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Vinod Kumar
Department of Chemistry, Kumaun University, Nainital- 263001, Uttarakhand, India

C. S. Mathela
Department of Chemistry, Kumaun University, Nainital- 263001, Uttarakhand, India

Amit Panwar
Deapartment of Biotechnology, Bhimtal, Kumaun University, Nainital- 263 001, Uttarakhand, India

Correspondence:
Dr. Vinod Kumar
Department of Chemistry, Kumaun University, Nainital- 263001, Uttarakhand, India
Email: drvkumar85[at]gmail.com

In vitro antibacterial activity of essential oils from Lamiaceae species

Vinod Kumar*, C. S. Mathela, Amit Panwar

ABSTRACT

Essential oils from *Calamintha umbrosa* and *Nepeta* species viz. *N. leucophylla*; *N. hindostana*; *N. ciliaris* and *N. clarkei* (family Lamiaceae), was tested against six bacterial strains. To evaluate the correlation between the antimicrobial activity and the essential oils, PCA and HCA analysis was done. PCA and HCA analysis of the antibacterial activity revealed that essential oils of *Nepeta* species had a strong and broad spectrum antibacterial effect against bacterial strains of *P. aeruginosa* and *S. scandidus*. The *N. leucophylla* oil showed higher activity against Gram-negative bacteria *P. aeruginosa* (10.5 mm, MIC 10 µL/mL) and *K. pneumonia* (9.1 mm, MIC 45 µL/mL) among all *Nepeta* oils which may be due to presence of active antimicrobial iridoids compounds.

Keywords: Lamiaceae; Essential oils, *Nepeta*; Antibacterial activity.

INTRODUCTION

Food borne diseases are common and contribute largely to contamination of food and drinking water [1]. Efforts are being made to search new alternatives to control these diseases, giving priority to those having no side effects on human health. In some cases herbal remedies provide safe alternative to synthetic drugs. The extracts including volatile constituents of many plants species have shown interesting biological activities leading to researches focused on the characterization of antimicrobial constituents of these plants [2-4].

The Lamiaceae species are one of the most diverse and widespread in terms of ethnomedicine and its medicinal value. Genus *Nepeta*, with approximately 300 species are widely used in folk medicine because of their biological activities [5-6]. Few *Nepeta* species and their constituents' *viz*. iridodial β-monoenol acetate, and actinidine have been reported to possess high antimicrobial activity [7-9]. *Calamintha* species have also been the subject of intensive studies over the last few years due to a great diversity in microbial activities against various bacteria [10-11].

This communication focuses on antimicrobial activity of essential oils from *Calamintha umbrosa* and *Nepeta* species *viz. N. leucophylla*; *N. hindostana*; *N. ciliaris* and *N. clarkei* (family Lamiaceae) against six bacterial strains.

MATERIALS AND METHODS

Plant materials

Aerial parts of *Nepeta* and *Calamintha* species were collected at the flowering stage from sub-Alpine Himalayan region. The plant specimens were identified from Botanical Survey of India, Dehradun.

Extraction of the essential oil

The fresh aerial parts (~2 kg) of each species were subjected to steam distillation. The distillate was treated with n-hexane followed by dichloromethane and organic phase separated. The n-hexane and dichloromethane extracts were combined and dried over anhydrous Na_2SO_4. The solvent was distilled off in vacuum to get residual oil which was stored at ~4 ºC.

GC and GC-MS analysis

The oil samples were analyzed on a gas chromatograph (Nucon 5765) with Rtx-5 non-polar fused silica capillary column (30 m × 0.32 mm, film thickness: 0.25 µm) and the temperature was programmed (60-210 °C) at 3 °C/min. The injector and detector temperatures were maintained at 210 °C. Injection (0.5 µL, using a 10% solution of the oil in *n*-hexane) was made on Thermo Quest Trace GC 2000 (Thermo

Quest/Finnigan, Germany) fitted with an Rtx-5 non-polar fused silica capillary column (30 m × 0.25 mm, film thickness: 0.25 µm) and interfaced with a Finnigan MAT Polaris Q ion trap mass spectrometer using above mentioned operating parameters. The MS were recorded (70 eV) within a mass range of 40-450 amu. Identification of components was done by comparison of their retention indices (RI) relative to a series of n-alkanes (C_9-C_{24}) indices on the Rtx-5 non-polar fused silica capillary column and with those of published data [12], further confirmed by NIST and WILEY mass spectral library data.

Bacterial strains

The *in vitro* antibacterial activity was evaluated against four gram negative bacteria *Pseudomonas aeruginosa* (MTCC No. 424), *Escherichia coli* (MTCC No. 443), *Aeromonas hydrophila* subsp. *hydrophila* (MTCC No. 646), *Klebsiella pneumonia* (MTCC No. 3384) and two gram positive bacterium [*Bacillus subtilis* (MTCC No. 441) and *Streptomyces scandidus* subsp. *azaticus* (MTCC No. 703)]. The test strains were provided by the Department of Biotechnology, Bhimtal, Kumaun University which were procured from the Institute of Microbial Technology, Chandigarh. Microbial technology culture collection (MTCC) numbers represent the standard strain numbers assigned to these microorganisms. The cultures of bacteria and fungi were maintained on their appropriate agar slants at 4 °C throughout and used as stock cultures.

Antimicrobial activity evaluation

Antimicrobial activity evaluation was done by the agar well diffusion method [13]. The samples were dissolved in dimethyl sulphoxide (DMSO) to prepare desired concentrations. Inoculums of the microbial strains (1×10^6 CFU/mL) were plated using sterile swabs into petri dishes (90 mm) with 20 mL of Mueller-Hinton agar, where 3 mm wells were cut and filled with 30 µL/mL of sample. Standard antibiotic gentamycin was used as positive control and DMSO as negative control. The petri dishes were pre-incubated for 3 h at room temperature, allowing the complete diffusion of the samples and then, incubated at 37±1 ^oC for 24 h [14]. The zones of inhibition were measured.

Determination of MIC

The evaluation of MICs was done using the agar dilution methods with slight modifications described by the National Committee for Clinical Laboratory Standards [15]. In these assays, equal volumes of each microbial strain culture, containg approximately 1×10^6 CFU/mL, were applied onto MHB supplemented with the essential oils at concentration ranging from (5-100µL/mL) in tubes. These cultures were then incubated at 37 ^oC for 24 h. Culture medium (100 µL) was taken from each micro broth assay tube and subculture in fresh MHA. After incubation at 37 ^oC for 24 h, the least concentration showing no visible growth on subculture was taken as the MIC.

Statistical analysis

The principal component analysis (PCA) and hierarchical cluster analysis (HCA) were performed using XLSTAT statistical computer software package, version 14 for evaluating correlation between antibacterial activity and essential oils.

RESULTS AND DISCUSSION

Qualitative and quantitative content of essential oils of *C. umbrosa* and four *Nepeta* species *viz. N. ciliaris*, *N. leucophylla*, *N. clarkei* and *N. hindostana* were determined by using GC and GC-MS after steam distillation of the fresh aerial parts. Major chemical constituents of these species have already published in our previous paper [8,16]. The GC and GC/MS of essential oils of different Lamiaceae species lead to identification of iridodial β-monoenol acetate and caryophyllene oxide as major constituents from *N. leucophylla* besides sesquiphellandrene and iridodial derivatives as minor constituents. β-Sesquiphellandrene, germacrene D, actinidine were the major constituents in addition to minor/ trace content of diastereomeric iridodial esters in the oil from *N. clarkei.* Chemical composition of oil of *N. ciliaris* was characterized by β-caryophyllene, β-sesquiphellandrene and caryophyllene oxide as main constituents. Major differences in terms of qualitative and quantitative composition of essential of *C. umbrosa* were noticed, such as β-caryophyllene, germacrene D and spathulenol were identified in the sample [16]. While the *N. hindostana* essential oil was found to contained β-Caryophyllene, δ-cadinene**,** caryophyllene oxide, 2,3-dihydrofarnesol as major constitients.

The results of antibacterial activity evaluation are presented in Tables 1and 2. Oils showed variable zones of inhibition (5.1-10.8 mm). The maximum zone of inhibition was recorded against *S. scandidus* (10.8 mm) and *B. subtilis* (10.5 mm) for Gram-positive, while *P. aeruginosa* (10.5 mm) and *K. pneumonia* (9.1 mm) for Gram-negative bacteria. The essential oil of *C. umbrosa* showed potent bactericidal activity against two strains *E. coli* and *S. scandidus* with inhibition zone 6.6 mm and 10.5 mm, respectively at MIC value 25 µL/mL. The results of antibacterial activity of *Nepeta* species *viz*., *N. leucophylla*, *N. hindostana*, *N. ciliaris* and *N. clarkei* showed higher activity against all the tested bacterial species. Among these, *N. leucophylla* showed highest inhibition zone against Gram-negative bacteria *P. aeruginosa* (10.5 mm, MIC 10 µL/mL) and *K. pneumonia* (9.1 mm, MIC 45 µL/mL) while *N. ciliaris* against *B. subtilis* (10.0 mm, MIC 20 µL/mL) and *P. aeruginosa* (9.6 mm, MIC 10 µL/mL). Furthermore, *N. hindostana* essential oil was more active against Gram-positive bacteria *B. subtilis* (8.3 mm, MIC 30 µL/mL) and *S. scandidus* (6.6 mm, MIC 10 µL/mL), and also able to inhibit the Gram-negative bacteria with (4.6-7.8 mm, MICs 35-50 µL/mL). While *N. clarkei* essential oil has weak antimicrobial activity against *S. scandidus* (10.8 mm, MIC 20 µL/mL) followed by *B. subtilis* > *P. aeruginosa* > *E. coli* > *K. pneumonia*> *A. hyrdophilla* (Table 1 and 2). On comparing inhibition zone and MIC values, *N. leucophylla* and *N. ciliaris* were found to be more effective than other *Nepeta* species against all the bacterial species.The results of MIC values are listed in Table 2. To evaluate the correlation between the antimicrobial activity and the essential oils, all the MIC values subjected to the PCA and HCA analysis. The PCA horizontal axis explained 77.6% of the total variance, while the vertical axis a further 17.7% (Fig. 1). The HCA indicate three groups (I, II and III) of bacteria (Fig. 2).

Group I, composed of the Gram-positive bacteria (*B. subtilis*) and two Gram-negative bacteria (*E. coli* and *P. aeruginosa*), are characterized by their average sensitivity to the essential oils; however, the variability between these groups of bacterial strain was also seen and divided into two main subgroups (Ia and IIb). Subgroup Ia consist of *E. coli* which showed the maximum for *N. ciliaris* oil (MIC 10 µL/mL), while lowest for *N. hindostana* and *N. clarkei* (MIC 40 µL/mL, each). Subgroup IIb was limited to *B. subtilis* and *P. aeruginosa* which was characterized by the sensitivity for *N. ciliaris*, *N. leucophylla* and *C. umbrosa* oils. (MICs 10-35 µL/mL). *B. subtilis* had the sensitivity for *C. umbrosa* (MIC 350 µL/mL), while *P. aeruginosa* to the *N. ciliaris* and *N. leucophylla* (MIC 10 µL/mL, each).

Group II is represented by *K. pneumonia* and *A. hyrdophilla*. These species were distinguishable in the PCA and formed a distinct group. It was considered as being the most registrnt to all the tested oils (MICs 30-60 µL/mL). The *A. hyrdophilla* was most resistant to the *N. clarkei* oil (MIC 60 µL/mL) and *K. pneumonia* to the *C. umbrosa* and *N. ciliaris* oils (MIC 55 µL/mL, each). Group III, which is formed by Gram-positive bacteria *S. scandidus*, was characterized by a relatively high sensitivity to all the oils, especially to *N. leucophylla* and *N. ciliaris* (MIC 5 µL/mL, each). This strain showed the low resistant to the other oils (MIC >20 µL/mL).

The *N. leucophylla* and *N. ciliaris* oils showed the highest activity against Gram-positive bacteria (*S. scandidus*) and Gram-negative bacteria (*P. aeruginosa*) than other *Nepeta* oils. The higher activity attributed to the presence of iridoid constituents [7].

Table 1: Antibacterial activity of essential oils extracted from the Lamiaceae species

Samples	Diameter of Inhibition Zone (mean ± SD) mm					
	Bacterial strains					
	P. aeruginosa	*B. subtilis*	*E. coli*	*A. hyrdophilla*	*K. pneumonia*	*S. candidus*
C. umbrosa	8.5 ± 0.8	10.5 ± 0.8	6.6 ± 0.5	6.8 ± 0.2	5.1 ± 0.2	10.5 ± 0.5
N. leucophylla	10.5 ± 0.8	6.3 ± 1.1	6.1 ± 0.7	5.6 ± 1.1	9.1 ± 0.7	6.6 ± 0.5
N. hindostana	7.8 ± 1.0	8.3 ± 1.1	6.8 ± 0.2	4.6 ± 0.5	5.3 ± 0.5	6.6 ± 0.5
N. ciliaris	9.6 ± 1.1	10.0 ± 1.0	5.3 ± 0.5	6.3 ± 1.1	5.6 ± 0.5	8.5 ± 0.8
N. clarkei	8.3 ± 1.1	9.6 ± 1.1	6.6 ± 0.5	7.0 ± 0.5	8.6 ± 0.5	10.8 ± 0.2
Gentamycin (Reference antibiotic)	20.3 ± 0.2	21.3 ± 0.1	27.7 ± 0.1	21.0 ± 0.3	25.3 ± 0.1	24.0 ± 0.1

Table 2: Minimum inhibitory concentration (MIC) (µL/mL) of essential oils extracted from Lamiaceae species against bacterial strains

Samples	MIC (µL/mL)					
	Bacterial strains					
	P. aeruginosa	*B. subtilis*	*E. coli*	*A. hyrdophilla*	*K. pneumonia*	*S. candidus*
C. umbrosa	30	35	25	30	55	25
N. leuchophylla	10	20	20	55	45	5
N. hindostana	35	30	40	50	40	10
N. ciliaris	10	20	10	45	55	5
N. clarkei	30	30	40	60	50	20
Gentamycin* (Reference antibiotic)	5	5	5	5	5	5

*MIC of antibiotic in µg/mL

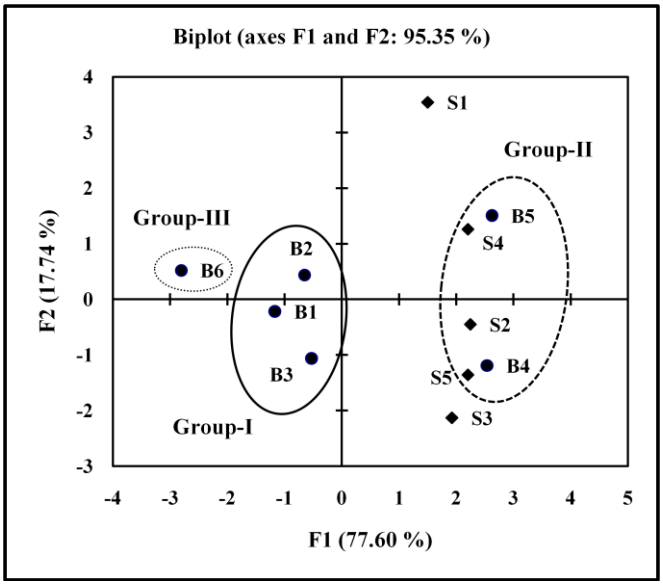

Fig. 1: PCA of the antimicrobial activity of essential oils of Lamiaceae plants against six bacteria *(*S1= *C. umbrosa;* S2= *N. leucophylla;* S3= *N. hindostana;* S4= *N. ciliaris;* S5= *N. clarkei;* B1= *P. aeruginosa;* B2= *B. subtilis;* B3= *E. coli;* B4= *A. hydrophila;* B5= *K. pneumonia;* B6= *S. scandidus)*

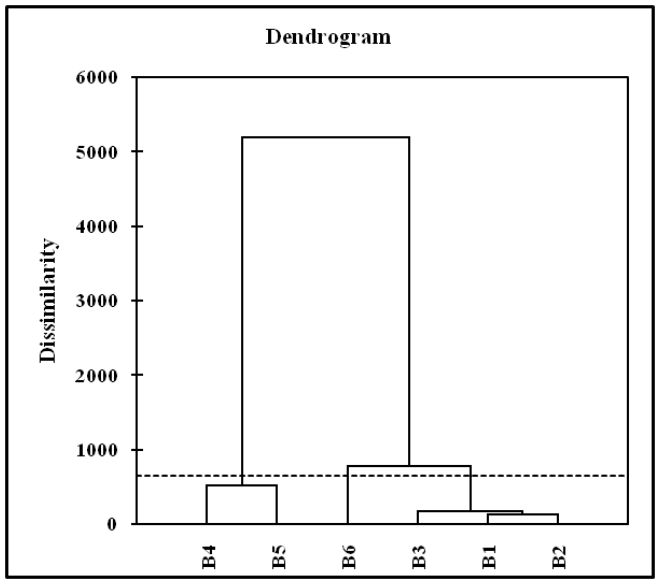

Fig. 2: HCA based on the Euclidean distance between groups of the antibacterial activity of essential oils of Lamiaceae species

CONCLUSION

The results of present study, PCA and HCA analysis of the antibacterial activity revealed that essential oils of *Nepeta* species had a strong and broad spectrum antibacterial effect against bacterial strains of *P. aeruginosa* and *S. scandidus*. The *N. leucophylla* oil showed higher activity among all *Nepeta* oils which may be due to presence of active antimicrobial iridoids compounds. The biological activities displayed by the essential oils showed their potential for the use of such species for traditional medicinal purposes. The species could also be a good natural source for the production of new antimicrobial chemicals.

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