ECOLOGIA BALKANICA
2024, Vol. 16, Issue 1 June 2024 pp. 167-173

Solvent-dependent antibacterial activity of Nepeta nuda leaf extracts

Detelina Petrova[1*], Tereza Georgieva[1], Ganka Chaneva[1], Miroslava Zhiponova[1]

[1]Sofia University, Faculty of Biology, Department of Plant Physiology, Dragan Tsankov Blvd. 8, 1164 Sofia, BULGARIA
*Corresponding author: detelina@biofac.uni-sofia.bg

Abstract. The medicinal plant *Nepeta nuda* L. (known as 'naked catmint') is characterized by various biological activities due to the high content of terpenes, iridoids and phenolic compounds. In the present study, we analysed the antimicrobial activity of *N. nuda* leaf extracts against 16 human pathogenic microorganisms. The antimicrobial efficiency of *N. nuda* extracts was investigated depending on their preparation by applying primary extraction solvents (chloroform/Chl and methanol/Met), and the respective secondary solvents for dissolving the dry primary extracts (Chl, Met), as well as H_2O and dimethyl sulfoxide/DMSO. The applied disk-diffusion method allowed evaporation of the solvent prior contact to the bacteria, and the effect of extracted *N. nuda* polar and non-polar secondary metabolites could be evaluated. The dry Met and Chl extracts showed good solubility in the respective secondary solvents Met, Chl and DMSO, while H_2O appeared as inappropriate solvent. The leaf extracts showed activity to 6 of the tested 15 bacteria and no antifungal activity was detected. High inhibitory effect was established when using Met and Chl solvents, while the H_2O extracts were inactive. The bacteria *Staphylococcus aureus* and *Acinetobacter calcoaceticus* showed the strongest sensitivity to Chl extracts. The most susceptible to exposure of Met extracts turned out to be *Bacillus subtilis* and *A. calcoacetisus*. We concluded that the use of solvents with different polarity succeeded to extract *N. nuda* antimicrobial substances. Therefore, the obtained extracts could be further explored for application as biocontrol agents, natural preservatives and for medicinal purposes.

Key words: *Nepeta nuda* extracts, antimicrobial activity, chloroform, methanol, solvents.

Introduction

Medicinal plants have been well-known as natural sources of remedies for the treatment of various diseases since ancient times. A range of medicinal plants parts – flowers, leaves, stems, fruits, roots, twigs exudates, possess medicinal properties and are used as raw drugs (Gowri et al., 2016). According to a report by the World Health Organization (WHO), nearly 20 000 plants species are currently being used for medicinal purposes (Zare et al., 2012).

The excessive use of antibiotics worldwide has resulted in increased resistance of bacteria to them, and on the other hand, many antibiotics can also cause numerous unfavorable side effects in humans (Fair & Tor, 2014). It has been reported that plants have been considered as one of the most searched sources for discovery of alternative antimicrobial agents (Ginovyan et al., 2017). Herbal-origin medicines are used by about 75-80% of whole population and involve plant extracts and their active constituents (Akerele, 1993), such as phenolics, alkaloids, flavonoids, terpenoids, tannins and others reported to have antibacterial activities (Adekunle & Adekunle, 2009; Lewis & Ausubel, 2006). Despite testing hundreds of plants

for antimicrobial properties, there is still a huge part of them that have not been effectively evaluated.

Nepeta is a large genus of the family Lamiaceae that comprise about 250 species, which are distributed in Europe, Asia and North Africa. In the literature, the *Nepeta* genus is characterized by the presence of monoterpene nepetalactones, other terpenoids, iridoids, steroids and flavonoids (Aćimović et al., 2022; Köskal et al., 2017; Nadeem et al., 2022; Petrova et al., 2022; Teber & Bursal, 2020; Zaharieva et al., 2023). *Nepeta nuda* subsp. *nuda* L. (known as 'naked catmint') is one of the most common species of genus *Nepeta* and it is a valuable medicinal plant distributed in Bulgaria. Due to the high content of terpenes, iridoids and phenolic compounds, *N. nuda* is characterized by various biological activities (Aćimović et al., 2022; Petrova et al., 2022; Zaharieva et al., 2023). Our previous studies demonstrated antibacterial activities of *N. nuda* against the Gram (-) bacteria *Acinetobacter calcoaceticus* and *Klebsiella pneumoniae*, and the Gram (+) bacteria *Bacillus cereus* and *Staphylococcus aureus* (Petrova et al., 2022). The extracts were prepared by the Soxhlet apparatus by subsequent extraction with chloroform (Chl) and methanol (Met). The Met extract enriched in phenolics and iridoids, was dissolved in 5% dimethyl sulfoxide (DMSO) and demonstrated to have antibacterial potential.

In the present study, we expanded our research by exploring the anti-microbial activity of *N. nuda* leaf extracts against a total of 16 human pathogenic microorganisms. The work was focused on investigating the efficiency of the extracts depending on their prepa-ration by applying primary extraction solvents (Chl, Met) and secondary solvents for dissolving the dry primary extracts (H_2O, Chl, Met, DMSO).

Materials and Methods

Plant material and preparation of plant extracts

Nepeta nuda subsp. *nuda* L. plants were collected from their natural habitat located in Bekovi skali (nearly 1320 a.s.l.), Rhodopes Mt. [41.99437188774722, 24.396310265460215], Bulgaria, during the flowering period. A voucher specimen, SO108229, has been deposited at the Herbarium of Sofia University "St Kliment Ohridski", Sofia, Bulgaria.

Nepeta nuda plants were dried at room temperature to constant weight and then ground to a powder. Extraction procedure by maceration was applied as 10 g of leaf material was placed in a flask and extracted initially with 100 ml of Chl for 24 h at a temperature of 60°C in a thermostat. After filtration, the dried plant material was incubated with 100 ml of Met in a thermostat at 60°C for 24 h. The resulting Chl and Met extracts were evaporated by a vacuum evaporator (VWR, IKA, RV 10 D S93, Staufen, Germany) at 40°C to dry weight. The obtained primary Chl and Met extracts were measured and dissolved by 5% DMSO to 200 mg/ml concentration for screening the antimicrobial activity. Then, to evaluate the antimicrobial efficiency after application of various secondary solvents (to 200 mg/ml final concentration), the dry Chl extracts were dissolved in Chl, DMSO or H_2O: a/ Chl (Chl); b/ Chl (DMSO); c/ Chl (H_2O). The Met extracts were dissolved in Met, DMSO or H_2O: a/ Met (Met); b/ Met (DMSO); c/ Met (H_2O).

Antimicrobial assay

The disk-diffusion method according to Essawi & Srour (2000) was applied to evaluate the antimicrobial properties of *N. nuda* leaf extracts. For the screening of the samples, 16 test-microorganisms (15 bacteria: Gr (+) - *Bacillus cereus* NBIMCC 1085, *Bacillus subtilis* NBIMCC 1709, *Enterococcus faecalis* NBIMCC 3360, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* NBIMCC 1093; Gr (-) - *Acinetobacter cloaceticus* NBIMCC 3730, *Enterobacter cloacae* NBIMCC 8570, *Escherichia coli* ATCC 25922, *Escherichia coli* UPEC NBIMCC 8954, *Escherichia coli* EPEC (clinical isolate), *Pseudomonas aeruginosa* NBIMCC 3700, *Salmonella enterica ser. Typhimurium* NBIMCC 3669 NBIMCC 3669, *Proteus hauseri* NBIMCC 1393, *Proteus mirabilis* NBIMCC 8747, *Klebsiella pneumoniae* NBIMCC 3670, and one fungal pathogen - *Candida albicans* NBIMCC 74) were investigated. The test microorganisms were obtained from the Bulgarian National Bank for Industrial Microorganisms and Cell Cultures (NBIMCC).

The *N. nuda* leaf extracts were dissolved in the relevant solvent, then 50 µl from initial concentration of each extract were loaded on sterile paper discs (6 mm in diameter). An agar medium (Müeller-Hinton agar for bacteria /

Sabouraud dextrose agar for fungi) was poured into sterile Petri dishes previously inoculated with bacterial/fungal suspension (1.5 x 108 CFU/ml).

The extract-impregnated paper discs after completely evaporation of the solvent were placed on the inoculated agar medium. The Petri dishes were left for pre-diffusion at 4°C for 2 h. Sterilized disks loaded with Chl, Met, DMSO served as negative controls. Amikacin (30µg/disk) and Nystatin (35 µg/disk) disks were used as positive controls for the studied bacteria and fungus, respectively. The inhibition of microorganism growth was measured after 24 h incubation period at 37°C for bacteria and at 30°C for the fungal strain. Antimicrobial activity was measured by the diameter of obtained inhibition zones. Three replicates were done for each *N. nuda* leaf extract.

Results

In our study it was carried out a two-step extraction on *N. nuda* leaves by starting with separation of non-polar compounds by Chl and finishing with extraction of polar compounds by Met. The dried extracts were dissolved in 5% DMSO secondary solvent and used to screen their antimicrobial activity against all the 16 human-pathogens (Table 1). After incubation, the diameters of the inhibition zones were measured (Table 1). The results revealed that both extracts were potentially effective in suppressing the growth of the tested strains with variable strength. The Met extract showed antibacterial activity against 8 out of 15 strains. The diameter of the zones of inhibition ranged between 7-11 mm. The Chl extract had activity against 4 out of 15 bacteria with zones of growth inhibition ranging between 8-10 mm. The data indicated significant antibacterial capacity of *N. nuda* extracts, as the Met extracts exerted a stronger and broader spectrum of action.

In summary, Met and Chl leaf extracts of *N. nuda* showed antimicrobial activity mainly to 6 bacterial strains with inhibition zones above 8 mm: 2 Gram (+) *B. subtilis* and *S. aureus* and 4 Gram (-) *P. mirabilis*, *A. calcoaceticus*, *P. aeruginosa* and *E. cloacae* (Table 1). The extracts did not have or demonstrated very low activity against the other tested bacteria. No antifungal activity against *C. albicans* was detected.

Table 1. Screening of *N. nuda* leaf extracts (Met and Chl) in 5% DMSO for antimicrobial activity against human pathogen test-microorganisms.

Test-microorganisms	***N. nuda* leaf extracts**	
	Chl	**Met**
Gram (+) bacteria	**Zones of inhibition (mm)**	
B. subtilis	0	9
S. aureus	10	9
Gram (-) bacteria	**Zones of inhibition (mm)**	
E. coli EPEC	0	8
P. mirabilis	8	11
P. aeruginosa	8	10
E. cloacae	0	9
A. calcoaceticus	8	9
P. hauseri	0	7
Test-microorganisms to which no antimicrobial activity has been established:		
Gram (+) bacteria	***E. faecalis,*** ***S. epidermidis,*** ***B. cereus*** ***E. coli ATCC*** ***E. coli UPEC***	
Gram (-) bacteria	*K. pneumoniae* *S. typhimurium*	
Fungus	*C. albicans*	

Since the use of 5% DMSO solvent caused formation of precipitates, we tested if pure secondary solvents (Met, Chl, DMSO, H_2O) would improve the antimicrobial effectiveness against the 6 highlighted bacteria that showed sensitivity in the prior screening. Complete dissolution of the Met

extract was achieved in Met and DMSO, while H_2O could not resolve substantial part of the dry weight. Similar results obtained with Chl – excellent solubility in Chl and DMSO, but H_2O was not able to dissolve the dry weight. The precipitates in H_2O indicated lack of complete dissolution, therefore these extracts in the following microbiological analysis were dropped out.

The results on the effect of the pure secondary solvent on the antibacterial effectiveness are summarized in Table 2. The data showed that both, Met (Met) and Met (DMSO) extracts, inhibited the growth of all 6 tested bacteria but with different efficacy. The Met (DMSO) sample appeared to be more effective than the Met (Met) since it caused larger inhibition zones. The best activities of Met (DMSO) extract were recorded against *A. calcoaceticus* and *S. aureus* – up to 14 mm inhibition zone, whereas for Met (Met) - against *B. subtilis* and *A. calcoaceticus* – up to 12 mm inhibition zone. The lowest antibacterial activity was against *P. mirabilis* by both Met extracts.

The Chl(Chl) and Chl(DMSO) extracts did not exhibit activity against all the 6 bacteria. Each Chl extract inhibited the growth of 4 but different bacterial strains. However, both Chl extracts demonstrated strong activity towards the Gram (+) *S. aureus* and Gram (-) *A. calcoaceticus* – up to 16 mm. No activities were recorded against *B. subtilis* and *P. aeruginosa* by the Chl (Chl) extract and to *B. subtilis* and *E. cloacae* by the Chl (DMSO) sample. Very weak inhibition was detected to *P. mirabilis* by both Chl extracts.

Table 2. Antimicrobial activity of *N. nuda* (Met and Chl) leaf extracts dissolved in different secondary solvents - Met, Chl, DMSO, H_2O.

***N. nuda* leaf extracts**	**Zones of inhibition (mm)**					
	Gram (+) bacteria		**Gram (-) bacteria**			
	S. aureus	***B. subtilis***	***E. cloacae***	***P. mirabilis***	***P. aeruginosa***	***A. calcoaceticus***
Met (Met)	9	12	9	9	8	11
Met (DMSO)	13	10	11	8	12	14
	-	-	-	-	-	-
Chl (Chl)	15	0	8	8	0	14
Chl (DMSO)	16	0	0	9	10	15
	-	-	-	-	-	-
Amikacin (30 µg/disk)	20	20	30	20	20	20
Negative controls Met, Chl, DMSO	-	-	-	-	-	-

Discussion

The screening for antimicrobial activity of plant extracts and phytochemicals is a starting point for antimicrobial drug discovery (Cseke et al., 2016). The object of this study is the medicinal plant *N. nuda* collected from its natural habitat in Rhodope Mountain, Bulgaria. Previous reports had been focused mainly on the antimicrobial properties of *N. nuda* essential oil fractions (Adiguzel et al., 2009; Alim et al., 2009; Ashrafi et al., 2019; Ghavam et al., 2022; Sonboli et al., 2017) while studies of extracts in polar and non-polar solvents had not been well investigated.

Solvents used for the extraction of biomolecules were reported to have an influence on the nature and the quantity of secondary metabolites extracted from medicinal plants (Abdullahi & Mainul, 2020). Whatever is the extraction method used, the chemical nature of the extraction solvent is of a primary importance to favor the compound solubility, i.e. the extraction selectivity (to extract the active constituent and leave the inert material) and the recovery after extraction (Altemimi et al., 2017). The solvents could be used sequentially to limit the number of analogous compounds in the extracts. For this reason, we applied a two-step extraction, by which we obtained and then analyzed a non-polar Chl fraction and a polar Met fraction. Notably, the obtained in this work *N. nuda* Chl and Met extracts exhibited varying

degrees of antibacterial activity against the tested bacterial strains. The data demonstrated a greater antibacterial potential of the Met extract (about 53%) than the Chl ones (about 27%). The effecttiveness of the respective antibacterial action of the extracts also differed.

The observed higher activity of Met extracts could be due to the presence of polar compound such as polyphenols and iridoids as reported in our previous study (Petrova et al., 2022). According to Anićić et al. (2021), *Nepeta iridoids* (nepetalactones and 1,5,9-eDLA) and the phenolic acid rosmarinic acid are also potent antimicrobial agents. Nadeem et al. (2022) reported that highest content of phenols was identified in *N. cataria* Met extract. The presence of iridoid components including non-volatile nepetalactones have been found in Met extracts of some *Nepeta* species in the literature (Galati et al., 2004; Kökdil et al., 1999), as well as in *N. nuda* (Petrova et al., 2022).

The effectiveness of the plant metabolites depends on their solubility in various solvents (Al-Daihan et al., 2013). Therefore, we investigated the effect of some secondary solvents on the obtained *N. nuda* extracts. The results showed excellent solubility of the Met and Chl extracts in DMSO and corresponding solvent (Met, Chl), whereas the water was unsuitable. This could be due to the high capacity of the organic solvents to dissolve organic and active antimicrobial compounds (Cowan, 1999). Conversely, water is a highly polar, however, it is a weak solvent for most organic compounds under ambient conditions. Raising the temperature significantly above ambient has a positive effect on solubility but this may cause the degradation of many heat-sensitive metabolites (Chemat & Vian, 2014).

Previous studies reported about antifungal activity of essential oils and aqueous extracts from different *Nepeta* species (Salehi et al., 2018). Aničić et al. (2021) established antifungal activity of N. *rtanjensis* and *N. argolica* methanol extracts against *Aspergillus* and *Penicillium* fungal species. In our study all *N. nuda* extracts showed no *C. albicans* inhibition.

The antibacterial activities towards the 6 susceptible strains varied depending on the solvent used. The Met extracts demonstrated an increase in antimicrobial activity against all the bacterial strains after dissolution in DMSO except *P. mirabilis.* While enhanced activities were observed only against *B. subtilis* and *A. calcoaceticus* at Met (Met) extract, compared to the screening results performed in 5% DMSO. Zaharieva et al. (2023) reported the identification by GC-MS analysis of metabolites that were more abundant in the polar fraction of *N. nuda* leaves: phenolic derivatives (hydroquinone, tyrosol, 4-coumaric acid, caffeic acid, vanillic acid, gentisic acid, isoferulic acid), as well as predominance of organic acids and sugars. We hypothesize that the activities of the Met extracts could be due to the action of these compounds.

The two Chl extracts indicated enhanced antibacterial potential against *S. aureus* and *A. calcoaceticus*, with a significant increase in the inhibitory zones compared to those obtained in the screening in 5% DMSO. No significant changes were observed in the activity of the extracts to the other four bacteria from the initially reported ones. Strikingly, the Chl extracts showed significantly increased activity in this antimicrobial assay, albeit against two bacteria. We assumed that this is due to the presence of hydrophobic substances related to the observed antimicrobial potential. Accordingly, the non-polar fraction of *N. nuda* was reported to be enriched in fatty acids, alkanes and sterols (Zaharieva et al., 2023). These data are an indication that the secondary solvents are of importance in the efficacy of the antimicrobial action and their application should not be neglected.

The present study indicated promising effect of *N. nuda* extracts as antibacterial agents towards *S. aureus* and *A. calcoaceticus* by the activity of Chl (Chl, DMSO) sample, and against *B. subtilis* and *A. calcoaceticus* by the action of Met (Met, DMSO) sample. These data are consistent with the results from our previous study, where Soxhlet Met extract of *N. nuda* had activity against *S. aureus*, *A. calcoaceticus*, *B. cereus* and *K. penumoniae* (Petrova et al., 2022). The lack of activity against *B. cereus* and *K. pneumoniae* in the present extracts is probably due to differences in extraction methods, as well as the different location of plant collection.

Conclusions

In conclusion, the choice of a suitable secondary solvent for plant extracts is an important step for their effectiveness. *Nepeta nuda* fractions showed promising antibacterial potential against some bacteria after being dissolved in them. The

obtained results can be a prerequisite for deepening the research in the direction of isolating and purifying the active molecules, and also for evaluating the possible synergism between them. Moreover, the use of natural raw extracts determines much lower probability of occurrence of bacterial resistance to a mixture of active components. In the search for a suitable solvent, it is possible, in addition to the classic solvents such as water, Met, Chl, hexane, acetone, ethanol, etc. to test others or a mixture of them. There is an increasing knowledge for the utilization of solvents with less toxicity, such as "green" solvents, ionic liquids, polymers, as well as CO_2 extraction, which is an advancing trend and necessity.

Acknowledgements: This work was financially supported by the Bulgarian National Science Fund (BNSF), Grant No. KP-06-N56/9/12.11.2021 and by the Ministry of Education and Science of Bulgaria.

References

Abubakar, A.R., & Haque, M. (2020). Preparation of medicinal plants: basic extraction and fractionation procedures for experimental purposes. *Journal of Pharmacy and Bioallied Science*, 12, 1-10. doi: 10.4103/jpbs.JPBS_175_19

Aćimović, M., Lončar, B., Pezo, M., Jeremić, J.S., Cvetković, M., Rat, M., & Pezo, L. (2022). Volatile compounds of *Nepeta nuda* L. from Tranj Mountain (Serbia). *Horticulturae*, 8(2), 85. doi: 10.3390/horticulturae8020085

Adekunle, A.S., & Adekunle, O.C. (2009). Preliminary assessment of antimicrobial properties of aqueous extract of plants against infection disease. *Biology and Medicine*, 1, 20-24.

Adiguzel, A., Ozer, H., Sokmet, M., Gulluce, M., Sokmet, A., Kilic, H., Sahin, F., & Baris, O. (2009). Antimicrobial and antioxidant activity of the essential oil and methanol extract of *Nepeta cataria*. *Polish Journal of Microbiology*, 58, 69-76.

Akerele, O. (1993). Summary of WHO guidelines for the assessment of herbal medicines. *HerbalGram*, 28, 13-19.

Al-Daihan, S., Al-Faham, M., Al-Shawi, N., Almayman, R., Brnawi, A., Zargar, S., & Bhat, R. (2013). Antibacterial activity and phytochemical screening of some medicinal plants commonly used is Saudi Arabia against selected pathogenic microorganisms. *Journal of King Sau University-Science*, 25, 115-120. doi: 10.1016/j.jksus.2012.11.003

Alim, A., Goze, I., Cetin, A., Atas, A.D., Cetinus, S.A., & Vural, N. (2009). Chemical composition and in vitro antimicrobial and antioxidant activities of the essential oil of *Nepeta nuda* L. subsp. *albiflora* (Boii.) gams. *African Journal of Microbiology Research*, 3, 463-467.

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, DG., & Lightfoot, DA. (2017). Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts. *Plants*, 6(4), 42(1-23). doi: 10.3390/plants6040042

Aničić, N., Gašic, U., Lu, F., Ćirić, A., Ivanov, M., Jevtić, B., Dimitrijević, M., Anđelkovi, B., Skorić, M., Živković, JN., Mao, Y., Liu, J., Tang, C., Soković, M., Ye, Y., & MiŠić, D. (2021). Antimicrobial and immunomodulating activities of two endemic *Nepeta* species and their major iridoids isolated from natural sources. *Pharmaceuticals*, 14(5), 414(1-22). doi: 10.3390/ph14050414

Ashrafi, B., Ramak, P., Ezatpour, B., & Talei, G.R. (2019). Biological activity and chemical composition of the essential oil of Nepeta *cataria* L. *Journal of Research in Pharmacy*, 23, 336-343. doi: 10.12991/jrp.2019.141

Chemat, F., & Vian, M.A. (Ed.) (2014). *Alternative solvents for naturally products extraction*. Springer, Berlin, Heidelberg, 315 p. doi: 10.1007/978-3-662-43628-8

Cowan, M.M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Review*, 12, 564-582. doi: 10.1128/cmr.12.4.564

Cseke, L.J., Kirakosyan, A., Kaufman, P.B., Warber, S.L., Duke, J.A., & Brielmann, H.L. (2016). *Natural Products from Plants*, 2nd Edition. Taylor & Francis Group, London, New York, 587 p. doi: 10.1201/9781420004472

Essawi, T., & Srour, M. (2000). Screening of some Palestinian medicinal plants for antibacterial activity. *Journal of Ethnopharmacology*, 70, 343–349. doi: 10.1016/s0378-8741(99)00187-7

Fair, R.J., & Tor, Y. (2014). Antibiotics and bacterial resistance in the 21st Century. *Perspectives in*

Medicinal Chemistry, 6, 25-64. doi: 10.4137/PMC.S14459

Galati, E.M., Miceli, N., Galluzzo, M., Taviano, M.F., & Tzakou, O. (2004). Neuropharmacological effects of epinepetalactone from *Nepeta sibthorpii* behavioral and anticonvulsant activity. *Pharmaceutical Biology*, 42, 391-395, doi: 10.1080/13880200490885059

Ghavam, M., Bacchetta, G., Castangia, I., & Manca, M.L. (2022). Evaluation of the composition and antimicrobial activities of essential oils from four species of Lamiaceae Martinov native to Iran. *Scientific Reports*, 12, 17044, 1-12. doi: 10.1038/s41598-022-21509-5

Ginovyan, M., Petrosyan, M., & Trchounian, A. (2017). Antimicrobial activity of some plant materials used in Armenian traditional medicine. *BMC Complementary Medicine and Therapies*, 17, 50(1-9). doi: 10.1186/s12906-017-1573-y

Gowri, J., Sahayaraj, P.A., & Amaladasan, M. (2016). Antimicrobial activity of the leaf, flower and stem extracts of *Sphenoclea zeylanica*. *International Journal of Applied Science and Biotechnology*, 4, 325-329. doi: 10.3126/ijasbt.v4i3.15760

Kökdil, G., Yalçin, S.M., & Topçu, G. (1999). Nepetalactones and other constituents of *Nepeta nuda* ssp. *albiflora*. *Turkish Journal of Chemistry*, 23, 99-104.

Köskal, E., Tohma, H., Kiliç, Ö., Alan, Y., Aras, A., & Gülçin, İ., B, E. (2017). Assessment of antimicrobial and antioxidant activities of *Nepeta trachonitica*: Analysis of its phenolic compounds using HPLC-MS/MS. *Scientia Pharmaceutica*, 85(2), 24(1-14). doi: 10.3390/scipharm85020024

Lewis, K., & Ausubel, F.M. (2006). Prospects for plant-derived antibacterial nature. *Biotechnology*, 24, 1504-1507. doi: 10.1038/nbt1206-1504

Nadeem, A., Shahzad, H., Ahmed, B., Muntean, T., Waseem, M., & Tabassum, A. (2022). Phytochemical profiling of antimicrobial and potential antioxidant plant: *Nepeta cataria*. *Frontiers in Plant Science*, 13, 969316(1-18). doi: 10.3389/fpls.2022.969316

Petrova, D., Gašić, U., Yocheva, L., Hinkov, A., Yordanova, Z., Chaneva, G., Mantovska, D., Paunov, M., Ivanova, L., Rogova, M., Shishkova, K., Todorov, D., Tosheva, A., Kapchina-Toteva, V., Vassileva, V., Atanassov, A., Mišić, D., Bonchev, G., & Zhiponova, M. (2022). Catmint (*Nepeta nuda* L.) phylogenetics and metabolic responses in variable growth conditions. *Frontiers in Plant Science*, 13, 866777(1-23). doi: 10.3389/fpls.2022.866777

Salehi, B., Valussi, M., Jugran, A.K., Martorell, M., Ramírez-Alarcón, K., Stojanović-Radić, Z.Z., Antolak, N., Kręgiel, D., Mileski, K.S., Sharifi-Rad, M., Setzer, W.N., Cádiz-Gurrea, M.L., Segura-Carretero, A., Şener, B., & Sharifi-Rad, I. (2018) *Nepeta* species: From farm to food applications and phytotherapy. *Trends in Food Science & Technology*, 80, 104-122. doi: 10.1016/j.tifs.2018.07.030

Sonboli, A., Saadat, M.H., Arman, M., & Kanani, M.R. (2017). Antibacterial activity and composition of the essential oil of *Nepeta hormozganica* Jamzad from Iran. *Natural Product Research*, 31 (23), 2806-2809. doi: 10.1080/14786419.2017.1295237

Teber, I., & Bursal, E. (2020). Phenolic compounds and antioxidant activity of *Nepeta nuda* subsp. *albiflora*. *International Letters of Natural Sciences*, 79, 1-8. doi: 10.56431/p-nln568

Zaharieva, A., Rusanov, K., Rusanova, M., Paunov, M., Yordanova, Z., Mantovska, D., Tsacheva, I., Petrova, D., Mishev, K., Dobrev, P.I., Lacek, J., Filepová, R., Zehirov., G, Vassileva, V., Mišić, D., Motyka, V., Chaneva, G., & Zhiponova, M. (2023). Uncovering the interrelation between metabolite profiles and bioactivity of In Vitro - and Wild-Grown Catmint (*Nepeta nuda* L.). *Metabolites*, 13 (10), 1099(1-18). doi: 10.3390/metabo13101099

Zare, Z., Majd, A., Sattari, T.N., Iranbakhsh, A., & Mehrabian, S. (2012). Antimicrobial activity of leaf and flower extracts of *Lippia nodiflora* L. (Verbenacea). *Journal of Plant Protection research*, 52 (4), 401-403. doi: 10.2478/v10045-012-0065-9

Received: 06.12.2023
Accepted: 09.05.2024