

Full Length Research Paper

Antioxidant activity and concentration of phenols and flavonoids in the whole plant and plant parts of *Teucrium chamaedrys* L. var. *glanduliferum* Haussk

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This paper deals with antioxidant activity of phenol and flavonoid contents in twenty different extracts obtained from the whole plant and plant parts of *Teucrium chamaedrys* L. var. *glanduliferum* Haussk. Antioxidant activity was determined *in vitro* using 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent and expressed as IC₅₀ values ranged from 1165.75 to 24.51 µg/ml. Total phenols were determined using Folin-Ciocalteu reagent and their amounts ranged from 15.98 to 208.17 mg/g. The amounts of flavonoids in plant extracts were in range from 16.42 to 110.13 mg/g. Concentrations of phenols in different plant parts compared with concentrations for the whole plant were of uneven value. The highest concentration of flavonoids was in all the leaf extracts, while slightly lower was found in the stem extracts and the lowest in the flowered ones. A significant linear correlation was noticed between the values of the concentration of phenols and antioxidant activity. Parallel to the analysis of *T. chamaedrys*, *Ginkgo biloba* and Green tea extracts were analyzed for comparison, and the results indicated that some extracts of *T. chamaedrys* were equal in activity with Ginkgo or Green tea and some appeared to have greater activity. Based on these results, *T. chamaedrys* is a potential source of phenols as natural antioxidant substance of high value.

Key words: *Teucrium chamaedrys* L., wall germander, antioxidant activity, phenols, flavonoids.

INTRODUCTION

Wall Germander, *Teucrium chamaedrys* L. var. *glanduliferum* Haussk belongs to the family Lamiaceae, subfamily Ajugoideae and section Chamaedrys. It is a perennial herbaceous plant with half-ligneous and shrub-like low stem up to 30 cm high. It has little-branched stem with oval serrated leaves and tiny blooms on branch-tops. The plant inhabits rocky limestone areas, dry mountain meadows and pastures, the edge of the sparse oak and pine forest up to 1000 meters above sea level in Central Europe, Mediterranean region and Western Asia (Tutin et

al., 1972; Josifovic, 1974). A large number of known medicinal species belonging to the genus *Teucrium* is used in folk medicine and pharmacy. Species of the genus *Teucrium* are very rich in phenolic compounds and exhibit very strong biological activity. The best popular species of this genus in the flora of Europe is *T. chamaedrys* which is used in the treatment of digestive disorders, abscesses, gout and conjunctivitis and in stimulation of fat and cellulite decomposition (Jurisic et al., 2003, Stankovic et al., 2010).

The products of secondary metabolism are not of essential importance, but are most often the result of environmental conditions. The main secondary metabolites in plants are glycosides, terpenoids, steroids, tannins and phenolic compounds (Acamovic and

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Brooker, 2005). Secondary metabolites perform a number of protective functions in the human body and are involved in important anti-oxidative, anti-allergic, antibiotic, hypoglycaemic and anti-carcinogen biological and pharmacological activities (Mulabagal and Tsay, 2004; Borneo et al., 2008; Kulisic-Bilusic et al., 2008).

Many pathological disorders in human organism such as atherosclerosis, arthritis, Alzheimer disease, cancers etc. may be the results of increased concentrations of free radicals in the body. Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) are the most frequent prooxidants either produced from normal metabolism or induced by ultraviolet (UV) radiation and different pollutants from the environment (Ognjanovic et al., 2008). Harmful effects resulted from the disequilibrium in the antioxidant-prooxidant balance can be largely prevented by the intake of antioxidant substances (Ghosh et al., 2008; Rattanachitthawat et al., 2010). Antioxidants can be of synthetic origin and a great number of secondary metabolites are isolated from plants, such as various phenolic compounds (Singh, 2002; Shahvar et al., 2010). Health promoting effects of antioxidants in plants have been well documented. Antioxidants work by donating an electron to a molecule that has been compromised by oxidation, bringing it back into a state of proper function (Zulkhairi et al., 2010). Due to their natural origin, the antioxidants obtained from plants are of greater benefit in comparison to synthetic ones. The use of natural antioxidants from plants does not induce side effects, while synthetic antioxidants were found to have genotoxic effect (Chen et al., 1992; Kahl and Kappus, 1993).

Some medicinal plants such as Ginkgo (*Ginkgo biloba* L., Ginkgoaceae) and Green tea (*Camellia sinensis* L. (Kuntze), Theaceae) are very important regarding the content of natural antioxidant substances and are widely used in folk medicine and pharmacy (Bridi et al., 2001; Anesini et al., 2008). In many studies of plant antioxidant substances, these plants serve as natural standards for evaluation of antioxidant activity and other pharmaceutical purposes of medicinal plants (Shrififar et al., 2003). Whole plant extracts were investigated in numerous studies of biological activity of medicinal plants (Couladis et al., 2003). The results of some authors who studied plants in particular have showed different activity of vegetative plant parts. Comparative studies of the composition of active substances and biological activity of extracts obtained from leaves, flowers, roots and stems showed different values. Separate examination of plant parts allows a significant contribution to medicinal plant study and their pharmaceutical applications (Mariod et al., 2007; Siddique et al., 2009).

The present study is prompted by the fact that no data on antioxidant activity, phenol concentration and flavonoid content of *T. chamaedrys* var. *glanduliferum* have been provided so far, and also in the literature there are no data concerning the comparative analysis of the antioxidant activity of the whole plant and different plant

parts of any species of the genus *Teucrium*. The basic aim of the presented research was to determine the contents of phenolics and concentrations of flavonoids in various extracts of the species *T. chamaedrys* using spectrophotometric methods, as well as to examine the antioxidant activity of plant extracts *in vitro* using standard model system. In addition, we compared the results obtained from the whole plant extracts with the results of analysis of extracts from different plant parts such as leaves, flowers and stems, and assessed the significance of particular analysis for effective use in pharmacy. We also compared the obtained values of antioxidant activity with the values of standard synthetic antioxidants. A parallel analysis of Ginkgo (*G. biloba*) and Green tea (*C. sinensis*) as most popular plants rich in natural antioxidants was carried out and compared with the values related to *T. chamaedrys*.

MATERIALS AND METHODS

Chemicals

Acetone, methanol, petroleum ether, ethyl acetate and sodium hydrogen carbonate were purchased from "Zorka pharma" Sabac, Serbia. Gallic acid, rutin hydrate, chlorogenic acid and 2,2-Dyphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma Chemicals Co., St Louis, MO, USA. Folin-Ciocalteu phenol reagent, 3-tert-Butyl-4-hydroxyanisole (BHA) and aluminium chloride hexahydrate were purchased from Fluka Chemie AG, Buchs, Switzerland. All other solvents and chemicals were of analytical grade. The samples of Green tea (*C. sinensis*) were purchased from a local pharmacy. A standardized extract of *G. biloba* was obtained from Pharmaceutical Company "Ivancic i Sinovi", Belgrade, Serbia.

Plant material

In June, 2009 aerial flowering parts of *T. chamaedrys* were collected from natural populations in the region of Trgoviste in south Serbia: (position: 42° 21' 53.93" N, 22° 05' 11.53" E, altitude: 697.68 m, exposition: SW, substratum: limestone). The voucher specimen of *T. chamaedrys* L. 1767, No 2-2210, Pcinja river gorge, Trgoviste, UTM 34 TEM 89, 12th June, 2009., det.: Milan Stankovic; rev.: Goran Anackov, were confirmed and deposited at the Herbarium of the Department of Biology and Ecology (BUNS Herbarium), Faculty of Natural Science, University of Novi Sad. The collected plant material was air-dried in darkness at ambient temperature (20°C). A part of the collected material was the whole dried plant and the other consisted of dried plant parts (leaves, flowers and stems). The dried plant material was cut up and stored in tightly sealed dark containers until needed.

Preparation of plant extracts

The air-dried plant material (10 g) was coarsely crushed in small pieces of 2 - 6 mm by using the cylindrical crusher and extracted with water and different organic solvents (methanol, acetone, ethyl acetate and petroleum ether), using the Soxhlet apparatus. The extract was filtered through a paper filter (Whatman, No. 1) and evaporated under reduced pressure by the rotary evaporator. The obtained extracts (Table 1) were stored in dark glass bottles for further processing.

Table 1. The yields of extracts of *T. chamaedrys* obtained from 10 g of dried plant material.

Type of extract	Water	Methanol	Acetone	Ethyl acetate	Petroleum ether
Whole plant	2.42	1.93	0.33	0.35	0.12
Leaves	2.75	2.44	0.63	0.42	0.08
Flowers	1.61	1.73	0.41	0.24	0.06
Stems	2.29	1.04	0.23	0.11	0.05

Determination of total phenolics in the plant extracts

The concentration of phenolics in the plant extracts was measured by using spectrophotometric method (Singleton et al., 1999). The methanol solution of the extract in concentration of 1 mg/ml was used in the analysis. The reaction mixture was prepared by mixing 0.5 ml of methanol solution of the extract, 2.5 ml of 10% Folin-Ciocalteu reagent dissolved in water and 2 ml of 7.5% NaHCO₃. The blank was concomitantly prepared containing 0.5 ml of methanol, 2.5 ml of 10% Folin-Ciocalteu reagent dissolved in water and 2 ml of 7.5% of NaHCO₃. The samples were thereafter incubated in the thermostat at 45°C for 45 min. The absorbance was determined using spectrophotometer at λ_{\max} = 765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid and the calibration line was construed. Based on the measured absorbance, the concentration of phenolics was read (mg/ml) from the calibration line; then the content of phenolics in the extracts was expressed in terms of gallic acid equivalent, GAE (mg of GA/g of extract).

Determination of total flavonoids in the plant extracts

The content of flavonoids in the examined plant extracts was determined using spectrophotometric method (Quettier et al., 2000). The sample contained 1 ml of methanol solution of the extract in concentration of 1 mg/ml and 1 ml of 2% AlCl₃ solution dissolved in methanol. The samples were incubated for an hour at room temperature. The absorbance was determined using spectrophotometer at λ_{\max} = 415 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of rutin and the calibration line was construed. Based on the measured absorbance, the concentration of flavonoids was read (mg/ml) on the calibration line; then, the content of flavonoids in extracts was expressed in terms of rutin equivalent, RUE (mg of RU/g of extract).

Evaluation of antioxidant activity

The ability of the plant extract to scavenge DPPH free radicals was assessed by the standard method (Tekao et al., 1994), adopted with suitable modifications (Kumarasamy et al., 2007). DPPH (20 mg) was dissolved in methanol (250 ml) to obtain the concentration of 80 µg/ml. The stock solution of the plant extract was prepared in methanol to achieve the concentration of 1 mg/ml. Dilutions were made to obtain concentrations of 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.90, 1.99, 0.97 µg/ml. Diluted solutions (1 ml each) were mixed with DPPH (1 ml). After 30 min in darkness at room temperature (23°C), the absorbance was recorded at 517 nm. The control samples contained all the reagents except the extract. The percentage inhibition was calculated using Equation (1), whilst IC₅₀ values were estimated from the percentage (%) inhibition versus

concentration plot, using a non-linear regression algorithm. The data were presented as mean values ± standard deviation (n = 3).

$$\% \text{inhibition} = \left(\frac{A \text{ of control} - A \text{ of sample}}{A \text{ of control}} \right) \times 100 \quad (1)$$

Statistical analysis

All experimental measurements were carried out in triplicate and are expressed as average of three analyses ± standard deviation. The magnitude of correlation between variables was done using a SPSS (Chicago, IL) statistical software package (SPSS for Windows, ver. 17, 2008).

RESULTS AND DISCUSSION

Twenty whole-plant and plant part extracts using water and four different organic solvents were prepared to examine the antioxidant activity and concentrations of phenols and flavonoids. The extraction solvents of different polarity were used to extract the active substances of different polarity. The yield of extract obtained from 10 g of dried plant material was measured for each extract (Table 1). Based on the yield values of the obtained extracts, it was found that the largest amount of yield was obtained using polar solvents for extraction such as water and methanol. The concentration of phenols in the examined plant extracts using the Folin-Ciocalteu reagent was expressed in terms of gallic acid equivalent, GAE (the standard curve equation: $y = 7.026x - 0.0191$, $r^2 = 0.999$) as mg of GA/g of extract (Table 2). The concentrations of phenols in the examined extracts ranged from 15.98 to 208.17 mg/g. The high concentration of phenols was measured in some aqueous, methanol and acetone extracts. The aqueous leaf extracts and methanol stem extracts had the highest concentrations of phenols. All ethyl acetate and petroleum ether extracts had considerably smaller concentration of phenols. The extracts obtained using more polar solvents had higher concentrations of phenols while the extracts obtained using low polar solvents contained small concentrations (Table 2).

The concentrations of phenols in different plant parts compared with those of the whole plant were not the same. Compared to other plant parts and the whole plant, leaf extracts contained the highest concentration of

Table 2. Total phenol contents¹ in the plant extracts of *T. chamaedrys* expressed in terms of gallic acid equivalent, GAE (mg of GA/g of extract).

Type of extract	Water	Methanol	Acetone	Ethyl acetate	Petroleum ether
Whole plant	168.46 ± 0.26	169.50 ± 0.22	143.95 ± 0.58	30.39 ± 0.27	42.64 ± 0.23
Leaves	208.17 ± 0.18	149.15 ± 0.24	153.67 ± 0.49	61.10 ± 0.15	56.73 ± 0.21
Flowers	150.04 ± 0.21	144.28 ± 0.23	127.50 ± 0.48	34.22 ± 0.14	15.98 ± 0.19
Stems	136.75 ± 0.19	175.46 ± 0.21	107.67 ± 0.59	70.18 ± 0.17	43.57 ± 0.16

¹Each value in the table was obtained by calculating the average of three analyses ± standard deviation.

Table 3. Flavonoid contents¹ in different plant extracts of *T. chamaedrys* expressed in terms of rutin equivalent (mg of RU/g of extract).

Type of extract	Water	Methanol	Acetone	Ethyl acetate	Petroleum ether
Whole plant	16.67 ± 0.21	61.80 ± 0.18	78.03 ± 0.94	87.17 ± 0.21	20.71 ± 0.51
Leaves	18.26 ± 0.37	71.21 ± 0.52	100.15 ± 0.59	110.13 ± 0.41	19.64 ± 0.71
Flowers	14.93 ± 0.16	27.39 ± 0.75	49.04 ± 0.46	43.61 ± 0.22	16.42 ± 0.26
Stems	16.33 ± 0.35	47.01 ± 0.26	80.96 ± 0.71	46.42 ± 0.43	16.58 ± 0.44

¹Each value in the table was obtained by calculating the average of three analyses ± standard deviation.

phenol in water, acetone and petroleum ether extracts; in the flower extract it was smaller and minimum concentrations were detected in the stem extracts. In methanol and ethyl acetate extracts the content of phenols in plant parts had different values because the stems contain the highest concentration of phenols, while in the leaves it was smaller and minimum concentration is in flowers. The approximate concentrations were measured in the whole plant extracts because the whole plant extracts contain different compounds present in all the plant parts, the only deviation was noted in the ethyl acetate extract (Table 2).

Our data confirm the results of other researchers who analyzed the concentration of phenols in the plant parts and measured the greatest value in the leaves (Siddique et al., 2009; Rafat et al., 2010). The content of phenols in plant extracts of the species *T. chamaedrys* depends on the type of extracts and polarity of solvents used for extractions. The reason for high concentrations of phenols in some stem extracts is in a large number of glandular hairs on the surface of stems which contain large amounts of phenolic components. Due to the presence of numerous glandular hairs, this variety is named *glanduliferum* (Josifovic, 1974). High solubility of phenols in polar solvents provides high concentration of these compounds in the extracts obtained using polar solvents for the extraction (Zhou and Yu, 2004; Mohsen and Ammar, 2008). The summary of experimental results regarding total phenolic compounds in different plant extracts tested is shown in Table 2.

In their research some authors use to compare medicinal plants with some well-known natural standards such as Green tea (Shrififar et al., 2003). In this research

Green tea and *G. biloba* were comparatively analyzed. The obtained values of total phenol content in Green tea varied from 16.02 to 233.68 mg/g (Table 6). In comparing with Green tea, some water, acetone, ethyl acetate and petroleum ether extracts of *T. chamaedrys* contained greater concentrations of phenols, while only methanol extracts of Green tea had higher concentration of phenols than methanol whole plant and plant parts extracts of *T. chamaedrys*. The value of phenol content in *G. biloba* standardized extract was 140.18 mg/g (GAE), (Table 7). In comparing with *G. biloba*, some water, methanol and acetone *T. chamaedrys* extracts contained greater concentrations of phenols, while all ethyl acetate and petroleum ether extracts were found to have smaller concentrations than *G. biloba* extract.

The concentration of flavonoids in various plant extracts of *T. chamaedrys* was determined using spectrophotometric method with aluminium chloride. The content of flavonoids was expressed in terms of rutin equivalent, RUE (the standard curve equation: $y = 17.231x - 0.0591$, $r^2 = 0.999$), mg of RU/g of extract. The summary of quantities of flavonoids identified in the tested extracts is shown in Table 3. The concentrations of flavonoids in plant extracts ranged from 16.42 to 110.13 mg/g. High concentrations of flavonoids were measured in ethyl acetate, acetone and some methanol extracts. Ethyl acetate and acetone extracts from leaves contained concentrations above 100 mg/g. The lowest flavonoid concentration was measured in aqueous and petroleum ether extracts. The concentration of flavonoids in plant parts differed greatly from the value obtained for the whole plant. The highest concentration of flavonoids was in all the leaf extracts, it was slightly lower in the stem

Table 4. Antioxidant (DPPH scavenging) activity of plant extracts of *T. chamaedrys* presented as IC₅₀ values¹ (µg/ml).

Type of extract	Water	Methanol	Acetone	Ethyl acetate	Petroleum ether
Whole plant	31.79 ± 1.16	29.46 ± 0.99	35.73 ± 0.89	269.41 ± 2.31	341.08 ± 2.87
Leaves	40.87 ± 1.44	47.07 ± 1.12	42.85 ± 1.51	156.22 ± 1.89	568.62 ± 3.24
Flowers	32.38 ± 0.96	29.33 ± 1.08	32.60 ± 1.11	362.70 ± 2.55	1165.75 ± 4.18
Stems	35.91 ± 1.28	24.51 ± 1.32	27.77 ± 0.54	112.64 ± 2.17	193.81 ± 1.81

¹Each value in the table was obtained by calculating the average of three analyses ± standard deviation.

Table 5. Values¹ of antioxidant (DPPH scavenging) activity of standard substances obtained for comparison with *T. chamaedrys*.

Substances	IC ₅₀ µg/ml
BHA	5.39 ± 0.31
Rutin	9.28 ± 0.27
Chlorogenic acid	11.65 ± 0.52

¹Each value in the table was obtained by calculating the average of three analyses ± standard deviation.

extracts flavonoid concentration values ranked in the same order: leaves, flowers and stems (Table 3). A large quantity of flavonoids in stem extracts was derived from dense glandular hairs on the surface of the stem. These data are in agreement with other studies reporting flavonoid concentration in different plant parts (Siddique et al., 2009).

The concentration of flavonoids in the extracts depends on the polarity of solvents and the type of plant material used for the extractions. The concentration of flavonoids in plant extracts depends on the polarity of solvents used in the extract preparation (Min and Chun-Zhao, 2005). Based on the obtained values of the concentration of flavonoids in the examined extracts of *T. chamaedrys*, it was found that the highest concentration of these compounds was in the extracts obtained using solvents of moderate polarity. The obtained values of the flavonoid concentrations in Green tea varied from 34.18 to 335.40 mg/g (Table 6). In the comparison of flavonoid concentrations between Green tea and *T. chamaedrys* only the methanol extracts showed equal values. Water, acetone, ethyl acetate and petroleum ether extracts of Green tea had greater concentration of flavonoids than the ones of *T. chamaedrys*. The value of flavonoid concentration in *G. biloba* standardized extract was 192.69 mg/g (RUE), (Table 7) higher than the values of all *T. chamaedrys* extracts, but ethyl acetate and acetone leaf extracts had the most approximate values of concentrations if compared with *G. biloba*. compared with *G. biloba*.

The antioxidant activity of different plant extracts of *T. chamaedrys* was determined using methanol solution of DPPH reagent. DPPH is a very stable free radical, unlike

in vitro generated free radicals, such as hydroxyl radical and superoxide anion, DPPH has the advantage of being unaffected by certain side reactions, such as metal-ion chelation and enzyme inhibition, brought about by various additives. Freshly prepared DPPH solution exhibited a deep purple colour with an absorption maximum at 517 nm. This purple colour generally fades/disappears when antioxidant molecules can quench DPPH free radicals (that is by providing hydrogen atoms or by electron donation, conceivably via a free-radical attack on the DPPH molecule) and converts them into a colourless/bleached product (that is 2, 2-diphenyl-1-hydrazine, or a substituted analogous hydrazine), resulting in a decrease in absorbance at 517 nm band (Amarowicz et al., 2003).

The antioxidant activity of twenty whole plant and different plant part extracts of *T. chamaedrys* is expressed in terms of IC₅₀ (µg/ml) values (Table 4). Parallel to the examination of the antioxidant activity of the plant extracts, the values for three standard compounds (Table 5) and two well-known medicinal plants - Green tea (Table 6) and *G. biloba* (Table 7) were obtained and compared to the values of the antioxidant activity. The standard substances used were rutin, chlorogenic acid and BHA. Summary display of obtained IC₅₀ values of antioxidant activity of the extracts is given in Table 4. The obtained values for antioxidant activity examined by DPPH radical scavenging activity ranged from 1165.75 to 24.51 µg/ml. The largest capacity to neutralize DPPH radicals was found in methanol extracts, especially the stem extracts, which neutralized 50% of free radicals at the concentration of 24.51 µg/ml. Similar activity was found in some aqueous and acetone extracts. The minute capacity to inhibit DPPH radicals was determined for ethyl acetate and petroleum ether extracts.

In comparison to IC₅₀ values of BHA, rutin and chlorogenic acid (Table 5), methanol, aqueous and acetone extracts of *T. chamaedrys* manifested the strongest capacity to neutralize DPPH radicals. The values of antioxidant activity of Green tea obtained for comparison with *T. chamaedrys* ranged from 14.50 to 238.25 µg/ml, (Table 6). Comparing the antioxidant activity of Green tea and *T. chamaedrys*, only the acetone and petroleum ether extracts showed higher values than Green tea. Other extracts appeared to have

Table 6. Values¹ of phenol and flavonoid contents and antioxidant activity of *Camellia sinensis* extracts obtained for comparison with *T. chamaedrys*.

Type of extract	Water	Methanol	Acetone	Ethyl acetate	Petroleum ether
Phenols (mg/g), (GAE)	140.11 ± 0.29	233.68 ± 0.18	147.77 ± 0.77	61.94 ± 0.19	16.02 ± 0.29
Flavonoids (mg/g), (RUE)	195.41 ± 1.04	47.80 ± 0.44	335.40 ± 0.71	242.95 ± 1.45	34.18 ± 0.85
Antioxidant activity (IC ₅₀)	20.62 ± 1.06	14.50 ± 1.69	28.59 ± 1.12	61.43 ± 1.16	238.25 ± 2.01

¹Each value in the table was obtained by calculating the average of three analyses ± standard deviation.

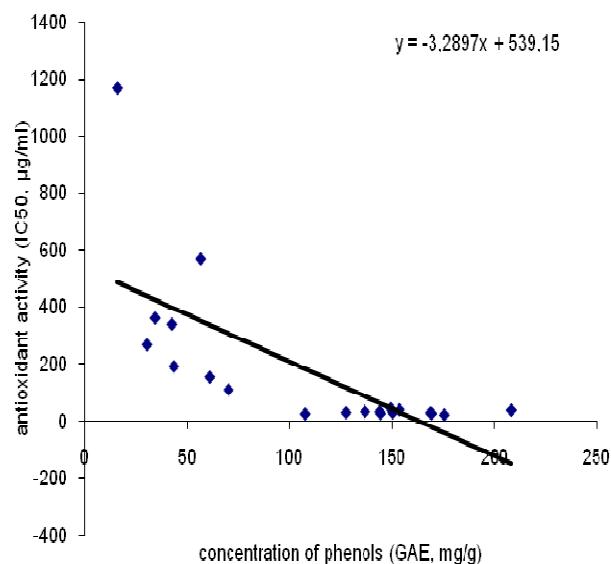
Table 7. Values¹ of phenol and flavonoid contents and antioxidant activity of *Ginkgo biloba* standardized extract obtained for comparison with *T. chamaedrys*.

Type of analysis	Value
Phenols (mg/g), (GAE)	140.18 ± 0.26
Flavonoids (mg/g), (RUE)	192.69 ± 0.72
Antioxidant activity (IC ₅₀ µg/ml)	33.91 ± 1.16

¹Each value in the table was obtained by calculating the average of three analyses ± standard deviation.

The extraction of antioxidant substances of different chemical structure was achieved using solvents of different polarity. Numerous investigations of qualitative composition of plant extracts revealed the presence of high concentrations of phenolics in the extracts obtained using polar solvents (Canadanovic et al., 2008). The extracts performing the highest antioxidant activity (Table 4) had the highest concentration of phenols (Table 2). Phenols are very important plant constituents because of their scavenging ability on free radicals due to their hydroxyl groups. Therefore, the phenolic content of plants may contribute directly to their antioxidant action (Tosun et al., 2009). Between the values of concentration of phenolic compounds (Table 2) and antioxidant activity of different plant extracts of *T. chamaedrys* a significant linear correlation has been proved (coefficient of correlation $r = 0.701$). Numerous investigations of the antioxidant activity of plant extracts have confirmed a high linear correlation between the values of phenol concentration and antioxidant activity (Katalinic et al., 2004; Borneo et al., 2008; Moein and Reza Moein, 2010). The results of statistical calculation of linear correlation are shown in Figure 1.

Flavonoids are class of secondary plant phenolics with significant antioxidant and chelating properties. Antioxidant activity of flavonoids depends on the structure and substitution pattern of hydroxyl groups (Sharififar et al., 2008). Methanol and acetone extracts of *T. chamaedrys* have high concentration of total phenols (Table 2) and flavonoids (Table 3), which is in correlation with the intense antioxidant activity of these extracts (Table 4).

**Figure 1.** Linear correlation between the amount of total phenols and antioxidant activity. Correlation coefficient $r = 0.701$, coefficient of determination (R^2) = 0.492. Correlation is significant at the 0.01 level (2-tailed).

Conclusions

Values for antioxidant activity and concentration of phenolic compounds of extracts from different plant parts of *T. chamaedrys* var. *glanduliferum* in relation to the values for extracts of whole plants vary according to the plant parts and the solvents used for extraction. The aqueous leaf extracts and methanol stem extracts had the highest concentrations of phenols. High concentrations of flavonoids were measured in ethyl acetate, acetone and some methanol extracts. The high phenolic content and significant correlation indicated that these compounds contribute to the strong antioxidant activity. These results indicate that some plant extracts have high concentrations of phenols and noticeable effect on the scavenging of free radicals equal with *G. biloba* and Green tea. The extracts of these plant can be regarded as promising candidates for plant sources antioxidant compound.

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