Original Article

The antibacterial effect of methanolic fraction of *Nepeta depauperataon* against *Pseudomonas aeruginosa* isolates from burn wound infections

Mojdeh Hakemi-Vala[1*], Zahra Saifiabolhassan[2], Fatemeh Bagheri[2,3]

[1] Department of Microbiology, Medical school, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2] Islamic Azad University, Pharmaceutical Sciences Branch, Department of Microbiology, Tehran, Iran
[3] Department of Biotechnology, Iranian Research Organization for Science and Technology, Tehran, Iran

Received: 15 April, 2017; Accepted: 5 October, 2017

Abstract

Background: In this study, the antibacterial effect of the methanolic fraction of *Nepeta depauperata* against 50 isolates of *Pseudomonas aeruginosa* from burn wound infections of patients who referred to Shahid Motahari hospital of Tehran in 2014 was evaluated.

Materials and Methods: All bacterial isolates were confirmed by standard bacteriologic methods. Their resistant to common antibiotics were evaluated by disk diffusion method based on CLSI 2014. The *Nepeta depauperata* aerial parts were collected from Hormozgan Province and identified. Methanolic extract was prepared by maceration method using percolator apparatus and concentrated by rotary evaporator. The antibacterial activity of methanolic extract were determined by two methods; cup plate diffusion agar for determination the zone diameter of inhibition and microdilution broth for minimum inhibitory concentration (MIC) and further minimum bacteriocidal concentration (MBC). Statistical analysis was done by SPSS software version 20.

Results: The percentage of resistance and susceptibility against nine different kinds of common antibiotic disk showed 83% resistance on average as evaluated by agar disk diffusion (Kirby–Bauer antibiotic test). Also, the mean of inhibition zone diameters has been measured in concentrations of 1000, 500, 250 mg/ml as follow: 12.58, 11.3 and 9.44 mm, respectively by cup plate and the amount of 87.93 and 104.78 mg/ml for MIC and MBC were determined, respectively, using the broth microdilution method. Statistic analysis was done with SPSS verssion19 software.

Conclusion: According to the satisfying results of the antibacterial effect of the testing methanolic extract against clinical isolates of *P. aeruginosa* isolates further in vitro and in vivo studies are recommended.

Keywords: *Pseudomonas aeruginosa*, *Nepeta depauperata*, drug resistant, burn unit

***Corresponding Author:** Mojdeh Hakemi-Vala, Department of microbiology, Medical School, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Tel/Fax**:** (+98) 21 23872556; Email: m.hakemi@sbmu.ac.ir, mojdeh_hakemi@yahoo.com

Please cite this article as: Hakemi-Vala M, Saifiabolhassan Z, Bagheri F. The antibacterial effect of methanolic fraction of *Nepeta depauperataon* against *Pseudomonas aeruginosa* isolates from burn wound infections. Novel Biomed. 2018;6(2):61-7.

Introduction

Burn patients are at risk of acquiring infection because of their damaged skin resulting in the broken immune system. A common gram-negative bacterium, *Pseudomonas aeruginosa (P.aeruginosa)*, is associated with nosocomial infections, especially in immune-compromised patients, which is a leading

cause of morbidity and mortality among these patients[1]. One of the major concerns in treating the burn patients is drug resistance among bacteria.

Lamiaceae family and Nepeta genus belongs to the subfamily Nepetoideae and tribe Mentheae, which comprises about 300 herbaceous perennial and rarely annual species[2]. Iran, is a center of origin of this genus with sixty-seven species which are described by the common Persian name of "Pune-sa" and about 53% of endemics[3]. Noteworthy, several *Nepeta* spp. are used in many treatment procedures such as, folk medicine as diuretic, diaphoretic, antitussive, anti-inflammatory, antispasmodic, anti-asthmatic, febrifuge and sedative agents, and also, for antiseptic and astringent properties as a topical remedy in children with cutaneous eruptions and for snake and scorpion bites[2]. Scientists have become fascinated to the genus *Nepeta* because of its diversity, species richness and variation, as well as its chemical properties. For instance, Nepetalactones, iridoids and their glucosides, diterpenes, triterpenes and flavonoids have been reported as major constituents of *Nepeta* species[4]. For our study, we used *Nepeta depauperata*, locally called *"Oryan Punesa"*, one of the endemic perennial species distributed just in the south of Iran. It has beautiful flowers with a pleasant odor and it grows up to a height of about 40-80 centimeters[5]. It is extensively exploited as a medicinal plant in Iranian traditional medicine, especially in the case of inflammation and pain for alleviation of patients suffering rheumatism and inflammatory disorders. Moreover, *N.depauperata* extract is used as a disinfectant agent to treat the infected wounds as an Ethno-Pharmaceutical oil products. After investigation on the composition of the plant, spathulenol (31.84%), beta-caryophyllene (12.93%) and caryophyllene oxide (10.27%) were identified as the major components[6]. Researchers showed that *N. depauperata* total extract and sub-fractions had antibacterial effect on some gram negative and positive standard strains of bacteria[7].

Due to antibacterial effect of the methanolic extract and different fractions of its, flowering aerial parts against standard bacteria[7] as well as, the widespread use of *N. depauperata* in the Iranian folk medicine to relieve and treatment of infective disorders. We were prompted to investigate the antibacterial effect of the methanolic fraction of flowering aerial parts *Nepeta depauperata* on *Pseudomonas aeruginosa* isolates from burn wound infections of patients who referred to Motahari hospital of Tehran in 2014.

Methods

Plant collection: In this descriptive study, fresh flowering aerial parts of *N. depauperata* were collected from the mountain areas of the Genow protected region in west-north of Bandar Abbas city, Hormozgan Province, south of Iran: (27° 24'6.62" N 56° 10' 46.57" E, 1800m) on March 2014. The specimen was identified by Dr. R. Asadpour and voucher were deposited in the Herbarium of Pharmaceutical Sciences Branch, Islamic Azad University (IAUPS), Tehran under code number 1030-AUPF.

Extraction Procedure: In the beginning, an air-dried grounded plant (One kg) was extracted by percolator apparatus using methanol during the maceration method. Then, it was concentrated by rotary evaporator apparatus to remove the solvent, which has resulted in a dark green gummy solid (50 g). The extract was partitioned between ethyl acetate (13g), chloroform (17g) and methanol (20g) to yield different fractions by liquid-liquid method.

Bacterial identification: *Pseudomonas aeruginosa* (50 isolates) were isolated from wound infections of burn patients admitted to the Burn Unit of Shahid Motahari Hospital (Tehran, Iran) during 2014. The wound exudates were collected by swabbing and immediately transported to the microbiology laboratory of the Department of Microbiology of Shahid Beheshti University of Medical Sciences, Tehran, Iran. Isolation and identification were done using standard methods[8-9], and confirmed by the Microgen identification kit (Microgen™, UK).One standard type of *Pseudomonas aeruginosa* (PTCC 1074) which was obtained from the Persian type culture collection (PTCC) of Iranian Research Organization for Science and Technology was tested, simultaneously.

Antimicrobial susceptibility testing (AST): Determination of an antibiotic sensitivity test of common antibiotics against 50 clinical isolates of *Pseudomonas aeruginosa* were determined by the Kirby-Bauer disk diffusion method on Mueller Hinton

agar (Merck, Germany), based on the Clinical Laboratory Standards Institute (CLSI) guidelines 2012[10]. The antibiotics included imipenem (IPM: 10µg), amikacin (AK: 30µg), piperacillin/tazobactam (PTZ: 100/10µg), ciprofloxacin (CIP: 5µg), cefepime (FEP:30µg), aztreonam (ATM: 30µg), gentamicin (GEN:10µg), Piperacillin (PIP: 100µg) and Ticarcillin (TIC: 75µg) all purchased from Mast company, UK. *P. aeruginosa* PTCC1074 was used as a control strain.

Antibacterial activity by Cup plate diffusion agar: The antibacterial activities of the methanolic fraction of *N. depauperata* were investigated against *Pseudomonas aeruginosa* isolates from burn patients by the cup plate method[11]. An overnight bacterial culture with turbidity equal to 0.5 McFarland standard (1.5 x 10^8 CFU/ml) was used to culture on Mueller-Hinton agar plates. The wells were made on agar plates with 5mm diameter. Different concentrations, including 1000, 500, 250 mg of the methanolic fraction were dissolved in 1 ml DMSO (10%) and then filtered and 80 µl of each solution was added to each well. Ciprofloxacin powder (17850 Sigma-Aldrich) were used as a positive control and pure DMSO (10%) as a negative control, simultaneously. The plates incubated at 35±2 ºC for 24h. The diameter of zone of inhibitions was detected in each plate. The experiments carried out three times and the results were presented as mean±standard déviation.

Minimum Inhibitory Concentration (MIC) and minimum bactericidal concentration (MBC): The MIC of the methanolic fraction of *N.depauperata* against 50 isolates of *Pseudomonas aeruginosa* was determined by testing eight concentrations by broth microdilution method. The methanolic fraction was diluted to give concentrations of 250 mg/ml, 125 mg/ml, 62.5 mg/ml, 31.25 mg/ml, 15.62 mg/ml, 7.81 mg/ml, 3.90 mg/ml and 1.95 mg/ml. The lowest concentration of the extract that could inhibit the bacterial growth was considered as MIC[12]. In these experiments, Ciprofloxacin and pure DMSO (10%) were used as a positive and negative control, respectively.

Minimum bactericidal concentration (MBC) is the lowest concentration of an antibacterial agent required to kill a particular bacterium. It was determined from broth dilution minimum inhibitory concentration (MIC) test by subculturing to agar plates that do not contain the test agent. Like MIC, Ciprofloxacin and pure DMSO (10%) were used as a positive and a negative control, respectively.

Results

Antimicrobial susceptibility testing (AST): As it is shown in Table 1, the maximum sensitivity (19.6%) were belonged to Piperacillin 100µg/Tazobactam 10µg and Imipenem 10µg. Also, maximum resistance detected against gentamicin 10µg (96.1%).

Table 1: Antibiogram among tested *Pseudomonas aeruginosa*.

Antibiotics	Susceptible	Intermediate	Resistance
Piperacillin 100µg	5.9%	11.8%	82.4%
Ticarcillin 75 µg	5.9%	7.8%	86.3%
Piperacillin100 µg/Tazobactam 10 µg	19.6%	2.0%	78.4%
Cefepim 30 µg	9.8%	3.9%	86.3%
Azteronam30 µg	7.8%	21.6%	70.6%
Imipenem10 µg	19.6%	0.0%	80.4%
Gentamicin10 µg	3.9%	0.0%	96.1%
Amikacin30 µg	3.9%	9.8%	86.3%
Ciprofloxacin5 µg	o.o%	13.7%	86.3%

methanolic fraction of *N.depauperata* in the cup plated method.

P-Value	Ciprofloxacin(500mg/ml) positive control	DMSO 10% (negative control)	Methanolic fraction of *N. depauperata* in dilution 1000mg/ml	Methanolic fraction of *N. depauperata* in dilution 500mg/ml	Methanolic fraction of *N.depauperata* In dilution 250mg/ml
<0.0001	25.56±1.59	0.0±0.0	12.58±1.01		
<0.0001				11.3±2.97	
<0.0001					9.44±3.93

Table 3: The mean and standard deviation of Minimum Inhibitory Concentration (MIC) of the methanolic fraction of *N.depauperata* and ciprofloxacin (as a positive control) on clinical isolates of P*. aeruginosa*.

P- Value	Ciprofloxacin positive control dilution series 0.003-0.5mg/ml		Methanolic fraction of *N. depauperata* in dilution series 3.91-500mg/ml		DMSO 10% (negative control)	
<0.0001	Standard deviation	mean	Standard deviation	mean	Standard deviation	mean
	0.016	0.048	37.1	87.93	0.0	0.0

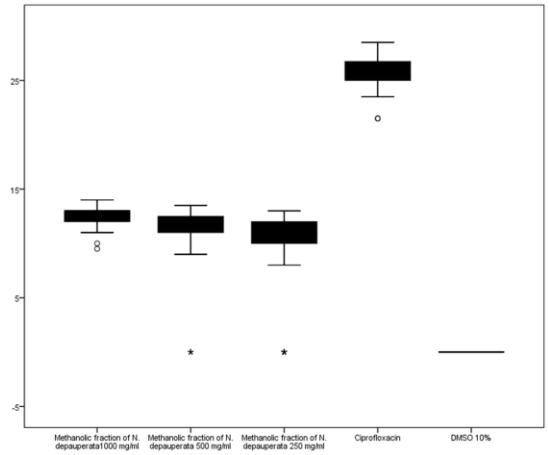

Figure 1. Comparison effect of methanolic fraction of *N.depauperata* in dilutions 1000, 500, 250 mg/ml and Ciprofloxacin in dilution 500 mg/ml as a positive control and DMSO 10% as a negative control in the well diffusion method.

The antibacterial activity of the methanolic fraction of *N. depauperata* was determined based on the cup plate method in a dilution series of 1000, 500, 250 mg/ml concentrations against fifty isolates of gram-negative *Pseudomonas aeruginosa*. The mean of inhibition zone diameters has been measured in concentrations of 1000, 500, 250 mg/ml as follow: 12.58, 11.3 and 9.44 mm, respectively. These results compared with Ciprofloxacin as the positive control were significant. As it is shown in both (Table 2, Figure 1), all prepared concentrations of methanolic fraction had an inhibitory activity on the growth of *Pseudomonas aeruginosa* isolates from burn patients.

MIC and MBC: The MIC and MBC of the methanolic fraction of *N. depauperata* against 50 clinical isolates of *P. aeruginosa* are presented in Tables 3 and 4 and Figures 2 and 5.

Mean and standard deviation of MIC and MBC of the methanolic fraction of *N. depauperata* on *P. aeruginosa* were 87.9337.1 and 104.7833.55 mg/ml,

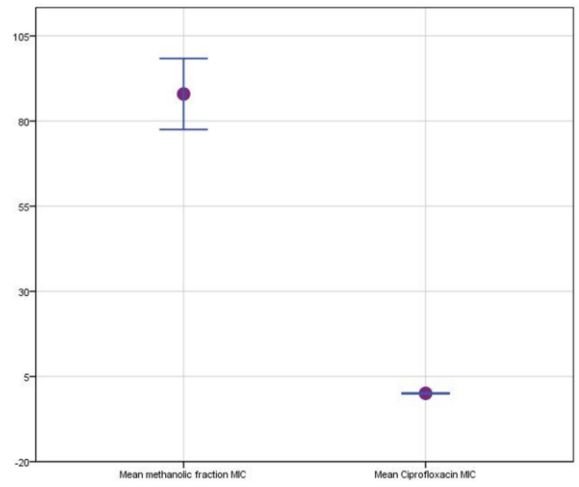

Figure 2. The mean and standard deviation of Minimum Inhibitory Concentration (MIC) of the methanolic fraction of *N.depauperata* using Ciprofloxacin as a positive control for *Pseudomonas aeruginosa*.

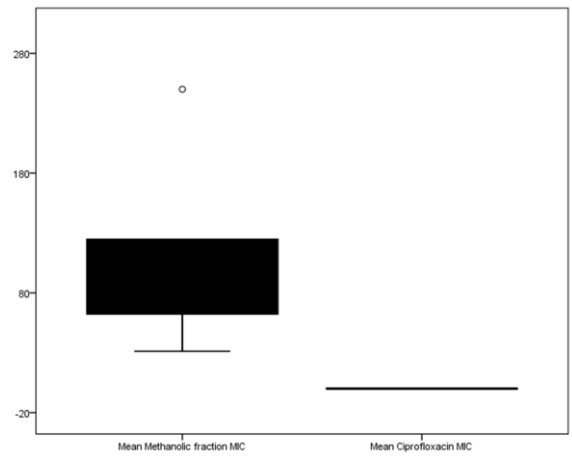

Figure 3. The minimum Inhibitory Concentration (MIC) of the methanolic fraction of *N.depauperata* using Ciprofloxacin as a positive control for fifty cells of *Pseudomonas aeruginosa* isolated from burn patients.

Assay antibacterial effect by Cup plate method:

respectively. Similarly, ciprofloxacin and DMSO 10% were tested as a positive control as a negative control, simultaneously.

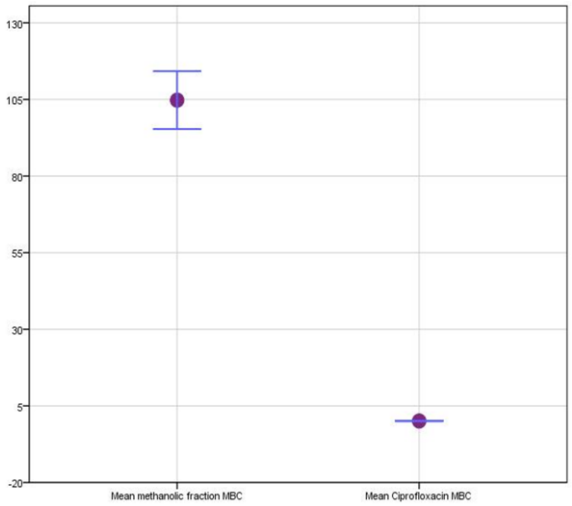

Figure 4. The mean and standard deviation of Minimum Bactriocidal Concentration (MBC) of the methanolic fraction of *N.depauperata* using Ciprofloxacin as a positive control for *Pseudomonas aeruginosa*.

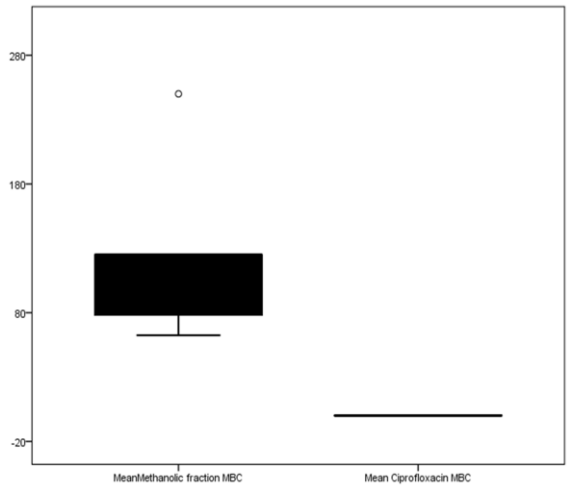

Figure 5. The minimum Bactericidal Concentration (MBC) of the methanolic fraction of *N. depauperata* and ciprofloxacin (as

Discussion

The occurrence of bacterial diseases is a serious problem of the present world, which is the result of an antibacterial drug resistance of the pathogens and the side effects exhibited by the drugs used for bacterial diseases. Hence, there is a great demand for safer, alternative and effective chemotherapeutic agents such as using medicinal herbs in the treatment of bacterial infections[13]. Plants contain a spectrum of secondary metabolites that their importance as an antimicrobial or antifungal agent has been emphasized by several works[14].

Literature surveys revealed that the other *Nepeta* species had significant antibacterial activities[15-20]. Phytobiological evaluation of *Nepeta* species displayed that its essential oil has an antibacterial properties.

All investigations have been done directly on the extracted oils and low concentrations of the oils had inhibition growth on the tested organisms by large inhibition zone diameter. Despite strong odor of oils, this is very low for *N. depauperat*, so that it makes it easier to use.

That is to say that the oil components extracted in methanol and non-polar and semi-polar solvents like chloroform so that the main compounds, typically responsible for antibacterial and antifungal properties in *Nepeta* species could be terpenoids. Regarding the previous phytochemical research done on *N. depauperata* essential oil, spathulenol characterized as the main component of the oil[6].

Based on assay of antibacterial effect by cup plate method, our results showed that *Nepeta depauperata* extract has an antibacterial effect like the other species of *lamiacea* so that it can be used as a potent antibiotic for the infection treatment. Moreover, we found that *Nepeta depauperata* extract was less effective on growth inhibition of *Pseudomonas aeruginosa* isolated from burn wound infections compare to standard

Table 4: The mean and standard deviation of Minimum Bactriocidal Concentration (MBC) of the methanolic fraction of *N.depauperata* and ciprofloxacin (as a positive control) against 50 clinical isolates of *Pseudomonas aeruginosa* from burn infections.

P- Value	Ciprofloxacin positive control dilution series 0.003-0.5mg/ml		Methanolic fraction of *N. depauperata* in dilution series 3.91-500mg/ml		DMSO 10% (negative control)	
<0.0001	Standard deviation	mean	Standard deviation	mean	Standard deviation	mean
	0.031	0.08	33.55	104.78	0.0	0.0

Pseudomonas aeruginosa as evidenced by the systematic lower mean of inhibition zone diameters. We hypothesized that it may be due to the acquired antibiotic resistance of *Pseudomonas aeruginosa* isolated from burn wound infections. Also, the antibacterial effect of methanolic extract of *Nepeta depauperata* was lower than ciprofloxacin even against standard strain .

This simply relates to purity of ciprofloxacin in comparison to the total non-pure methanolic extract of this herb.

Conclusion

According to the satisfying results of antibacterial effect of tested methanolic extract against clinical isolates of *Pseudomonas aeruginosa*, further analytical techniques like mass spectrometry as well as *in vivo* complementation studies are recommended to characterize the effective chemical component(s) of the extract and the mechanism of their antibacterial activity.

Acknowledgment

The authors like to say their thanks to the personnel of Motahari hospital, for their assistance during bacterial collection. Also, our thanks to Pharmaceutical sciences branch, Islamic Azad university for their support during the extract preparation and foundation of this student project.

Also, the authors like to say their thanks to Mr. Peyman Kavakeb for his assistance during the grammer revision. This study was supported by a grant of Pediatric Infectious Research Center, Shahid Beheshti University of Medical Sciences and approved by their ethical committee on 2013 (ID; 390).

References

1. Vahdani M, Azimi L, Asghari B, Bazmi F, Rastegar Lari A. Phenotypic screening of extended-spectrum ß-lactamase and metallo-ß-lactamase in multidrug-resistant Pseudomonas aeruginosa from infected burns. Ann Burns Fire Disasters. 2012;25:78-81.

2. Formisano C, Rigano D, Senatore F. Chemical constituents and biological ctivities of Nepeta species. Chem Biodivers. 2011;8:1783-818.

3. Jamzad Z, Grayer RJ, Kite GC, Simmonds MSJ, Ingrouille M, Jalili A. Leaf surface flavonoids in Iranian species of Nepeta (Lamiaceae) and some related genera. Biochem Syst Ecol. 2003;31:587-600.

4. Asgarpanah J, Sarabian S, Ziarati P. Essential oil of Nepeta genus (Lamiaceae) from Iran: a review. J Essent Oil Res. 2014;26(1):1-12.

5. Mozaffarian V. A Dictionary of Iranian Plants Names. Tehran: Farhang Moaser Press; 1995.

6. Mehrabani M, Asadipour A, Saber-Amoli S. Chemical constituents of the essential oil of Nepetadepauperata from Iran. Daru J Pharm Sci. 2004;12:98-100.

7. Kariminejad S, Abdnikfarjam M, Hosseini Doust R, Hakemi-Vala M, Asgarpanah J. Antibacterial and antifungal activities of the endemic species Nepeta depauperata Benth from Iran. Ethno-Pharmaceutical products. 2014:9-13.

8. Shahcheraghi F, Abbasalipour M, Feizabadi M, et al. Isolation and genetic characterization of metallo-beta-lactamase and carbapenamase producing strains of Acinetobacter baumannii from patients at Tehran hospitals. Iran J Microbiol. 2011;3:68-74.

9. Fallah F, Borhan RS, Hashemi A: Detection of bla(IMP) and bla(VIM) metallo-beta-lactamases genes among Pseudomonas aeruginosa strains. Int J Burns Trauma. 2013;3:122-4.

10. Wayne PC. Clinical and Laboratory Standards Institute (CLSI): Performance standards for antimicrobial susceptibility testing. Twenty-second informational supplement. Document M100-S22; Pennynsylvania, USA; 2012.

11. Fazly-Bazzaz BS, Khajehkaramadin M, Shokooheizadeh HR. Antibacterial activity of Rheum ribes extract obtained from various plant parts against clinical isolates of Gram-negative pathogens. Iran J Pharm Res. 2005;2:87-91.

12. Mehregan H, Mojab F, Pakdaman SH, Poursaeed M. Antibacterial activity of Thymus pubescencemethanolic extract. Iran J Pharm Res. 2008;7(4):291-5.

13. Irobi ON, Darambolo SO. Antifungal activity of crude extracts of Mitracarpusvillosus (Rubiaceae). J Ethnopharmacol. 1993;40:137-40.

14. Vaijayantimala J, Rajendra-Prasad N, Pugalendi KV. Antifungal activity of oils. Ind J Microbiol. 2001;41:325-8.

15. Sonboli A, Gholipour A, Yousefzadi M, Mojarrad M. Antibacterial activity and composition of the essential oil of Nepetamenthoides from Iran. Nat Prod Commun. 2009;4(2):283-6.

16. Gautam SS, Navneet S, Kumar S, Prabhat A. Screening of antibacteial activity of NepetaciliarisBenth. against respiratory tract pathogens. Kathmandu Uni J SciEngeen Technol. 2012;8(1):100-3.

17. Nezhadali A, Masrornia M, Bari H, Akbarpour M, Joharchi MH, Nakhaei-Moghadam M.Antibacterial activity and composition of essential oil of Nepetapungens Benth from Iran. J Essent. Oil Bear Pl. 2011;14(2):241-4.

18. Grbic ML, Stupar M, Vukojevic J, Sokovic M, Misic D, Grubisic D, Ristic M. Antifungal activity of Nepeta rtanjensis essential oil. J Serb Chem Soc. 2008;73(10):961-5.

19. Sexena J, Mathela CS. Antifungal activity of new compounds

from Nepetaleucophylla and Nepetaclarkei. Appl Environ Microbiol. 1996;62(2):702-4.
20. Kordali S, Usanmaz A, Cakir A, Cavusoðlu A, Ercisli S. In Vitro antifungal effect of essential oils from Nepeta meyeri Benth. Egyp J Biol Pest Cont. 2013;9(3):404-18.