Antioxidant properties of hydro-alcoholic extract and extract of nepeta crispa in Lorestan province

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Original Article

Abstract

Introduction: Antioxidants protect body against the oxidative stress caused by free radicals. This study aims at comparing the antioxidant properties of hydro-alcoholic extract and extract of Nepeta crispa (from its shoot part).

Methods: The study was carried out in Lorestan University of Medical Sciences in 2012. Both hydro-alcoholic extract and essence of Nepeta crispa were prepared and antioxidant properties of samples were assessed using different methods. The ability to inhibit free radicals production was measured through 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method. Total antioxidant capacity was measured using Phosphomolybdate Method. Likewise, phenol and flavonoid compounds of samples were measured using folin-ciocalteu Zishen method.

Results: The results showed that the total antioxidant capacity of hydro-alcoholic extract and essence of Nepeta crispa were equal to 0.23 ± 0.12 and 0.82 ± 0.40 Nmol ascorbic acid per gram extract or essence. The phenol content of hydro-alcoholic extract and essence of Nepeta crispa were 254.63 ± 15.75 and 6.94 ± 1.37 mg gallic acid per gram extract or essence, respectively. Flavonoid content of hydro-alcoholic extract and essence of Nepeta crispa were equal to 5.53 ± 0.34 and 0.36 ± 0.06 mg quercetin per gram extract or essence, respectively. IC_{50} value (concentration that will inhibit 50% of free radicals production), hydro-alcoholic extract, essence of plant and Butylated hydroxytoluene (BHT), as positive control, were equal to 2.09 ± 0.11, 6.05, and 3.88 ± 1 µg/ml, respectively.

Conclusion: The results showed that the hydro-alcoholic extract has better antioxidant properties in contrast to essence of Nepeta crispa. As a result, hydro-alcoholic extract of Nepeta crispa is a good and available source of antioxidant compounds which can be used, if useful, in food, pharmaceutical and industrial products.

Key words: Essential Oil – Antioxidants – Nepeta Crispa

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Introduction:

Antioxidants are molecules or compounds that act as inhibitors of free radicals. Free radicals damage or counteract molecules. The initial protection against such oxidative inhibitions is carried out by antioxidants (1).

Free radicals, higher than 1 percent, will result in atherosclerosis, arthritis, anemia and tissue damages including central nervous system trauma, gastritis and even cancer in human beings (2-5). Lipid oxidation disturbs functions and structure of biological membranes and brings about different cell and tissue damages and also plays a very effective role in pathogenesis of many diseases such as atherosclerosis (6,7). In general, lipids peroxidation is considered as the symptom of cytotoxicity caused by the free radicals (8).

Today, synthetic antioxidants are used widely in food industries; however, most of them include detrimental components for human, as some of them are carcinogenic (9,10).

In living organisms, peroxidation of lipids across the live cell walls is amongst the most targets of free radicals. In such condition, not only cell wall structure and function are influenced, but also some byproducts of oxidation process, e.g. Malondialdehyde, can react with biomolecules and show cytotoxic and genotoxic properties. Hence, high concentration of free radicals, especially peroxides, plays a key role in pathogenicity of some diseases such as diabetes, cardiovascular diseases, cancers, senescence, etc. (11).

To eliminate negative effects of free radicals and oxidative stress reactions, presence of antioxidant compounds that can protect body against the oxidative stress seems necessary. Antioxidants play a special role in prevention and treatment of diseases and studies suggest that some plants with special properties such as controlling the lipid oxidation content are able to show antioxidant properties (12,13).

Regarding what mentioned, natural antioxidants, which are mostly found in pharmaceutical plants, fruits and vegetables, have become very popular among people and it seems that they play a very important role in preventing many diseases. Pharmaceutical plants are important and rich sources of natural antioxidants (14).

Crispa is a species of Nepeta genus. This genus is found in west and southwestern Iran, in Lorestan Province. Because of its pharmaceutical and aromatic properties, this species is known locally as *Mifrah* in northern Lorestan. Napeta *cri* is an aromatic plant which is found plentifully in Iran. Mifrah is used broadly in the Iranian traditional medicine, particularly in Hamedan Province (15).

Nepeta genus has 250 different species, out of which 67 species are available in Iran and out of which 37 species are exclusively found in Iran (15).

Because of their medical properties, most species of this genus have been used in the traditional medicine (16). Various species of Nepeta have medical usages such as anti-spasm, anti-cough, diuretic, disinfectant and anti-asthma. Likewise, some species of Nepeta are prescribed as anti-inflammatory, antifungal, hypoallergenic, pain relief, febrifuge, carminative, antibacterial and a medicine for treating cold, stomach ache (17).

Regarding its useful properties and wide range of usages, and also since its antioxidant properties have not studied so far, we decided to study antioxidant properties of hydro-alcoholic extract and essence of Nepeta through the ability of inhibiting free radicals production, total antioxidant capacity, phenolic compounds amount, and flavonoid content.

Methods:

Ssodium ethylene diaminetetraacetic acid (Na_2EDTA), sodium chloride, Butylated hydroxytoluen, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, quercetin, disodium hydrogen phosphate (Na_2HPO_4) were purchased from Sigma Company. This experimental study was conducted in Pharmaceutical Drugs Research Center in Khoramabad City.

Preparing hydro-alcoholic extract and essence of Nepeta:

Nepeta was collected from Northern Lorestan in June, 2012. The hydro-alcoholic extract of Nepeta was prepared in the Pharmaceutical Drugs Research Center of Lorestan University of Medical Sciences and the essence was prepared in Lorestan University. Initially the plant was dried in the shadow and then was powdered for preparing both hydro-alcoholic extract and essence of Nepeta. Hydro-alcoholic extract was prepared using Soxhlet extractor and essence of Nepeta was extracted using Clevenger apparatus with distillation method. The hydro-alcoholic extract and essence of Nepeta

contents were equal to 10.04% and 0.200%, respectively.

Total antioxidant capacity:

For analyzing antioxidant capacity of hydro-alcoholic extract and essence of Nepeta, initially different concentration of hydro-alcoholic extract and essence of Nepeta were prepared and then three test tubes were used for each concentration and each tube was filled 1 ml phosphotungstic acid and 0.1 ml hydro-alcoholic extract and essence of Nepeta with different concentrations, then the tubes were kept in boiling temperature for 90 minutes. After tubes become cold, the absorption of samples was measured at a wavelength of 695 nm against reagent blank using a Cecil spectrophotometer, Model 9000 UK. Using the same method, the standard curve for the total antioxidant capacity of ascorbic acid, absorption at a wavelength of 695 nm, in terms of ascorbic acid concentration in the concentration range of 0.01 to 15 nm ascorbic acid was depicted. Total antioxidant capacity of hydro-alcoholic extract and essence of Nepeta was measured in terms of Nmol ascorbic acid per gram hydro-alcoholic extract and essence of Nepeta (18).

Phenol Content:

For measuring the phenol content of hydro-alcoholic extract and essence of Nepeta, initially various concentrations of hydro-alcoholic extract and essence of Nepeta were prepared. Then, three test tubes were prepared for each concentration and they were filled with 2 ml distilled water and 100 µl folin-ciocalteu phosphotungstic acid 7% and 20 µl hydro-alcoholic extract and essence of Nepeta in various concentrations. After three minutes 300 µl sodium carbonate was added and the solutions were shaken for 2 hours. Finally, absorption of solutions was measured at a wavelength of 765 nm by the spectrophotometer. Using the same method, the standard curve for gallic acid at the concentration range of 10 to 3000 mg/ml gallic acid was depicted. The total content of phenolic compounds of hydro-alcoholic extract and essence of Nepeta was measured in terms of mg gallic acid per gram hydro-alcoholic extract and essence (19).

Flavonoid content:

For measuring the flavonoid content of hydro-alcoholic extract and essence of Nepeta, initially various concentrations of hydro-alcoholic extract and essence of Nepeta were prepared. Then, three test tubes were prepared for each concentration and 500 µl Aluminum chloride - ethanol solution 2% and 500 µl hydro-alcoholic extract and essence of Nepeta with various concentrations were added to each tube. Solutions were kept in a room temperature for 1 hour. Finally, absorption of solutions was measured at a wavelength of 420 nm by the spectrophotometer. Using the same method, the standard curve for quercetin at the concentration range of 5 to 60 mg/ml quercetin was depicted. The total content of flavonoid compounds of hydro-alcoholic extract and essence of Nepeta was measured in terms of mg quercetin per gram hydro-alcoholic extract and essence (20).

Analyzing 2, 2-diphenyl-1-picrylhydrazyl (DPPH):

For analyzing the antioxidant activity of hydro-alcoholic extract and essence of Nepeta, inhibition of free radicals production was measured using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) in terms of percentage (21). Initially different concentrations of hydro-alcoholic extract and essence of Nepeta were prepared and then three test tubes were prepared for each concentration. 200 µl of samples with various concentrations were mixed with ml of 90 µmol DPPH and its volume was increased to 4 liter with 95% methanol and it was shaken in darkness for 60 minutes. Absorptions of samples containing the extract, essence and control were measured at a wavelength of 517 nm using a spectrophotometer instrument. Inhibition of free radical production percentage was measured with the following equation:

Inhibition of free radical production percentage $= 100 \times (\text{A blank} - \text{A sample}/\text{A blank})$

A blank and A sample represent absorption of control and absorption of sample, respectively. Antioxidant activity of hydro-alcoholic extract and essence of Nepeta are represented by IC50, which is a concentration of the compound that inhibits radical capacity as large as 50%.

DPPH is a stable free radical which becomes stable by accepting an electron and hydrogen free

radical. Its maximum absorption is seen at 517 nm. The capacity of inhibiting free radicals of DPPH is seen at 517 nm. The capacity of inhibiting free radicals of DPPH is measured by reducing absorption at 517 by antioxidants. Antioxidants react with free radicals and inhibit them. As a result, absorption of DPPH is reduced by antioxidants and its purple color changes into yellow. Accordingly, DPPH test is used to assess anti-oxidative activity of antioxidants and pharmaceutical plants (22,23).

Statistical Analysis:

The results were expressed in the form of mean+SD. Significance results in terms of statistics and difference between groups were assessed using SPSS software and independent t-test.

Results:

Total antioxidant capacity:

Figure 1 shows the standard curve of ascorbic acid used for measuring antioxidant capacity of hydro-alcoholic extract and essence of Nepeta. Total activity of hydro-alcoholic extract and essence of Nepeta are 0.23±0.12 and 0.82±0.4, respectively, which are equal to Nmol ascorbic acid per gram hydro-alcoholic extract and essence.

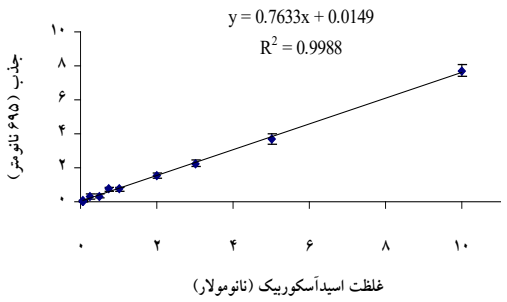

Figure 1. Standard curve of total antioxidant capacity of ascorbic acid

Total content of phenol:

Figure 2 shows the standard curve of gallic acid used for measuring phenol content of hydro-alcoholic extract and essence of Nepeta. The measured phenol content by folin-ciocalteu method in hydro-alcoholic extract and essence of Nepeta are 254.63±15.75 and 6.94±1.37, respectively, which are equal to mg gallic acid per gram hydro-alcoholic extract and essence; which they showed a significant difference (Table 2).

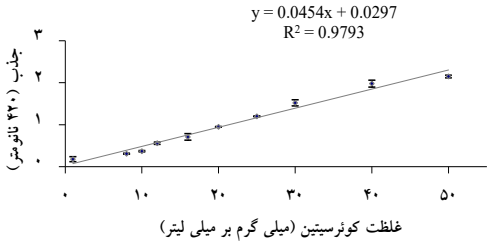

Figure 2. Standard curve of flavonoid quercetin

Total Flavonoid content:

Figure 3 shows the standard curve of quercetin used for measuring flavonoid content of hydro-alcoholic extract and essence of Nepeta. The measured flavonoid content by folin-ciocalteu method in hydro-alcoholic extract and essence of Nepeta are 5.53±0.34 and 0.36±0.069, respectively, which are equal to mg quercetin per gram hydro-alcoholic extract and essence (Table 2).

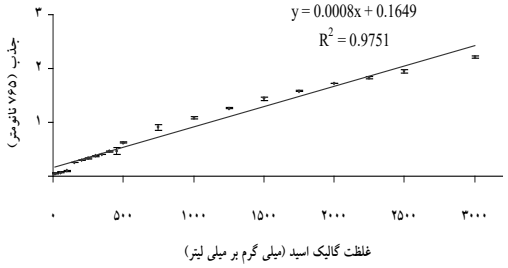

Figure 3: Standard curve of gallic acid

Analyzing 2, 2-diphenyl-1-picrylhydrazyl (DPPH):

The ability of inhibiting free radicals of hydro-alcoholic extract and essence of Nepeta was achieved in terms of percent of inhibiting free radicals percentage. As IC50 value of hydro-alcoholic extract and essence of Nepeta and BHT, as the positive control, are equal to 2.09±0.11; 6.05±0.99; and 3.88 µg/ml (Table 1).

Table 1. IC50 value of hydro-alcoholic extract and essence of Nepeta and BHT

IC 50 content	Mean	SD
Hydro-alcoholic extract of shoot of Nepeta	2.09	0.11
Essence of shoot of Nepeta	6.05x	0.99
BHT	3.88	1.00

*: Significant rather extract ($P<0.05$) # significant rather essence ($P<0.05$)

Table 2. Total antioxidant capacity, total phenol and flavonoid of hydro-alcoholic extract and essence of Nepeta

Total antioxidant capacity (Nmol ascorbic acid per gram extract)	0.23±0.12	0.82±0.4X
Total phenol content (mg gallic acid per gram extract)	254.63±15.75	6.94±1.37x
Total flavonoid (mg quercetin per gram extract)	5.53 ± 0.34	0.36 ± 0.06x

Conclusion:

Total antioxidant activity of hydro-alcoholic extract and essence of Nepeta are 0.82±0.4 and 0.23±0.12, equal to Nmol ascorbic acid per gram hydro-alcoholic extract and essence whose difference was significant. The measured phenol content of Nepeta by folin-ciocalteu method in hydro-alcoholic extract and essence was 254.63±15.75 and 6.94±1.37 mg hydro-alcoholic extract and essence whose difference was significant. The flavonoid contents measured by folin-ciocalteu method in hydro-alcoholic extract and essence of Nepeta were 5.53±0.34 and 0.36±0.06 mg quercetin per gram hydro-alcoholic extract and essence, whose difference was significant. The inhibition of free radicals production percent in hydro-alcoholic extract and essence of Nepeta was measure in terms of percentage of inhibition of free radicals production. As IC50 value of hydro-alcoholic extract and essence of Nepeta and BHT, as the positive control, were 20.09±0.11; 6.05±0.99 and 3.88±1 µg/ml. The value of IC50, positive control of BHT is more than that of essence of Nepeta and their difference is significant. Similarly, the value of IC50 of essence is more than that of hydro-alcoholic extract of Nepeta and their difference is significant.

Regarding studies carried out on N. cataria using DPPH, IG50 value for ethanol extract and essence was measured 16.4% and 0.275% which is incompatible with our results. In this study, researchers carried out on ethanol extract and root extract of N. cataria, showed that the antioxidant capacity of phenol is 22.6±2.07 µg/mg which represents a very weak antioxidant capacity and is inconsistent with our results (23). According to studies made on N. persica using DPPH, IC50 values of extract and essence are 0.03 and 2.98, respectively which are consistent with the results of our study (24). Studies about N. Cilicia, N. italica and N. Caesarea showed that their phenol contents, measured by folin-ciocalteu method, in methanol extract are 21.4, 24.8 and 17.3 µg/ml. And in this study, inhibition of free radicals production percent in methanol extract were 33.4±1.25, 25.5±0.55 and 39.1±0.76 (25). A study on N. meyeri showed that inhibition of free radicals percent in acetone extract using DPPH was 672.2 µg/ml and its total phenol amount was 12.86 µg/ml which indicates the high antioxidant capacity of this plant (26). In a study, the ability of inhibition of free radicals in the methanol extract of N. sibthorpii using DPPH was reported 97.18 g/ml which was incompatible with this study (27).

Comparing this study and previous studies indicated that many species of Labiatae family are recognized as the plants containing antioxidants compounds (28). The difference lies in the antioxidant ability of Nepeta in this study and other studies because of genetic and environmental factors impact. On the one hand, comparing antioxidant properties of hydro-alcoholic extract and essence of Nepeta in this study indicated that the antioxidant factors of hydro-alcoholic extract of Nepeta were more than that in its essence; it suggested that antioxidant compounds in the hydro-alcoholic extract are more than that in the shoot essence.

The results indicate that Nepeta, particularly its hydro-alcoholic extract, acts as a primary antioxidant through exchanging hydrogen and reacting with high-capacity free radicals in

removing free radicals. The results of previous studies on Nepeta and this study indicated that Nepeta has antioxidant properties. Further studies, especially analyzing constituents and extracting its effective materials and using them to treat the relevant diseases of oxidative stress such as diabetes, cardiovascular diseases and cancers in lab animals is very important. Further studies will result in ideal results to be used in food, pharmaceutical and industrial products.

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