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Antibacterial and antifungal activities of the endemic species *Nepeta depauperata* Benth

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Abstract

Nepeta depauperata belongs to the Lamiaceae family and is one the Persian endemic plants which has not been investigated biologically. In the present paper we had focused on the assessment of the antibacterial and antifungal activities of the total methanolic extract and different sub-fraction of the flowering aerial parts of it. The Antibacterial and antifungal activities were investigated by cup plate method and disc diffusion assay, respectively. The minimum inhibitory concentrations and minimum bactericidal concentrations of the active extract or subfraction were determined by micro plate dilution method. The crude extract and chloroform sub-fraction of *N. depauperata* had inhibition activity on the growth of *Staphylococcus aureus, Bacillus subtilis* and *Pseudomonas aeruginosa* while no antibacterial activity observed against *Staphylococcus epidermidis, Escherichia coli* and *Salmonella typhi*. It was concluded from the antifungal assay that just the yeast *C. albicans*, showed a high sensitivity to all the extract and related sub-fractions. No activity was seen against *Aspergillus niger, A. fumigatus, A. flavus* and *Fusarium oxysporum*. These findings demonstrate that the *N. depauperata* is effective against *S. aureus, B. subtilis* and *P. aeruginosa* and could be a natural source of effective natural antifungal compounds against *C. albicans*.

Introduction

One of the largest genera of the Lamiaceae family, *Nepeta* genus belongs to the subfamily Nepetoideae and tribe Mentheae, which comprises about 300 herbaceous perennial and rarely annual species (Formisano et al., 2011). Iran, particularly, is one of the centers of origin of the genus with sixty-seven species, described by the common Persian name of 'Pune-sa' and about 53% of endemics (Jamzad et al., 2003). Several Nepeta spp. are used in folk medicine as diuretic, diaphoretic, antitussive,antiinflammatory, antispasmodic, anti-asthmatic, febrifuge and sedative agents, and for antiseptic and astringent properties as a topical remedy in children with cutaneous eruptions, and for snake and scorpion bites (Formisano et al., 2011). The diversity, species richness and variation, as well as the chemical properties have led many scientists do research on the genus *Nepeta*. Nepetalactones, iridoids and their glucosides, diterpenes, triterpenes and flavonoids have been reported as major constituents of Nepeta species (Asgarpanah et al., 2014).

*Nepetadepauperata*Benth.is one of the endemic perennial species distributed just in south of Iran. It has beautiful flowers with a pleasant odor and it grows up to a height of about 40–80 cm (Mozaffarian., 1995).

N. depauperata which is locally called "Oryan Pune-sa" is one of the endemic ones in south of Iran and extensively exploited as a medicinal plant in Iranian traditional medicine (Mozaffarian., 1995). It is used to relieve of snake, scorpion and wasp bites, and for wound healing. It is also believed to be very effective in the treatment of inflammation and relieve of pain and is used orally and topically by patients suffering rheumatism and inflammatory disorders. *N. depauperata* extract is used as a disinfectant agent to treat the infected wounds. The essential oil chemical

composition of this plant has been investigated and spathulenol (31.84%), beta caryophyllene (12.93%) and caryophyllene oxide (10.27%) were identified as the major components of the oil (Mehrabani et al., 2004).

Due to the widespread use of *N. depauperata* in the Iranian folk medicine to relieve and treatment of infective disorders, we were prompted to evaluate the antibacterial and antifungal activities of the methanolic extract and different sub-fractions of its flowering aerial parts and investigate the biological basis for the folkloric use of this plant as an antiseptic and antifungal agent.

Materials and Methods

- **Plant material**

Fresh flowering aerial parts of *N. depauperata* were collected in March 2013 from the mountain areas of the Genow protected region in 30 Km west north of Bandar Abbas, Hormozgan Province, Iran: (27° 24' 6.62" N 56° 10' 46.57" E, 1800m). Specimen was identified by R. Asadpour and voucher was deposited in the Herbarium of Pharmaceutical Sciences Branch, Islamic Azad University (IAU), Tehran under code number 1030-AUPF.

- **Extraction Procedure**

1 kg of the air-dried grounded plant was extracted by percolator apparatus using methanol. The extraction was repeated for 3 times. The extract was concentrated by rotary evaporator apparatus and the solvent was removed to produce a dark green gummy solid (75g). The adequate part of the resulting extract was kept in a sterile vial in a dark and cool place for further tests and the remains were partitioned between water (20g), chloroform (38g) and methanol (17g) to yield different fractions. We also tried to have ethyl acetate sub-fraction but the plant did not yield it.

- **Test Organisms**

Gram-positive bacteria including *Staphylococcus aureus* (PTCC25923), *Staphylococcus epidermidis* (PTCC12228) and *Bacillus subtilis* (PTCC6633), and Gram-negative bacteria including *Escherichia coli* (PTCC25922), *Salmonella typhi* (PTCC1609) and *Pseudomonas aeruginosa* (PTCC25823) were obtained from the Persian type culture collection (PTCC) of Iranian Research Organization for Science and Technology.

Fungal strains including *Aspergillusfumigatus, A. flavus, A. niger, Fusariumoxysporum* and the yeast *Candida albicans* were the five different clinical isolates of pathogenic fungi and yeast taken for this study.

- **Antibacterial activity**

Antibacterial activities of the methanolic crud extract and sub-fractions of *N. depauperata* were investigated against 6 bacterial strains by the cup plate method (Fazly-Bazzaz et al., 2005). An overnight bacterial culture equal to 0.5 McFarland standard (1.5 x 108 CFU/ml) was used to culture on Muller-Hinton agar plates. The wells were made on agar plates with 5mm diameter. 1000, 500, 250 and 125 mg of the methanol extract and the fractions were separately dissolved in 1 ml DMSO (10%) and then filtered and 80 µl of each solution was added to each well. Ciprofloxacin (40 mg/ml) and Gentamycin (40 mg/ml) were used as positive controls for Gram-positive and Gram-negative microorganisms, respectively. 80 µl of pure DMSO (10%) served as negative control. The plates incubated at37ºC for 24h .The diameter of zone of inhibitions was detected in each plate. The experiments carried out 3 times and the results were presented as mean±sd.

- **Minimum Inhibitory Concentration (MIC)**

After confirmation the antibacterial activity in the extract and the obtained sub-fractions, MIC of each was determine by testing 10 concentrations of the extract and every fractions against sensitive Gram-positive and Gram-negative tested bacteria by the micro plate dilution method. The reconstituted extract 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, 1.95 mg/ml, 0.97 mg/ml and 0.48 mg/ml. The lowest concentration of the extract that could inhibit the bacterial growth was considered as MIC (Mehregan et al., 2008). As the same, Erythromycin and Gentamycin, and pure DMSO (10%) were used as positive and negative controls, respectively.

- **Antifungal assay**

The total extract and sub-fractions were prepared by dissolving in their specific solvents (methanolic, chloroform and water), then they were loaded into blank paper disks at omit concentrations of 8 mg/disc, 4 mg/disc, 2 mg/disc, 1 mg/disc and 0.5 mg/disc. Ketoconazole (10 µg/disc) was used as positive control.

The isolates were transferred from DW (distilled water) stocks to Mycosel agar and then sub-cultured to Potato dextrose agar (Merck, Germany) to enhance sporulation. Seven day-old cultures were covered with 1ml DW and the colonies were probed with the tip of a sterile Pasteur pipette to obtain a mixture of mycelium and conidia. The suspensions were transferred to sterile tubes and allowed to sediment for 30 minutes and it was then adjusted with a spectrophotometer set at 65% transmittance and 530nm (Esteban et al., 2005).

All the tests were performed according to Esteban et al. (Esteban et al., 2005). The inoculum was evenly spread on the surface of 10cm Petri dishes containing Sabouraud dextrose agar medium (Merck, Germany) and exposed to air dry. Then, the antifungal disks were applied to the plates, after which the plates were incubated at 25°C for 5-10 days. After the colonies grew, the zones of inhibition around the disks were measured and recorded. All tests were performed in triplicate and, Microsoft SPSS was used for data analysis (Pakshir et al., 2009).

Results

As the endemic species *N. depauperata* is used widely as a disinfectant agent in the Iranian traditional medicine, we prompted to evaluate the antibacterial and antifungal activities of the plant against eleven bacterial or fungal strains. The crude extract and the chloroform sub-fraction had inhibition activity on the growth of *S. aureus*, *B. subtilis* and *P. aeruginosa* while no antibacterial activity observed against *S. epidermidis*, *E. coli* and *S. typhi*. Methanolic sub-fractions showed activity against the *P. aeruginosa*. The inhibition zone diameters of the active ones were measured in the range of 10-18 mm comparing with the positive standards, Gentamicine and Erythromycin (30-40 mm) (Table 1).

According to the table 3 all the crude extract and the sub-fractions were completely inactive against the investigated fungal strains except the yeast *C. albicans*. It can be observed that *C. albicans* showed a high sensitivity to all of the tested extract and sub-fractions by inhibition zone diameters 4.5-18.3 mm.

Table1. The inhibition zone diameter (mm) of total extract and sub-fractions of *N. depauperata*[a].

	Diameter of zone of inhibition (mm)																	
	MTE (mg/ml)				CSF (mg/ml)				MSF (mg/ml)				ASF (mg/ml)				Gen.	Eryth.
	1000	500	250	125	1000	500	250	125	1000	500	250	125	1000	500	250	125		
S. aureus	12.7	12.3	11.7	10.0	13.0	12.3	12.0	12.0	-	-	-	-	-	-	-	-	-	40.0
S .epidermidis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	30.0
B. subtilis	13.3	12.3	11.7	11.0	12.3	12.3	10.0	10.0	-	-	-	-	-	-	-	-	-	35.0
E. coli	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	40.0	-
S. typhi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	40.0	-
P. aeruginosa	13.3	13.3	13.0	11.7	18.0	17.7	17.0	16.7	14.3	11.0	10.7	8.0	-	-	-	-	40.0	-

[a]Zone of inhibition, including the diameter of the well (6mm); mean value of three independent experiments.
MTE=Methanol total extract; CSF=Chloroform sub-fraction; MSF=Methanol sub-fraction; ASF=Aqueous sub-fraction
Gen.=Gentamycin; Eryth.=Erythromycin

MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) values of the active extract and fractions determined for the sensitive strains (Table 2). The results of antifungal assay revealed that all the investigate extract and fractions were active just against *C. albicans* and the result was that the plant is totally inactive against *A. niger*, *A. fumigatus*, *A. flavus* and *F. oxysporum* (Table 3). MIC and MFC values of the extract and all three sub-fractions against *C. albicans* are presented in table 4.

Table 2. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of total extract and sub-fractions of *N. depauperata*[a].

	MIC (mg/ml)					**MBC** (mg/ml)				
	MTE	CSF	MSF	Gen.	Eryth.	MTE	CSF	MSF	Gen.	Eryth
S. aureus	500	1000	ND	-	250	>1000	>1000	ND	-	500
B. subtilis	1000	1000	ND	-	250	>1000	>1000	ND	-	500
P. aeroginosae	1000	1000	500	1000	-	>1000	>1000	>1000	>1000	-

[a]All determinations were done in triplicate.
MTE=Methanol total extract; CSF=Chloroform sub-fraction; MSF=Methanol sub-fraction; ASF=Aqueous sub-fraction.
ND=Not determined

Table 3. The inhibition zone diameter of total extract and sub-fractions of *N. depauperata* [a].

	Diameter of zone of inhibition (mm)																				
	MTE (mg/disc)					CSF (mg/disc)					MSF (mg/disc)					ASF (mg/disc)					ketoc. (µg/disc)
	8	4	2	1	0.5	8	4	2	1	0.5	8	4	2	1	0.5	8	4	2	1	0.5	10
C. albicans	17.3	15.1	13.0	11.5	4.5	18.3	18.0	17.3	16.6	15.7	18.1	17.3	15.2	14.3	11.8	16.0	14.8	11.3	9.5	8.6	55

[a]Zone of inhibition, including the diameter of the well (6mm); mean value of three independent experiments.
MTE=Methanol total extract; CSF=Chloroform sub-fraction; MSF=Methanol sub-fraction; ASF=Aqueous sub-fraction
Ketoc.=Ketoconazol

Table 4. Minimum inhibitory concentration (MIC) and Minimum fungicide concentration (MBC) of total extract and sub-fractions of *N. depauperata*[a].

	MIC (mg/ml)					**MFC** (mg/ml)				
	MTE	CSF	MSF	ASF	Ketoc.	MTE	CSF	MSF	ASF	Ketoc.
C. albicans	2.5	0.6	1.2	10	0.004	20	10	10	20	0.06

[a]All determinations were done in triplicate.
MTE=Methanol total extract; CSF=Chloroform sub-fraction; MSF=Methanol sub-fraction; ASF=Aqueous sub-fraction

Discussion

Occurrence of bacterial and fungal diseases is a serious problem of the present world. This is because of the development of antibacterial and antifungal drug resistance of the pathogens and side effects exhibited by the drugs used for bacterial and fungal diseases. Hence there is a great demand safer, alternative and effective chemotherapeutic agents. Use of medicinal herbs in the treatment of bacterial and fungal infections is an old practice in many parts of the world (Irobi&Darambolo., 1993). Plants contain a spectrum of secondary metabolites that their importance as antimicrobial or antifungal agents has been emphasized by several works (Vaijayantimala et al., 2001).

Literature survey revealed that the other *Nepeta* species had significant antibacterial and antifungal activities (Sonboli et al., 2009; Gautam et al., 2012; Nezhadali et al., 2001; Grbic et al., 2008; Sexana& Mathela.,1996; Kordali et al., 2013). Phytobiological evaluation of *Nepeta* species displayed that the essential oil contained in these plants have marked antibacterial and antifungal properties. All investigations have been done directly on the extracted oils and low concentrations of the oils had inhibition growth on tested organisms by large inhibition zone diameter (12-17mm). Despite of strong odor made by *N. depauperata*, oil bearing in this plant is very low and we had to investigate the activities of the extract and related sub-fractions. It was expectable because the oil components could be extracted in methanol and non-polar and semi-polar solvents like chloroform. It could be concluded that the main compounds, typically responsible for antibacterial and antifungal properties in *Nepeta* species were terpenoids and the oil components. Regarding to the previous phytochemical research done on *N. depauperata* essential oil, spathulenol characterized as the main component of the oil (Mehrabani et al., 2004). It could be considered as one of the active components responsible for the antibacterial or antifungal activities but regarding the low amount of essential oil yielded from the aerial parts of the plant, future bioassay-guided phytochemical investigations suggests to determine the main antibacterial and antifungal compound(s) in the plant.

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References

1. 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.

2. Esteban A, Abarca ML, Cabanes FJ. Comparison of disk diffusion method and broth microdillution method for antifungal susceptibility testing of dermatophytes. Med Mycol. 2005; 43: 61- 66.

3. 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.

4. Formisano C, Rigano D, Senatore F. Chemical constituents and biological activities of *Nepeta*species. Chem Biodivers. 2011; 8: 1783–1818.

5. Gautam SS, Navneet S, Kumar S, Prabhat A. Screening of antibacterla activity of *Nepetaciliaris*Benth. against respiratory tract pathogens. Kathmandu Uni J SciEngeen Technol. 2012; 8(1): 100-103.

6. Grbic ML, Stupar M, Vukojevic J, Sokovic M, Misic D, Grubisic D, Ristic M. Antifungal activity of *Nepetartanjensis* essential oil. J Serb Chem Soc. 2008; 73(10): 961-965.

7. Irobi ON, Darambolo SO. Antifungal activity of crude extracts of *Mitracarpusvillosus* (Rubiaceae). J Ethnopharmacol.1993; 40: 137-140.

8. 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.

9. Kordali S, Usanmaz A, Cakir A, Cavusoğlu A, Ercisli S. In Vitro antifungal effect of essential oils from *Nepetameyeri* Benth. Egyp J Biol Pest Cont. 2013; 23(2): 209.

10. 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.

11. Mehregan H, Mojab F, Pakdaman SH, Poursaeed M. Antibacterial activity of *Thymus pubescence*methanolic extract. Iran J Pharm Res. 2008; 7(4): 291-295.

12. Mozaffarian V. A Dictionary of Iranian Plants Names. Tehran: Farhang Moaser Press, 1995.

13. 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): 241-244.

14. Pakshir K, Bahaedinie L, Rezaei Z, Sodaifi M, Zomorodian K. In vitro activity of six antifungal drugs against clinically important dermatophytes. Jundishapur J Microbiol. 2009; 2(4): 158-163.

15. Sexena J, Mathela CS. Antifungal activity of new compounds from *Nepetaleucophylla* and *Nepetaclarkei*. Appl Environ Microbiol.1996; 62(2): 702-704.

16. 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): 283-286.

17. Vaijayantimala J, Rajendra-Prasad N, Pugalendi KV. Antifungal activity of oils. Ind J Microbiol. 2001; 41: 325-328.