Progress in Nutrition 2017; Vol. 19, Supplement 1: 121-129 DOI: 10.23751/pn.v19i1-S.5279 © Mattioli 1885

Original Article

Appraisal of biological activities of crude extracts with sub-fractions and phytochemical content of endemic *Nepeta nuda* L. subsp. *lydiae* from Turkey

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Summary. Antioxidant activity of crude water extract (CWE) of this plant and its five sub-fractions (namely, hexane fraction (HF), chloroform fraction (CFF), ethyl acetate fraction (EAF), n-butyl alcohol fraction (BAF) and rest of the fractions (RF) were measured using *in vitro* two antioxidant assays: DPPH and ferrous ion chelating. Also, quantitative content of total flavonoids and phenols were carried out by colorimetric methods, using aluminum chloride method and Folin Ciocalteu reagent respectively. CWE and five sub-fractions possessed different antioxidant activities in antioxidant assays. EAF showed the most potent activity on DPPH radicals and ferrous ion chelating activities with IC_{50} values of 10.45 and 16.76 µg /ml respectively. EAF had the highest total phenolics (145.06 µg GAE/mg dried extract or fraction) and total flavonoids (50.16 µg QEE/mg dried extract or fraction) contents. The essential oil of the aerial parts of this plant was analyzed by headspace gas chromatography mass spectroscopy (HS-GC/MS) for the first time and fifty nine components were identified representing 95.91% essential oil. 1,8-cineole (28.79%), nepetalactone (9.93%), sabinene (7.88%) and α-pinene (5.71%) were identified as the major components. The essential oil analyzes results have given some clues on the commercial usable, renewable resources and potentials usable of the *Nepeta* taxa.

Key words: antioxidant, endemic, essential oil, *Nepeta*, phytochemistry

Introduction

In many crude extracts, especially phenolic-rich plants, which are responsible for antioxidant activity, are increasingly of interest in the food industry, because they may prevent oxidative degradation of lipids and consequently, improvable quality and value of food (1). Medicinal plants are promising in many ways and represent the main source of antioxidants. So numerous medicinal plant species is investigated in detail because of its antioxidant properties. Especially those belonging to the family of Lamiaceae that have demonstrated with studies contained many effective natural antioxidant molecules. Located in the family of Lamiaceae *Nepeta* species in folk medicine is used for intensive healing disease such as diuretic, expectorant, antiseptic, antitussive, antiasthmatic and febrifuge efficiencies (2). A great deal functional and biochemical stages in the human body may compose oxygen-centered free radicals and different reactive oxygen species as byproducts. Excessively production of such free radicals can reason oxidative detriment to biomolecules (e.g. lipids, proteins, DNA), ultimately give rise to many chronic diseases, like cancer, atherosclerosis, diabetes, aging, and other degenerative illness in humans (3). Plants, fruits, vegetables and medicinal herbs are comprise, unique and different antioxidant molecules such as phenolic compounds, nitrogen compounds, vitamins, terpenoids and some other endogenous metabolites, which are rich in antioxidant activity (4). Many studies have proven that phenolic compounds exhibits a tremendous antioxidant activity as a consequence of their eliminate free radi-

cals. Furthermore phenolic compounds can be served by preventing radical formation, improving the antioxidant endogenous system and chelating metal ions. An enthusiastic manner we are continuing to research in the biological activity of aromatic, medicinal, edible and drinkable plants and their components and, more specifically, as a continuance of a previously published report (5-7), we'll continue to research not reported endemic, chemical composition and biological activity of plant material. *N. nuda* subsp. *lydiae* is an endemic plant in Flora of Turkey (8) and no phytochemical and antioxidant activity studies about this plant has been reported.

Although there are chemical studies of essential oils of some *Nepeta* taxa; the main constituents so far identified β-caryophyllene, caryophyllene oxide, 1,8-cineol, camphor, citronellol, geraniol, nepetalactone, nerol, spathulenol, β-elemene, geranyl acetate, borneol and germacrene D; the plant also contained nepetalactones and alkaloids, such as, actinidine and iridomyrmecine (9-11), *N. nuda* L. subsp. *lydiae* has not been studied so far in Turkey. To the best of our knowledge this is the first report on essential oil, phenol and flavonoid contents, and antioxidant activity of *N. nuda* subsp. *lydiae* which is grown in Turkey. We report on the essential oil composition of *N. nuda* subsp. *lydiae* for the first time from Turkey with HS-SPME and GC-MS methods. Here in this study, we focused on antioxidant properties of CWE extracts and subfraction of *N. nuda* subsp. *lydiae* as well as in the phytochemical composition.

Material and Methods

Plant source

N. nuda subsp. *lydiae* was collected from Saban and Yelesen villages, edge of *Quercus* forest, 24.06.2013, 1500-1600 m., Kilic 5325. The voucher specimens kept in the Yıldırımlı Herbarium from Ankara. The identification of plant sample was made according to volume seven of Flora of Turkey (7).

Chemicals

Gallic acid, α-tocopherol (Vit E), Butylated hydroxyanisole (BHA), sodium carbonate (Na_2CO_3), Butylated hydroxytoluene (BHT), hexane, ethyl acetate, chloroform, 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) and quercetine were supplied by Sigma Chemical Co. (St. Louis, USA). Ethylenediaminetetraacetic acid (EDTA), Folin-Ciocalteau's phenol reagent, ferrous chloride ($FeCl_2$), sodium hydroxide (NaOH), aluminium chloride ($AlCl_3$), sodium nitrite ($NaNO_2$), n-butanol, and methanol were all supplied by Merck (Germany). All chemicals were of analytical grade.

Extraction and fractionation process

For extraction, the dried aerial parts of plant were ground into fine powder inthelaboratory type mill; then the finely powdered plant was extracted by reflux with distilled water for 3 h at 90°C. The water extract was allowed to cool to laboratory conditions filtered through Whatman No.1 filter paper, then the dried water crude extract was obtained with evaporation of water by rotary evaporation under reduced pressure to calculate yield. Finally, from 118 g of the dried plant, the final yield of CWE was 7.11 g of the 7.11 g of dry extract, 0.50 g was redissolved in methanol to a concentration of 100 mg/ml and stored in the dark at 4°C for further use. For fractionation, 6.61 g of crude water extract (CWE) was redissolved in hot distilled water (700 ml) subjected to sequential liquid–liquid extraction with Hexane (HF), chloroform (CFF), ethyl acetate (EAF), n-butyl alcohol (BAF). These fractions were evaporated to dryness *in vacuo*. The yield of these fractions was 0.64 g, 1.22 g, 2.51 g and 0.97 g respectively. The four sub-fractions were redissolved in their appropriate solvents accordingly, to a concentration of 100 mg/ml and stored in the dark at 4°C until used to determine their antioxidant activities and component.

Determination of in vitro antioxidant activities of the extract and sub-fractions of NNL DPPH free radical-scavenging activity

CWE and its four sub-fractions were tested with DPPH free radical according to the method previously defined by Zovko Koncic *et al.* (2010), with some modifications (12). Shortly, 0.5 ml of extracts, sub-fractions and synthetic antioxidant compounds (BHA, BHT and Vit-E) prepared at different concentrations (12.5–400 μg/ml) were taken into test tubes and stirred with of 2.5 ml 2 mM DPPH solution. The mixture was stirred thoroughly and incubated for 30

min in the dark in laboratory conditions. The absorbance at a wavelength of 517 nm was measured by UV spectrophotometry. To determine DPPH free radical scavenging activity (FRSA) was used the following equation. FRSA (%)=((A_0-A_1)/A_0)x100 A_0 is the absorbances value without specimen and A_1 the absorbance in the presence of specimen. As opposed to increasing concentration of specimens decline of absorbance is an indication that destroyed DPPH radical. Antioxidant activity results are expressed as IC_{50} value (μg extract/ml) that reduces by half the effective concentration of DPPH radicals and was calculated by interpolation from linear regression analysis.

Ferrous chelating capacity

The ferrous chelating capacity of samples and the reference compound (EDTA) was conducted following the method used by Decker and Welch (13) with slight modifications. 2 ml of extract, sub-fractions and reference compound (EDTA) of concentration labeling between 25 and 250 μg/ml were added to 50 μl of 2 mM $FeCl_2$ and well mixed. The reaction mixture was incubated in laboratory conditions. The reaction occurred by the addition of 100 μl 5 mM ferrozine. After 10 min of incubation period, the absorbance of the solution was measured at 562 nm using a UV–visible spectrophotometer. For the ferrous-chelating activity, IC_{50} values were calculated using the equation as described above is used for DPPH free radical scavenging activity.

Determination of phytochemical content

Determination of total phenolic content

Total phenolic content of the extract and its sub-fractions were determined using the Folin-Ciocalteu reagent according to the method by Moulehi *et al.*, (2012) with some alterations (14). Volumes of 100 μL of dissolved in suitable solvents of CWE and sub-fractions taken into test tubes and were added to 4.5 mL of distilled water and 100 μL Folin-Ciocalteu reagent and fully mixed. After incubation for 10 min at laboratory conditions, on the test tubes 3 mL of 2% (m/v) Na_2CO_3 adding to this solution was stirred and placed in a water bath at 40ºC for 20 min. Samples cooled at laboratory conditions of absorbance at 760 nm were recorded by UV-VIS spectrophotometer. Experiments were repeated three times for each sample. The content of total phenolic was expressed as μg of gallic acid equivalents (GAE)/g dried extract and sub-fractions.

Determination of total flavonoid content

For the total flavonoid content (TFC), the method based on Dewanto *et al.*, (2002) with a minor modifications was used (15). One milliliter of diluted with suitable solvents of CWE and its sub-fractions were mixed with 150 μl $NaNO_2$ (15%) and left at laboratory conditions for 5 min before adding 75 μl of $AlCl_3$ (10%). After 5 min, 1 ml of NaOH (4%) was added. Total volume was adjusted to 5 ml with the addition of distilled water and in a good way mixed. The total flavonoid content in the examples was measured spectrophotometrically (UV-Vis) at 510 nm. Different concentrations of quercetin (15-480 μg/mL) were used for calibration. The quantification was carried out using a calibration curve. The results were expressed in μg quercetin equivalents (QEE)/mg dried extract and sub-fraction as mean of tree replicates.

Determination of the volatile oils of NNL

HS-SPME procedure

Five grams aerial part of plant sample was made powder with a blender. Five grams powder of plant sample put inside a 40 ml vial and carried out by a (HS-SPME) head space solid phase micro extraction method using a divinyl benzene/carboxen/polydimethylsiloxane fiber, with 50/30 μg film thickness; before the analysis the fiber was preconditioned in the injection port of the gas chromatography (GC) as indicated by the producer. 5 g of aerial parts of plant sample previously homogenized, was weighed into a 40 ml vial; the vial was equipped with a "mininert" valve. The vial was kept at 35°C with permanent internal stirring and the sample was left to equilibratefor 30 min; then, the SPME fiber was exposed for 40 min to the headspace while maintaining the sample at 35°C. After sampling, the SPME fiber was introduced into the GC injector, and was left for 3 min to allow the analyzes thermal desorption. In order to optimize the technique, sample volume, sample headspace volume, sample heating temperature and extraction time were studied on the

extraction efficiency as previously reported (16).

GC-MS analysis

A Varian 3800 gas chromatograph directly inter faced with a Varian 2000 ion trap mass spectrometer (Varian Spa, Milan, Italy) was used with injector temperature, 260°C; injection mode, splitless; column, 60 m, CP-Wax 52 CB 0.25 mm i.d., 0.25 µg film thickness. The oven temperature was programmed; 45°C held for 5 min, then increased to 80°C at a rate of 10°C/min, and to 240°C at 2°C/min. The transporter gas was helium, used at a constant pressure of 10 psi; the transfer line temperature, 250°C; the ionisation mode, electron impact (EI); acquisit ion range, 40 to 200 m/z; scan rate, 1 us-1 (17).

Results and Discussion

Yield of extraction and fractions

Aerial parts of *NNL* plant was extracted by traditional reflux method using distilled water. Extraction yield for CWE was found to be 6.02%. The fractions of HF, CFF, EAF, BAF and RF sub-fractions of CWE extracts were yielded 9.68%, 18.46%, 37.97%, 14.67%, and 19.21%, respectively. The fraction yields, in increasing order, were: EAF > RF > CFF > BAF > HF. This shows that ethyl acetate was the best solvent for fractionation of components from CWE. Extraction and fractionation process for the most decisive factor is the selection of suitable solvents, because of the variability of chemical structure and composition in extract, in terms of the biological activity the each extract-solvent mixture (fraction) may exhibit unpredictable behavior (18). For this reason, in this work, the extract and sub-fraction yield, total phenol contents, total flavonoid contents and antioxidant properties of CWE extracts and its sub-fraction obtained were compared.

Chemical composition of the essential oil

The compounds were identified using the NIST (National Institute of Standards and Technology) and mass spectral library and verified by the retention indices which were calculated as described by Van den Dool and Kratz (17). The relative amounts were calculated on the basis of peak-area ratios. The identified constituents of *NNL* is listed in Table 1. Fifty nine components were identified representing 95.91% essential oil. 1,8-cineole (28.79%), nepetalactone (9.93%), sabinene (7.88%) and α-pinene (5.71%) were identified the major components of studied sample (Table 1). The chemical structure essential oil of aerial parts of *N. baytopii, N. cataria* and *N. fissa* were invastigated by GC and GC-MS in a previous study; eventually forty six, forty seven and forty nine components were found to be in *N. baytopii, N. cataria* and *N. fissa* accounting from 92.4%, 90.2% to 92.5% of the whole oil, respectively. In *N. baytopii* (an endemic plant in Flora of Turkey) 1,8-cineole (23.2%) and nepetalactone (12.8%); in *N. fissa* 1,8-cineole (24.3%) and nepetalactone (17.6%); in *N. cataria* nepetalactone (27.5%) - 1,8-cineole (10.8%) and germacrene D (9.2%) were reported as major constituents (10). In our study, 1,8-cineole (28.79%) and nepetalactone (9.93%) were determined the main constituents of *NNL* too. On the other hand, germacrene D (3.93%) was identified low amounts in *NNL*.It is noteworthy that sabinene (7.88%) and α-pinene (5.71%) were found high amount only in *NNL* oil (Table 1). Moreover, *Nepeta* spp. are well known in the literature for their produce of nepetalactone isomers, and this sufficient secondary metabolism has led to the commercial production of nepetalactones, nepetalactones have repellant activity against different types of insects; which finds different commercial industry and expected uses, from *N. cataria* , grown as a non-food crop (19). In our study, however, *N. nuda* subsp. *lydiae.*was shown to contain much nepetalactones (9.93%), so *NNL* usable in commercial and anticipated uses. The comparison between *NNL* and previous studies evidenced a similarity, at least with reference to the presence of the 1,8-cineole and nepetalactone among the principal constituents. The percentages of some constituents are comparable (Table 1). The chemical composition results of this study have given some clues potential usefulness and renewable resources of *Nepeta* genus.

Total phenolic and flavonoid content

Medicinal and aromatic plants are an irrevocable source of phenolic compounds. Figure 1 presents total phenolic content (expressed as µg GAE/mg dried extract or sub-fraction) varied from 12,21±0,192 to

Table 1. The identified compounds of *N. nuda* subsp. *lydiae*

No	Compounds	RRI[a]	Composition (%)
1	α-thujene	925	0.36
2	1-propene	933	0.75
3	α-terpinene	1015	0.23
4	α-pinene	1022	5.71
5	Camphene	1052	0.25
6	β-pinene	1064	1.74
7	δ-3-carene	1073	0.25
8	β-myrcene	1092	0.12
9	3-buten-2-ol	1128	0.70
10	β-ocimene	1143	0.09
11	α-terpinolene	1150	0.48
12	*p*-cymene	1179	1.21
13	α-cubebene	1258	0.82
14	Bicycloelemene	1297	0.46
15	α-cedrene	1321	0.69
16	β-bourbonene	1334	0.13
17	Cyclopropane	1345	0.20
18	Sabinene	1351	7.88
19	Sabinenehydrate	1355	0.80
20	γ-terpinene	1375	0.86
21	Linalool	1385	3.46
22	β-cedrene	1390	0.06
23	β-phellandrene	1405	0.70
24	Nepetalactone	1413	9.93
25	β-caryophyllene	1435	1.87
26	Copaene	1455	0.21
27	Epi-bicyclosesquiphellandrene	1469	0.76
28	Bornylacetate	1480	0.34
29	α-muurolene	1488	0.91
30	1,8-cineole	1495	28.79
31	Camphor	1506	1.05
32	δ-cadinene	1512	1.03
33	β-selinene	1519	0.38
34	α-amorphene	1523	0.47
35	Ethanol	1527	0.97
36	Germacrene D	1535	3.93
37	γ-cadinene	1547	4.18
38	3-cyclohexene-1-methanol	1560	0.07
39	Bicyclogermacrene	1569	0.74
40	Borneol	1577	0.43
41	Myrtenal	1588	1.49
42	Nerolidol	1595	0.05
43	Bicyclo (3.1.1) hept-2-ene	1629	0.36
44	Myrtenol	1638	0.43
45	Benzoicacid	1657	0.53
46	2-cyclohexan-1-ol	1682	0.62
47	Cyclohexene	1795	0.09
48	Isoledene	1805	0.12
49	α-farnesene	1823	0.42
50	Linalool oxide	1912	1.02
51	Caryophylleneoxide	1934	1.06
52	Hexadecanoicacid	1946	0.21
53	Cedrol	1955	0.38
54	Spathulenol	1965	1.92
55	Methanoazulene	1977	0.09
56	α-cadinol	2028	1.96
57	Cis-calamenene	2138	0.84
58	Diethylphthalate	2281	0.08
59	Methanone	2331	0.23
		Total	95.91

[*] *RRI: Relative Retention Index.*

145,06±1,397 µg GAE/dried extract or fraction. As can be seen from Figure 1. The fractionation solvents significantly affected the quantity of total phenolic components with the following order: EAF> BAF > CWE > CFF > RF > HF. Phenolic compounds in extracts are the most powerful antioxidant compounds and might be related directly with radical sweeping motion. We have tested CWE extract and its sub-fractions to reveal the antioxidant activities and tried to establish a relationship between phenolic content with the tests performed. Due to many physiological functions of natural foods is contributing to the antioxidant activity and may be responsible for phenolic components this contribution (20). Some studies reveals that a favorable correlation in between total phenolic content and antioxidant activity of natural foods such as medicinal plants (21). However some researchers showed that substances such as proteins and polysaccharides in plants may have a significant contribution to the antioxidant activity (22). Moreover several research papers illustrate that consumption of natural foods like dietary plants rich in phenolic compounds have a critical role in the prevention of dangerous diseases such as cardiovascular diseases and certain cancers.These positive effects on health are attached to the phenolic compound to contain of medicinal and aromatic plants (23). The total flavonoid content in CWE and sub-fractions determined as quercetin equivalents

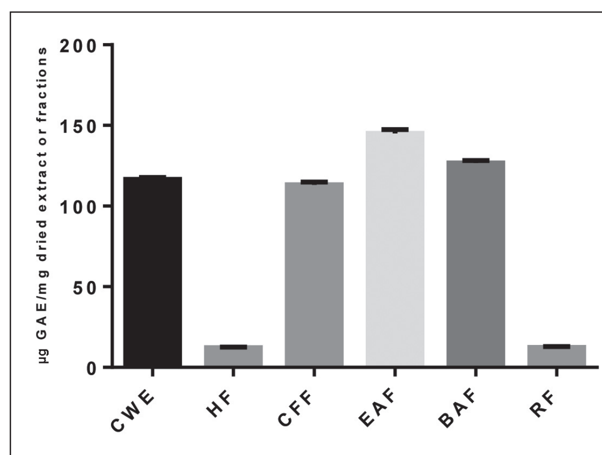

Figure 1. Quantity of total phenolic compounds in NNL extracts and sub-fractions (expressed as µg GAE/mg dried extract or sub-fraction) Data were the means of three replicates ± standard error (SE). Abbreviations: CWE, water extract, HF, hexane fraction, CFF, chloroform fraction, EAF, ethyl acetate fraction, BAF, butyl alcohol fraction and RF.

(QEE) is presented in Figure 2. The greatest quantity of flavonoids content was found in EAF (50.16±1.446 µg QEE/mg dried extract or fraction), followed by BAF (33.78±1.430 QEE/mg dried extract or fraction), CWE (28.98±0.606 µg QEE/mg mg dried extract or fraction) and RF (4.00±0.202 µg QEE/mg dried extract or fraction). The quantity of total flavonoids was not found in HF. The solvent used in the fractionation and extraction of the polarity of the differences may affect the solubility of the chemical component in the extract of plant and their yields. So, as flavonoid compounds in the plant secondary metabolites and the different antioxidant compounds fractionated or extracted by suitable solvent or solvent system, these process is the most essential steps in gaining those components (24). We found in our experiments, comparatively close relationship between the antioxidant activity and the total flavonoid content of the extract and its fractions and our results are supported by some studies (25). Also compounds of flavonoid have been shown as antioxidant agents that stop lipid peroxidation and interfering different types of reactive oxygen species (26). These exclusive compounds, found in plants divided into too many classes, represent a significant part of bioactive seconder metabolites and have earned the reputation of being the cure for many diseases as cases of severe cancer (27). Furthermore, some researchers

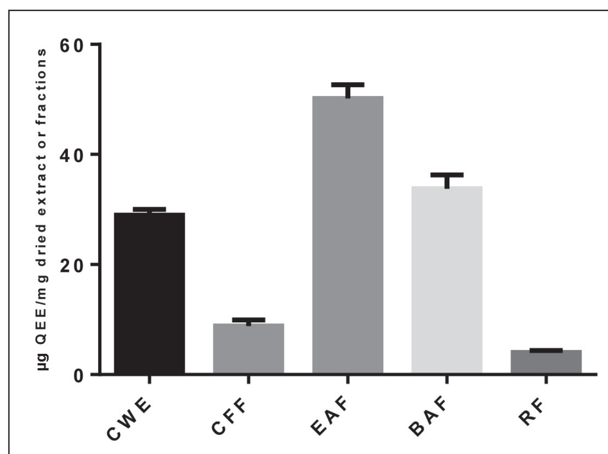

Figure 2. Quantity of total flavonoids in NNL extracts and sub-fractions (expressed as µg quercetin/mg dried extract or sub-fractions)Data were the means of three replicates ± standard error (SE). Abbreviations: CWE, water extract, CFF, chloroform fraction, EAF, ethyl acetate fraction, BAF, butyl alcohol fraction and RF.

have been reported to show a high antioxidant activity of flavonoids or flavonoid-rich fractions and extensive pharmacological use of flavonoids may be due to their antioxidant properties (28, 29).

Antioxidant activity

Antioxidant activity of CWE extract and its sub-fractions was measured using DPPH free-radical. The free-radical, DPPH is only one of organic radicals and the experiment is quick and simple so that likely account for its excessive use in antioxidant activity tests (30). In this experiment purple color of solution of DPPH radical turns to the color of pale yellow after upon addition antioxidant agents/reducing compound in the extract or fraction (31). The results of free radical scavenging activity of CWE extract, its sub-fractions and positive controls are presented in Figure 3. Both the CWE extract and its sub-fractions, the remarkably scavenged the DPPH radical with increasing concentrations. EAF sub-fraction (IC_{50}=10.45±0.393 µg/ml) exhibited the strongest DPPH radical scavenging activity among all the tested sub-fraction and extract, followed by BAF (IC_{50}=39.19±1.134 µg/ml), CFF (IC_{50}=48.95±0.687 µg/ml), HF (IC_{50}=79.28±0.398 µg/ml) and RF (IC_{50}=162.70±1.729 µg/ml). The ferrous ion (Fe^{2+}) reacts with the ferrozine to magenta-colored complexes (Fe^{2+}–ferrozine) which giving rise to occurrence and spread of numerous radical reactions.This complex does not occur in the presence of CWE extracts or its sub-fractions and standards such as EDTA. Many plant extracts and natural products obtained from plant extracts prevents the formation of complexes of ferrous and ferrozine, it is preferred that they chelating and can capture ferrous ion forming a more stable complex than ferrozine (32). CWE and its sub-fractions were assayed for their Fe^{2+} chelating activity at different concentrations, and this activity was compared with the chelating activity of the synthetic metal chelator EDTA. The plot of iron-chelating capacity is shown in Fig. 4. While in HF, the iron-chelating activity was not determined, CWE extract and its four sub-fractions (CFF, EAF, BAF and RF) exhibited moderate iron-chelating capacities with IC_{50} values of 90.30±1.453, 9.94 ± 0.516, 16.76 ± 0.439, 20.63 ± 0.586 and 114.20 ± 0.881 µg/ml, respectively, which was less than the positive control (EDTA;

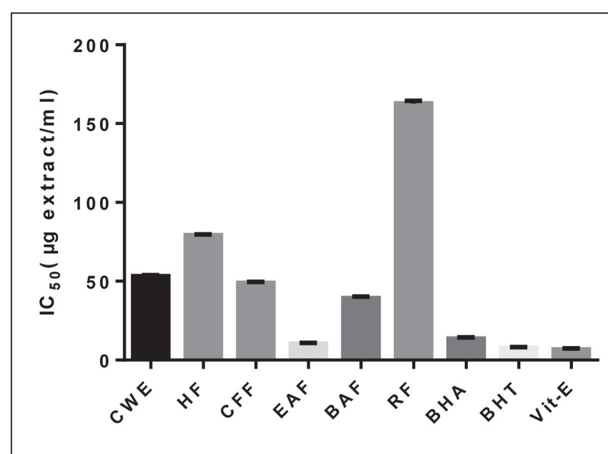

Figure 3. Free radical scavenging activities of NNL extracts and sub-fractions by DPPH radical. Data were the means of three replicates ± standard error (SE). Abbreviations: CWE, water extract, HF, hexane fraction, CFF, chloroform fraction, EAF, ethyl acetate fraction, BAF, butyl alcohol fraction, RF, rest fraction, Vit-E, Vitamin E, BHA, butylated hydroxyanisole, and BHT.

8.540±0.1172 µg/ml), which is excellent chelator. The CFF from CWE exhibited the strongest iron-chelating capacity (9.94 ± 0.516 µg/ml) among all the fractions.

Our results showed that the CWE extracts and sub-fractions contained phenolic compounds and flavonoids except for quantity of total flavonoid of HF.

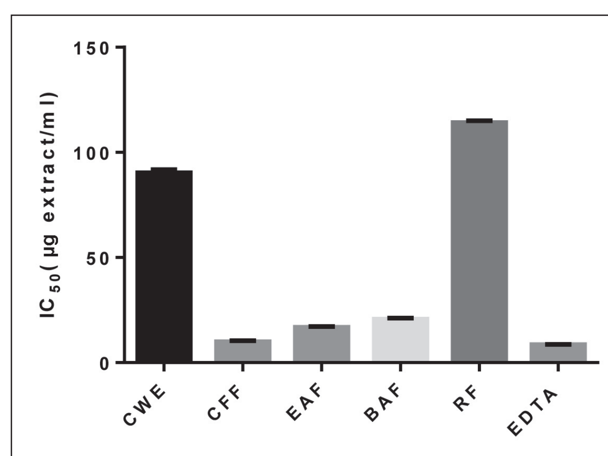

Figure 4. Metal chelating effect of NNL extracts and sub-fractions Data were the means of three replicates ± standard error (SE). Abbreviations: CWE, water extract, HF, hexane fraction, CFF, chloroform fraction, EAF, ethyl acetate fraction, BAF, butyl alcohol fraction, RF, rest fraction and EDTA.

CWE extract and all sub-fractions in this research exhibited different extent of antioxidant activity. EAF sub-fraction, with the highest phenolic and flavonoid contents, had the most active radical scavenger activity and CFF sub-fraction the highest chelating activity of all samples. EAF fraction had the highest amount of total flavonoids and possessed good radical scavenger activity and chelating power. The Chelating power activity of CFF was higher than that of all extract and sub-fractions. The correlation between activity of DPPH radical removing and phenolic content was higher than that between activity of DPPH radical removing and total flavonoid contents. At the same time, this study indicates that total phenolic and flavonoid contents may play important roles in antioxidant activity. This may be related to the high amount of flavonoid and phenolic compounds in this sub-fraction. EAF sub-fraction showed a higher potency than BHA in scavenging of DPPH free radical.

Conclusion

Antioxidants can performed to task through three main mechanisms; firstly by transferring hydrogen atom, the second single electron transfer, and finally the metal ion chelation (33). Therefore, we tested and compared the antioxidant activities of the extract and its sub-fractions both by metal chelating and hydrogen atom transfer. We have tried to reveal the strongest antioxidant activity of the endemic *NNL* plant by means of our performed tests. This plant can be imparted to the industry and next isolation and characterization exercises can result of active secondary metabolites and different biological activities, because of secondary metabolites are used due to medical and pharmacological properties. As in our previous study (34), we constantly strive to find and gained to industry and pharmacology of the new natural bioactive compounds from different drug source such as plant, fruits, vegetables and seaweed. Our study suggest that the endemic *NNL* plants can be utilized as an effective and safe antioxidant source and all the findings of the present study warrant further research wherein CWE and sub-fractions need to be isolated to natural product and characterized to chemical structure. Indeed, there

is a current need for availability of new plant derived bioactive molecules; thus *NNL* may be a great natural source for the development of new drugs.

Acknowledgement

The authors thank for financial support the projects of State Planning Organization (DPT); (Projects No: 2010K120630 and 2010K120720), the Scientific and Technological Research Council of Turkey (TUBITAK; 111T560) and Scientific Research Project Department of Bingol University (BÜBAP; 2010-07).

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