

Original Article

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

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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 *Staphylococcus epidermidis*, *Staphylococcus saprophyticus* and *Staphylococcus aureus* were sensitive against 50% methanol extract of *N. menthoides* even more than the standard antibiotics (10 μ g). 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]. Flavonoids 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 antibacterial activity [5-13].

Amongst various plant families which have been known for their medicinal values, Labiateae 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

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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 saprophyticus*, *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 AlCl₃ colorimetric method. Flavonoids as one of the most diverse and widespread group of natural compounds, posses a broad spectrum of chemical and biological activities.

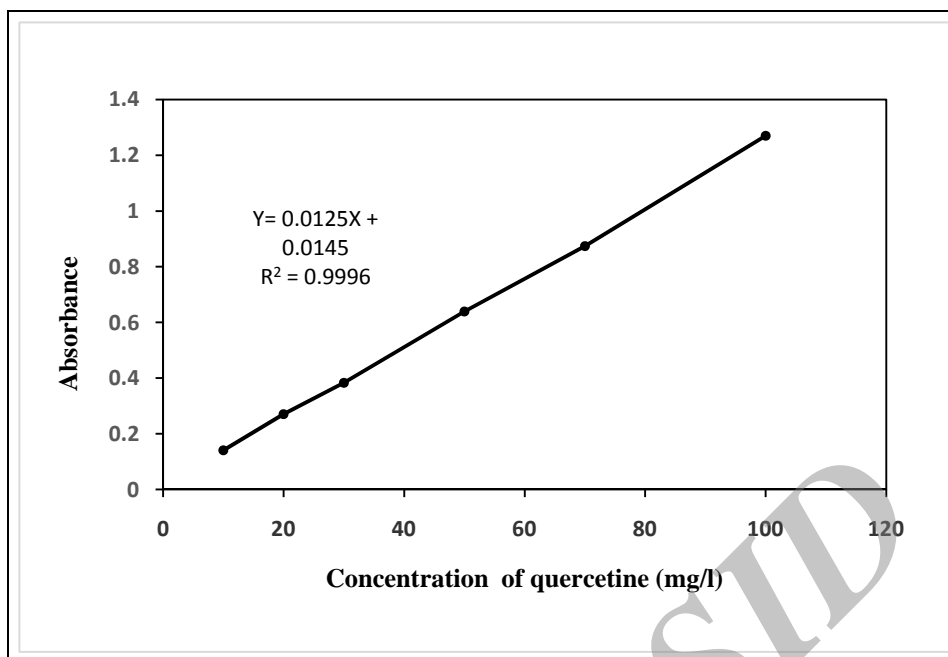


Fig. 1 Calibration plot for flavonoid determination

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].

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)
<i>Nepeta menthoides</i>	aqueous	0.90	71.08	0.71
	chloroform	1.12	88.44	0.88
	50% methanol	2.90	230.84	2.30
<i>Thymus trautvetteri</i>	aqueous	1.78	141.24	1.41
	chloroform	1.85	146.84	1.46
	50% methanol	2.61	207.64	2.07

^aTotal flavonoids contents of extracts in terms of quercetine equivalent (mg of quercetin/l of extract).

^bTotal 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.

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

*Inhibition zone diameter (mm); **NT: Not tested

a: *Nepeta menthoides* Boiss. & Buhse; b: *Thymus trautvetteri* 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.

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