Journal of Medicinal Plants and By-products (2015) 1: 77-82

Original Article

Total Flavonoids Contents and Anti Bacterial Activity of the Extracts of two Labiateae Species*: Nepeta menthoides* and *Thymus trautvetteri*

Shadi Ghandchi and Mina Jamzad*

Department of Chemistry, Shahr-e-Qods Branch, Islamic Azad University,Tehran, Iran

Article History: Received: 09 February 2015/Accepted in revised form: 30 May 2015
© 2013 Iranian Society of Medicinal Plants. All rights reserve

Abstract

Total flavonoids contents and antibacterial activity of the extracts from the aerial parts of two Labiateae species, *Nepeta menthoides* Boiss. & Buhse and *Thymus trautvetteri* Klokov & Desj.-Shost. were determined. Quantitative determination of flavonoid contents was calculated in terms of quercetin equivalent in various extracts (50% methanol, chloroform and distilled water) by $AlCl_3$ colorimetric method. It was found that total flavonoids contents of *N. menthoides* extracts in different solvents were (2.308%, 0.884% and 0.710%) mg/g and (2.076%, 1.468% and 1.412%) mg/g for *T. trautvetteri* respectively. Antibacterial activity of the extracts was also determined against 6 gr (+/-) bacteria by disc diffusion method. The results showed that 50% methanol extracts of two species displayed better inhibitor activity against the tested bacteria. The bacteria *Stapylococcus epidermidis*, *Staphylococcus saprophyticus* and *Staphylococcus aureus* were sensitive against 50% methanol extract of *N. menthoides* even more than the standard antibiotics (10µg). Therefore a good correlation between total flavonoids contents and antibacterial activity of the studied extracts is demonstrated.

Key words: *Nepeta menthoides, Thymus trautvetteri,* Flavonoids contents, Antibacterial activity

Introduction

Aromatic and medicinal plants have been regarded as foremost source of secondary metabolites. These compounds have been recognized as versatile source of biologically active drugs. Many kinds of diseases have been treated with herbal remedies since ancient times. Nowadays researchers and pharmaceutical industries are considering medicinal plants as a good choice, because these natural resources have ordinarily fewer side effects [1]. They are also costless and effective against a broad spectrum of antibiotic resistant micro organisms [2]. The increasing occurrence of antimicrobial resistance represents worldwide major concern to discover new antimicrobial agents. Flavonoids, the most common group of polyphenolic compounds that are found ubiquitously in plants, are widely distributed in plant fulfilling many functions. They are important in plant for normal growth development and defense against infection and injury [3]. Flavonids are the most important pigments for flower coloration. These plant secondary metabolites were also shown anti-allergic, anti- inflammatory, anti-microbial and anti- cancer activity [4]. Increasingly, this class of natural products is becoming the subject of anti- infective research and many phytochemical preparations with high flavonoid content have been reported to exhibit antibacterical activity [5-13].

Amongst various plant families which have been known for their medicinal values, Labiatae family remains quite important. The genus *Nepeta* L. and *Thymus* L. are the large groups of plants in the family Labiateae, which have been used for medicinal purposes in folk medicine. The genus *Nepeta* comprises of about 300 species widely

***Corresponding author: Department of Chemistry, Islamic Azad University, Shahr-e-Qods branch, Tehran, Iran
E-mail Address: Minajamzad@yahoo.com, M.jamzad@shahryariau.ac.ir

Archive of SID
www.SID.ir

distributed in the Eurasia. In Iran 75 species have been identified growing almost in all parts of country and about 46 species are endemic [14]. Anti-bacterial, fungicidal and anti-viral activities of some *Nepeta* species have been reported [15-16].The genus *Thymus* includes about 350 species, distributed over the Eurasia continent, the northern part of Africa and southern Greenland, although it has been spread by man all over the world [17]. In Iran 14 species are present, among which 4 are endemic. Because of high phenol content, *Thyme* oil has germicidal and antiseptic properties [18].

The aim of the present work is to determine total flavonoids contents of the extracts obtained from the aerial parts of *Nepeta menthoides* Boiss. & Buhse and *Thymus trautvetteri* Klokov & Desj.-Shost. and to evaluate the possible antibacterial properties of the extracts. To the best of our knowledge this is the first report in the subject of our study.

Material and Methods

Plant material: Aerial parts of *N. menthoides* and *T. trautvetteri* were both collected from the Sabalan mountains, located in Ardabil province, Iran in July 2012 during the flowering stage. Plant materials were identified by Research Institute of Forests and Rangelands (TARI), Tehran, Iran , where the voucher specimens are deposited.

Preparation of the extracts: The extracts were prepared by maceration method. Dried and powdered plant (7.5g) was used for preparation of each extract. Plant materials were mixed with 75 ml of each solvent separately (distilled water, 50% methanol and chloroform) (Merck, Germany) and stored at room temperature for 1 week. All the infusions were filtrated through Whatman paper No. 41. The solvents were removed below 60°C by a rotary evaporator (Heidolph, Germany) and stored at 4°C for further uses.

Determination of flavonoids concentration in the extracts**:** Total flavonoids contents were determined by aluminum chloride colorimetric method, a reported spectrophotometric procedure by Chang *et al*. [19], which has been used with slight modification. This method is based on the formation of a complex flavonoid-aluminum having the absorption maximum at 415 nm, after remaining at room temperature for 30 min. Briefly, 0.5 ml of each extract (1:10 g/ml) was dissolved in methanol (1.5 ml) and then 10% aluminum chloride (0.1 ml) and 1.0 M sodium acetate (0.1 ml) were added to the solutions. Finally distilled water (2.8 ml) was added and the solutions were incubated at room temperature. After half an hour the absorbance of the reaction mixtures were measured at 415 nm by a UV-Visible spectrophotometer, (Unico UV-2100, China). Each reported absorbance was the average of 3 independent measurements. The calibration curve was plotted (Fig.1) by employing the same procedure for the standard solutions of quercetin (0-100 ppm). Then the content of flavonoids in extracts was expressed in terms of quercetine equivalent (mg of quercetin/g of dried plant).

Antibacterial activity: The extracts were tested against 4 Gram positive bacteria namely *Staphylococcus epidermidis, Staphylococcus saprophytycous, Staphylococcus aureus, Bacillus cereus* and 2 Gram negative bacteria: *Klebsiella oxytoca and Pseudomonas aeruginosa.* Microorganisms were identified by Research Center of Biotechnology and Industrial center of fungi and bacteria collections, Iran. The in vitro antibacterial activity was evaluated by the disc diffusion method (DDM) according to the standard method by Bauer *et al*. [20], to assess the presence of antibacterial activities of the plants extracts. A bacteria culture (which has been adjusted to 0.5 McFarland standard), was used to lawn Mueller-Hinton agar plates evenly using a sterile swab. The plates were dried for 15 minutes and then used for the sensitivity test. The discs which had been impregnated with the plants extracts were placed on the Muller-Hinton agar surface. The standard antibiotics gentamicin, vancomycin and penicillin (10 µg) (Hindi Co., India) were used as positive controls. The plates were then incubated at 37°C for 24 h. After incubation, the growth inhibition zones were measured. Each test was carried out in duplicate and the average was calculated for inhibition zone diameters.

Results and Discussion

In this study we evaluated total flavonoids contents of different extracts of *N. menthoides* and *T. trautvetteri* by $AlCl_3$ colorimetric method. Flavonoids as one of the most diverse and widespread group of natural compounds, posses a broad spectrum of chemical and biological activities.

Archive of SID

www.SID.ir

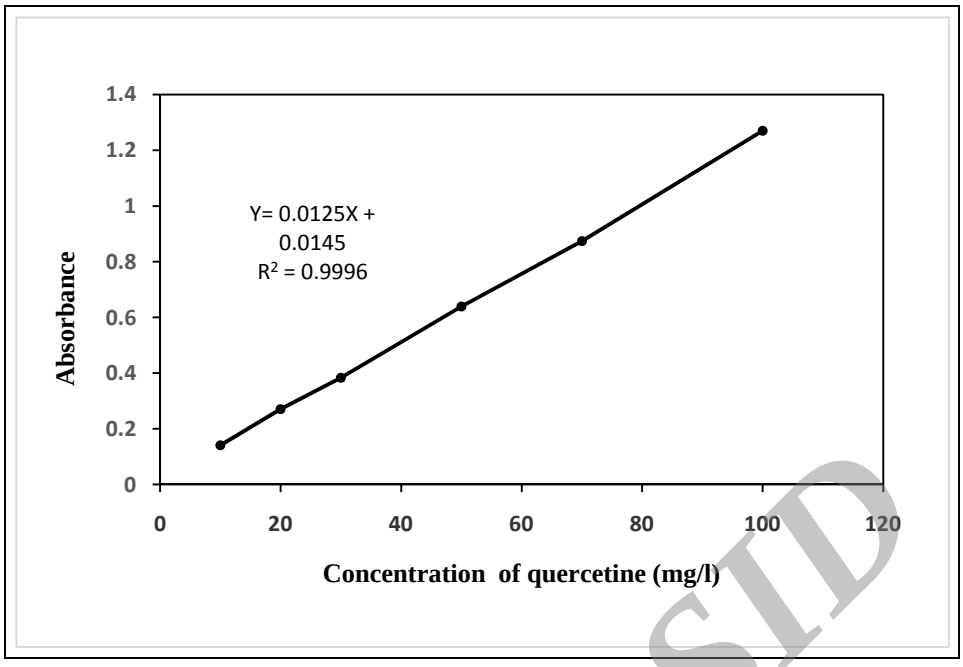

Fig. 1 Calibration plot for flavonoid determination

Using the standard plot of quercetin (Y=0.0125X+0.0145, R^2=0.9996) (Fig. 1), the flavonoids contents of *N. menthoides* and *T. trautvetteri* in different extracts were found ranging from (0.71 to 2.308) and (1.412 to 2.076) mg quercetin equivalent/g of dry plants respectively.

The results showed that flavonoids contents of 50% methanolic extracts of both plants aerial parts was superior to the other studied extracts in this study (Table 1).

The flavonoids contents of the extracts are related to the solvent polarity. It was reported that plant species extracted with methanol were usually higher phenolic content. As far as our literature survey could ascertain, several studies have been carried out with the *Nepeta* and *Thymus* species and their total phenolic and flavonoids contents results support our findings [21-28].

In the next part of our study, antibacterial activities of different extracts from the aerial parts of *N. menthoides* and *T. trautvetteri* were evaluated against 4 gram (+) and 2 gram (-) bacteria by disc diffusion method (Table 2). Results indicated that 50% methanolic extract of *N. menthoides* has strong antibacterial activity against the gram positive bacteria: *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Staphylococcus aureus* and *Bacillus cereus* from which the two former bacteria were more sensitive to *N. menthoides* 50% methanolic extract in comparison with the antibiotics vancomycin and gentamycin as positive controls. The 50% methanolic extract of *T. trautvetteri* showed moderate activity against the same bacteria. Aqueous extracts of the plants did not showed antibacterial activity against tested bacteria at all. Crude extracts from plants with a history of use in folk medicine have been screened in vitro antibacterial activity by many research groups. Broad empirical screening of chemical entities for antimicrobial activity represents a strategy for development of novel drugs.

Resistance to antimicrobial agents has become increasingly important and natural products have been a particularly rich source of anti-infection agents. *Nepeta* and *Thymus* species have been mentioned for their antibacterial activities many times before [24, 28-38]. *N. menthoides* one of the species which we studied, have been screened for anti-inflammatory and anti-nociceptive effects [39].

The effects of *N. menthoides* aqueous extract on retention and retrieval of memory in mice [40] and its neuroprotective effect in rats [41] have also been evaluated before. The essential oil composition and antioxidant activity of *T. trautvetteri* the other species in this study, have also been mentioned [42,43].

Archive of SID

www.SID.ir

Table 1 Total flavonoids contents of different extracts of *Nepeta menthoides* Boiss. & Buhse and *Thymus trautvetteri* Klokov & Desj.-Shost.

Species	Extract	Absorbance (nm)	[a]Flavonoids contents(mg/l)	[b]Flavonoids contents(mg/g)
Nepeta menthoides	aqueous	0.90	71.08	0.71
	chloroform	1.12	88.44	0.88
	50% methanol	2.90	230.84	2.30
Thymus trautvetteri	aqueous	1.78	141.24	1.41
	chloroform	1.85	146.84	1.46
	50% methanol	2.61	207.64	2.07

[a]Total flavonoids contents of extracts in terms of quercetine equivalent (mg of quercetin/l of extract).
[b]Total flavonoids contents of extracts in terms of quercetine equivalent (mg of quercetin/g of dried plant)

Table 2 Antibacterial activity of different extracts from the aerial parts of *Nepeta menthoides* Boiss. & Buhse and *Thymus trautvetteri* Klokov & Desj.-Shost.

		Zone of inhibition(mm)*										
		Chloroform extract		50% methanol extract		aqueous extract		negative controls		positive controls		
Gram(+/-) Bacteria	PTCC	a	b	a	b	a	b	methanol	chloroform	P	V	G
Staphylococcus aureus (+)	1431	12	17	40	28	-	-	-	14	40	NT**	NT
Staphylococcus epidermidis (+)	1114	13	-	36	24	-	-	-	-	NT	28	NT
Staphylococcus saprophyticus (+)	1440	11	10	35	17	-	-	-	-	28	NT	NT
Pseudomonas aeruginosa (-)	1310	-	-	-	-	-	-	-	-	NT	NT	18
Escherichia coli (-)	1395	-	-	-	-	-	-	-	-	-	-	-
Bacillus cereus (+)	1154	-	11	21	15	-	-	-	-	20	NT	NT
Klebsiella oxytoca (-)	1402	10	-	-	-	-	-	-	10	NT	NT	17

*Inhibition zone diameter (mm); **NT: Not tested
a:Nepeta menthoides Boiss. & Buhse*; b: Thymus trautveltter* Klokov & Desj.-Shost.
P: Penicillin; V: Vancomycine; G: Gentamicin
PTCC: Persian type culture

The present study revealed that the 50% methanolic extract of *N. menthoides* contain significant amount of flavonoids and also shows strong antibacterial activity against gram positive bacteria. We found that there is a good correlation between total flavonoids contents and antibacterial activity of the studied extracts and *N. menthoides* can be a potential source of natural bioactive chemicals. Further studies are needed to confirm the in-vivo antibacterial activity and subsequent isolation and chemical characterization of the active molecules.

Acknowledgement

Authors are thankful to Dr. Ziba Jamzad (Research Institute of forests & Rangelands, Tehran, Iran) for identifying the plant material.

References

1. Zargari A. Medicinal Plants. Vol.3, 6th ed. Tehran University Publication, Tehran, 1996.
2. Mohammadi A, Malek F, Dehpour A, Nazari H. Chemical composition and antibacterial activity of the essential oils of some medicinal plants. Afr. J. Agric. Res. 2013;8:3151-3158.
3. Kohkonen MP, Hopia AL, Vuorela JH, Rauha JP, Pihlaja K, Kujala TS, Heinonen M. Antioxidant activity

Archive of SID

www.SID.ir

of plant extracts containing phenolic compounds. J. Agric. Food Chem. 1999;47:3954-3962.
4. De Sousa RR, Queiroz KC, Souza AC, Gurgueira SA, Augusto AC, Miranda MA, Peppelenbosch MP, Ferreira CV, Aoyama H. Phosphoprotein levels, MAPK activities and NF kappa B expression are affected by fisetin. J. Enzyme Inhib. Med. Chem. 2007;22:439-444.
5. Tereschuk ML, Riera MV, Castro GR, Abdala LR. Antimicrobial activity of flavonoids from leaves of *Tagetes minuta*. J. Ethnopharmacol. 1997;56:227-232.
6. Aladesanmi AJ, Sofowora A, Leary JD. Preliminary, biological and phytochemical investigation of two Nigerian medicinal plants. Int . J. Crude Drug Res. 1986;24:147-153.
7. Al-Saleh FS, Gamal El-Din AY, Abbas JA, Saeed NA. Phytochemical and biological studies of medicinal plants in Bahrain: Family Chenopodiaceae. part 2. Int J. Pharmacogn. 1997;35:38-42.
8. Mahamoud MJ, Jawad AL, Hussain, AM, AL-Omari M, AL- Naib A. IN vitro antimicrobial activity of *Salsola rosmarinus* and *Adiantum capillus-veneris*. Int. J. Crude Drug. Res. 1989;27:14-16.
9. Quarenghi MV, Tereschuk ML, Baigori MD, Abdala LR. Antimicrobial activity of flowers from *Anthemis Cotula*. Fitoterapia. 2000;71:710-712.
10. Rauha JP, Remes S, Heinonen M, Hopia A, Kähkönen M, Kujala T, Pihlaja K, Vuorela H,Vuorela P. Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. Int. J. Food Microbial. 2000;56:3-12.
11. Singh RK, Nath G. Antimicribial activity of *Elaeocarpus sphaericus*. Phytother. Res. 1999;13:448-550.
12. Tarle D, Dvorzak I. Antimicrobial activity of the plant *Crisium oleraceum* (L.). Scop. Acta Pharm. Jugosl. 1990;40:569-571.
13. Torrengra RD, Ricardo AA, Pedrozo JP, Fuentes OC. Flavonoids from *Gnaphalium gracile* H.B.K. Int. J. Crude Drug Res. 1989;27:22-24.
14. Rechinger KH. *Nepeta*. In: Rechinger KH (ed.) Flora Iranica. No.150. Akademische Druck and Vertagsanstatt. Graz, Austria, 1982,108.
15. Jamzad Z, Grayer RJ, Kite GC, Simmonds MSJ, Ingrouille M. Leaf surface flavonoids in Iranian species of *Nepeta* L. (lamiaceae) and some related genera. Biochem. Syst. Ecol. 2003;31:587-600.
16. Bourrel C, Perineau F, Michel G, Bessiere JM. Catnip (*Nepeta cataria* l) essential oil: analysis of chemical constituents. Bacteriostatic and fungistatic properties. J. Essent Oil Res. 1993;5: 159-167.
17. Jalas J. *Thymus*. In: Rechinger KH. (ed.) Flora Iranica No. 150. Akademische Druck and Vertagsantsatt,Graz, Austria, 1982, 532.
18. Sattar A, Bankova V, Kujumgiev A, Galabov A, Ignatova A, Todorova C, Popv S. Chemical composition and biological activity of leaf exudates from some Lamiaceae plants. Pharmacie. 1995;50:62-65.
19. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J. Food Drug Anal. 2002;10:178-182.
20. Bauer AW, Kirby WMM, Sherris JC, Truch M. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 1966;45:493-496.
21. Tepe B, Daferera B, Tepe A, Polissiou M, Sokmen A. Antioxidant activity of the essential oil and various extracts of *Nepeta flavida* Hub.-Mor. From Turkey. Food Chem. 2007;103:1358-1364.
22. Modincki D, Tokar M, Klimek B. Flavonoids and phenolic acids of *Nepeta cataria* L. Var. citrodora (Becker) Balb. (Lamiaceae). Acta Pol. Pharm. 2007;64:247-250.
23. Adiguzel A, Ozer H, Sokmen M, Gulluce M, Sokmen A, Kilic H, Sahin F, Bans O. Antimicribial and antioxidant activity of the essential oil and methanol extract of *Nepeta cataria*. Polish J. Microbiol. 2009;58:69-76.
24. Cigremis Y, Ulukanliz Z, Ilcim A, Akgoz M. In vitro antioxidant and antimicrobial assays of acetone extracts from *Nepeta meyeri* Bentham. Eur. Rev. Med. Pharmacol. Sci. 2010;14:661-8.
25. Kraujalis P, Venskutonis PR, Ragazinskiene O. Antioxidant activities and phenolic composition of extracts from *Nepeta* plant species. Food Balt. 2011;79-81.
26. Ozbek Yazici S, Ozmen I, Celikoglu U, Ozcelik H, Genc H. In vitro Antioxidant activities of extracts from some *Nepeta* species. Int. J. Health & Nutri. 2012;3:8-12.
27. Amzad Hossain M, Salim Al-Raqmi KA, Hamed Al-Mijizi Z, Mohammed Weli A, Al-Riyahi Q. Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown *Thymus vulgaris*. Asian Pac. J. Trop. Biomed. 2013; 3(9): 705-710.
28. Seladji M, bekhechi C, Beddou F, Dib H, Bendimerad N. Antioxidant activity and Phytochemical screening of *Nepeta nepetella* aqueous and methanolic extracts from Algeria. J. Appl. Pharmacol. Sci. 2014;4:12-16.
29. Dulger B, Gonuz A. Antimicrobial activity of some Turkish medicinal plants. J. Pak. J. Biol. Sci. 2004;7:1559-1562.
30. Parekh J, Chanda SV. In vitro antimicrobial activity and phytochemical analysis of some Indian medicinal plants. Turk. J. Biol. 2007;31:53-58.
31. Al-Bayati FA. Synergistic antibacterial activity between *Thymus vulgaris* and *Primpinella anisum* essential oils and methanolic extracts. J. Ethnopharmacol. 2008; 166:403-406.
32. Mehrgan H, Mojab F, Pakdaman SH, Poursaeed M. Antibacterial activity of *Thymus pubescens* methanolic extract. Iran. J. Pharm. Res. 2008;7:291-298.
33. Zeghad N, Rachid M. Antioxidant and antibacterial activities of *Thymus vulgaris* L. Med. Aroma. Plant Res. J. 2013;1:5-11.

Archive of SID

www.SID.ir

34. Fareed G, Afza N, Malik A, Versiani MA, Fareed N, Lateef M, Iqbal l, Mughal UR, Tareen RB.Phytochemical screening, total phenolic contents and biological evaluation of aerial parts of *Nepeta praetervisa*. J. Chem. Soci. Pak. 2013;35: 1364-1368.
35. Nadia Z, Rashid M. Antioxidant and antibacterial activities of *Thymus vulgaris* L. Med. and Aroma. Plant Res. J. 2013;1:5-11.
36. Hamdy Roby MH, Sarhan MA, Selim KAH, Khalel KI. Evaluation of antioxidant activity, total phenols and phenolic compounds in thyme (*Thymus vulgaris* L.), sage (*Salvia officinalis* L.) and marjoram (*Origanum majorana* L.) extracts. Indust. Crops Prod. 2013;43:827-831.
37. Nikolic M, Glamoclija J, Ferreira ICFR, Calhelha R, Fernandes A, Markovic T, Markovic D, Giweli A, Sokovic M. Chemical composition, antimicrobial, antioxidant and antitumor activity of *Thymus serpyllum* L., *Thymus algerensis* Boiss. and Reut and *Thymus vulgaris* L. essential oil. Indust. Crops Prod. 2014;52:183-190.
38. Soares JR, Dinis TC, Cunha AP, Almedia LM. Antioxidant activities of some extracts of *Thymus zygis*. Free Rad. Res. 1997;26:469-478.
39. Asadi Balsin Sharif Abad S, Nasri S, Amin G, Bidaran S. Anti-inflammatory and anti-nociceptive effects of hydroalchoholic extract of *Nepeta menthoides* on pain in aerial parts in male mice. J. Jahrom Univ. Med. Sci. 2013;11:1-9.
40. Sarahroodi S, Jafari-Najafi R, Nasri S, Rohampour K, Maleki-Jamshid A, Esmaeili S. Effects of *Nepeta menthoides* aqueous extract on retention and retrieval of memory in mice. Pak. J. Biol. Sci. 2012;15:1085-9.
41. Azizzadeh Delshad A, Parvizi M. The Neuroprotective effects of *Nepeta menthoides* on Axotomized Dorsal Root Ganglion sensory neurons in neonate rats. J. Basic Clin. Pathophysiol. 2014; 2:13-20.
42. Farjam MH, Mohammadi M, Izadi S, Barzegari F. Composition and antioxidant activity of the essential oils of *Thymus trautvetteri* Klokov &Desi-Shost, *Thymus migricus* Klokov&Des.-Shost and *Thymus caespititus* Brot. From Iran. Nat. Prod. 2013; 9:345-349.
43. Shahnazi S, Khalighi Sigaroodi F, Ajani Y, Yazdani D, Ahvazi M, Taghizad F. Study on chemical composition and antimicrobial activity of the essential oil of *Thymus trautvetteri* Klokov & Desi-Shost. J. Med. Plants. 2007;6:80-88.

Archive of SID

www.SID.ir

Archive of SID

www.SID.ir