, *J. Serb. Chem. Soc.* **75,** 739–747.

[7] A.U. Ahmad, S. Bano and F.V. Mohammad (1985). Nepehinol: a new triterpene from *Nepeta hindostana*, *Planta Med.* **51**, 521-523.

[8] A.U Ahmad, M. Noorwala, F.V. Mohammad, M.G. Shah and A. Parvez (1993). Nepehinal: a new triterpenoidal aldehyde from *Nepeta hindostana*, *Planta Med.* **59**, 366-368.

[9] K.H.C Baser, B. Demircakak and H. Duman (1998). Composition of essential oil of *Nepeta sulfuriflora* P.H. Davis, *J. Essent. Oil Res.* **10**, 179-181.
[10] J.F. Clevenger (1928). Apparatus for the determination of volatile oil, *J. Am. Pharm. Assoc.* **17**, 345-349.
[11] R.P. Adams (2007). Identification of essential oil components by Gas Chromatography/Mass Spectrometry,4th Ed. Allured Business Media, Carol Stream, IL, U.S.A.
[12] S. Dordevic, S. Petrovic, S. Dobric, M. Milenkovic, D. Vucicevic, S. Zizic and J. Kukic (2007). Antimicrobial, anti-inflammatory, anti-ulcer and antioxidant activities of *Carlina acanthifolia* root essential oil, *J. Ethanopharmacol*. **109**, 458-463.
[13] H. Miraliakbari and F. Shahidi (2008). Antioxidant activity of minor components of tree nut oils, *Food Chem.* **111**, 421–427.
[14] J.M. Andrews (2001). BSAC standardized disc susceptibility testing method, *J. Antimicrob. Chemother.* **48 (Suppl. S1),** 43-57.
[15] O.E. Bocher (1938). Antibiotics In: modern methods of plant analysis Eds. Peach K. and Tracey M.V.; Vol III Springer Verlag Berlin p 651.
[16] National Committee for Clinical Laboratory Standards (NCCLS): (2003). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; Approved Standard - NCCLS document 6th ed., M7-A6, Wayne, PA, USA 2003
[17] Clinical and Laboratory Standards Institute (CLSI): (2009). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; Approved Standard - CLSI document 8th ed., M07-A8, Wayne, PA, USA.
[18] A.K. Mishra, A. Mishra, S. Tripathi and N.N. Tripathi (2008). Susceptibility of *Enterococcus faecalis* to the plant volatile oils, *J. Microb. World* **10**, 108-112.
[19] O. Delespaul, V.G. de Billerbeck, C.G. Roques, G. Michel, C. Marquiervinuales and J.M. Bessiere (2000). The antifungal activity of essential oils as determined by different screening methods, *J. Essent. Oil Res*. **12**, 256-266.
[20] G. Gkinis, B. Bozin, N. Mimica-Dukic and O. Tzakou (2010). Antioxidant activity of *Nepeta nuda* L. ssp. *nuda* essential oil rich in Nepetalactones from Greece, *J. Medicinal Food.* **13,** 1176-1181.
[21] K.H.C. Baser, B. Demircakak, A. Altintas and H. Duman (1998). Essential oil of *Nepeta flavida* Hub.-Mor., *J. Essent. Oil Res*. **10,** 299-300.
[22] M. Younes (1981). Inhibitory action of some flavonoids on enhanced spontaneous lipid peroxidation following glutathione depletion, *Planta Med.* **47,** 240-245.
[23] D. Fraternale, L. Giamperi, A. Bucchini, D. Ricci, F. Epifano, G. Burini and M. Curini (2006). Chemical composition, antifungal and *in vitro* antioxidant properties of *Monarda didyma* L. essential oil, *J. Essent. Oil Res.* **18,** 581-585.
[24] F. Lu and L.Y. Foo (2001). Antioxidant activities of polyphenol from sage *(Salvia officinalis*). *Food Chem.***75**, 197–202
[25] M.L. Grbic, M. Stupar, J. Vukojevic and D. Grubisic (2011). Inhibitory effect of essential oil from *Nepeta rtanjensis* on fungal spore germination, *Centr. Eur. J. Biol.* **6,** 583-586.
[26] Z. Laila, B. Houssine, H. Amina, B. Amina, A. Hassan, I. Abdelkader, E.A. Rajae, B. Youssef and B. Abdelaziz (2008). The essential oils and antimicrobial activity of four *Nepeta* species from Morocco, *J. Med. Plants Res.* **2,** 111-114.
[27] H.J.D. Dorman and S.G. Deans (2004). Chemical composition, antimicrobial and *in vitro* antioxidant properties of *Monarda citriodora* var. *citriodora, Myristica fragrans, Origanum vulgare* ssp. *hirtum, Pelargonium* sp. and *Thymus zygis* oils, *J. Essent. Oil Res.* **16,** 145-150.
[28] S. Pattnaik, V.R. Subramanyam, M. Bapaji and C.R. Kole (1997). Antibacterial and antifungal activity of aromatic constituents of essential oils, *Microbios.* **89,** 39-46.
[29] A.A. Galal (2003). *Erwinia herbicola* subsp. *anans*, a new pathogen to tomato plants. Tenth Congress of Phytopathol., Giza, Egypt, 325-337.
[30] Z. Shunying, Y. Yang, Y. Huaidong, Y. Yue and Z. Guolin (2005). Chemical composition and antimicrobial activity of the essential oil of *Chrysanthemum indicum*, *J. Ethanopharmacol*. **96,** 151-158.

[31] C.D. Bishop and I.B. Thornton (1997). Evaluation of antifungal activity of the essential oils of *Monarda citriodora* var. *citriodora* and *Melaleuca alternifolia* on post-harvest pathogens, *J. Essent. Oil Res*. **9,** 77-82.
[32] M. Marino, C. Bersani and G. Comi (2001). Impedance measurement to study the antimicrobial activity of essential oils from Lamiaceae and Compositae, *Int. J. Food Microbiol*. **67,** 187-195.
[33] M.M. Cowan (1999). Plant products as antimicrobial agents. *Clin. Microbiol. Rev*. **12,** 564-582.
[34] S.D. Cox, C.M. Mann, J.L. Markham, H.C. Bell, J.E. Gustafson, J.R. Warmington and S.G. Wyllie (2000). The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (Tea tree oil), *J. Appl. Microbiol.* **88,** 170-175.