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***J. Nat. Prod. Plant Resour.*, 2013, 3 (3):29-36**
(http://scholarsresearchlibrary.com/archive.html)

ISSN : 2231 – 3184
CODEN (USA): JNPPB7

Phytochemical screening and antioxidant activity of *Centranthus longiflorus* L.

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ABSTRACT

In our work, the primary phytochemical screening of the plant Centranthus longiflorus grown in Lebanon has been accomplished. Also, aqueous and methanolic extracts from this plant have been studied for their scavenger activity using three different in vitro tests, the DPPH, H_2O_2 and the iron chelating. The obtained results of the phytochemical screening showed the presence of flavonoids, phenols, essential oils, alkaloids and terpenoids. On the other hand, the DPPH test of the C. longiflorus showed a high antioxidant potential reaching 80 %. Moreover, the H_2O_2 test showed that this maximal antioxidant activity was 70 %. The iron-chelate test showed an antioxidant activity reaching 50 %. In conclusion, the obtained results of all of these tests demonstrated that this plant might be used in the prevention and in the treatment of different diseases related to oxidative stress.

Keywords: *Centranthus longiflorus*, phytochemical screening, antioxidant activity.

INTRODUCTION

Medicinal plants are the richest bioresource of drugs for traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. Several studies have demonstrated pharmacological properties of medicinal plants and their isolated constituents such as antioxidant, antidiabetic, antibacterial, antiviral and antiulcer [1-9].

Oxidative stress is involved in many acute and chronic diseases including cancer, cardiovascular disorders and neurodegenerative diseases. The balance between antioxidation and oxidation is believed to be critical in maintaining a healthy biological system [10-12]. Medicinal plants contain a wide variety of natural antioxidants, such as phenolic acids, flavonoids and tannins, which possess more potent antioxidant activity [4-9, 13].

This study aimed first to investigate the chemical composition of the aqueous and methanolic extracts from *C. longiflorus* L.. On the other hand, *in vitro* anti-free radical property of these extracts using different tests such as DPPH, H_2O_2 and iron chelating tests has been evaluated.

MATERIALS AND METHODS

Plant materials

Fresh plant was gathered from Mount Lebanon on spring season between March and Jun in 2012 and the biological authentication was carried out by Professor George Tohme, president of C.N.R.S of Lebanon. The whole plant was

left on air at room temperature for two weeks to be very well dried. After that, it was crushed up and ground to get homogeneous fine powder by a grinder and then kept in a dark place at room temperature till its use in the different studies.

Chemicals

Absolute methanol, n-hexane and sodium hydroxide n-butanol, petroleum ether, ammonium hydroxide, acetic acid and sodium chloride were purchased from BDH England. Aluminium chloride and $FeSO_4.7H_20$ were purchased from Merck, Germany. Sodium carbonate and hydrogen peroxide were purchased from Unichem, India. Ascorbic acid, gallic acid and rutin, Folin-Ciocalteau reagent, ferrozine and DPPH were purchased from sigma Aldrich, USA. Phosphate buffer solution (PBS) was purchased from Gibco, UK.

Sample Preparation

10 grams of powdered plant were putted into a flask with 500 mL of methanol, and the mixture was then extracted by agitation for five hours at 25 °C. Then, a maceration of the extracts was done overnight for 24 h at room temperature. After, the methanolic layer containing the extract was taken. The extraction was repeated on the remaining amount of the precipitate using 150 mL of methanol and all extracts were filtered by using a 0.45 millipore filter paper. After that, the two fractions of MeOH extract were mixed together and then concentrated using a rotary evaporator at 40 °C. Then, the extracts were stored at -20 °C till their usage in the different tests.

Aqueous extract has been prepared using the same steps of the methanolic extraction except for the concentration using a rotary evaporator was at 60 °C.

Phytochemical screening

The preliminary phytochemical screening of various active compounds of *C. longiflorus* was accomplished according to the method of Muanda et al. [14].

Evaluation of the antioxidant activity

DPPH radical scavenging activity [9]

1 mL of different concentrations (100, 200, 300, 400 and 500 µg/ml) of diluted extracts of the plant in methanol was added to 1 mL of DPPH (0.15 mM in methanol) and at the same time, a control consisting of 1 mL DPPH with 1 mL methanol was prepared. The reaction mixtures were mixed very well by hand and then incubated in the dark at room temperature for 30 min and the absorbance was measured at 517 nm by a Gene Quant 1300 UV-Vis spectrophotometer. The ascorbic acid was used as a positive control and the methanol was used as blank. The DPPH scavenging ability of plant extracts was calculated using the following equation:

***% scavenging* activity = [(Abs control − Abs sample)]/ (Abs control)] ×100**

The Abs control is the absorbance of DPPH + methanol; Abs sample is the absorbance of DPPH radical + sample. Also, three controls have been prepared.

Chelating effects on ferrous ion [6]

0.5 ml of various concentrations of all extracts of the plant was mixed with 0.5 mL of $FeSO_4$ (0.12 mM), and with 0.5 mL of Ferrozine (0.6 mM). The mixture was allowed to stand for 10 min at room temperature. After incubation, the absorbance was measured by Gene Quant 1300 UV- Vis spectrophotometrically at 562 nm. Ultra-pure water of sample solution was used as a control without extracts. Ultra-pure water instead of Ferrozine solution was used as a blank. EDTA-Na_2 was used as reference standard. All measurements were performed in triplicate. The ability of the sample to chelate ferrous ion was calculated relative to the control (consisting of iron and ferrozine only) using the formula:

$$\textit{Ferrous ion - chelating ability (\%)} = [(\textit{Abs control} - \textit{Abs sample}) / \textit{Abs control}] \times 100$$

H_2O_2 Radical Scavenging Activity [8]

A solution of H_2O_2 (40 mM) was prepared in PBS (pH 7.4) and concentrations were determined spectrophotometrically at 230 nm. Different concentrations(100, 200, 300, 400 and 500 µg/mL) of the two extracts were added to a H_2O_2 solution (0.6 mL, 40 mM) and the absorbance of H_2O_2 was determined at 230 nm after 10 min against a blank solution containing the plant extracts without H_2O_2. Ascorbic acid was used as standard reference.
The percentage scavenging of H_2O_2 was calculated using the following equation:

$$\textit{\% scavenged } [H_2O_2] = [(\text{Abs control} - \text{Abs sample}) / \text{Abs control}] \times 100.$$

Total phenolic content

Determination of total phenolic extracts quantities was determined using the Folin–Ciocalteau reagent method. Three concentrations have been prepared for each extract and 100 µL of various concentrations of plant extracts dissolved in 1 mL distilled water with 0.5 ml of Folin–Ciocalteau reagent (1/10 dilution) and 1.5 mL of Na_2CO_3 2% (w/v) were added and mixed well. The blend was incubated in the dark at room temperature for 15 min. The absorbance of blue-colored solution of all samples was measured at 765 nm using a Gene Quant 1300 UV-Vis spectrophotometers. The results were expressed in mg of Gallic acid equivalent of g dry weight of plant powders.

STATISTICAL ANALYSIS

All analyses were carried out in triplicates. The results of scavenger activity, were performed from the averages of all samples reading Mean ± SD used Excel 2003.

RESULTS AND DISCUSSION

In order to study the phytochemical composition of different extracts prepared from *C. longiflorus*, a phytochemical screening was performed allowing to consider the possible medical uses that may have this plant as several studies have demonstrated the positive correlation between the phytochemical composition of plants and their medicinal uses [6-9, 15].

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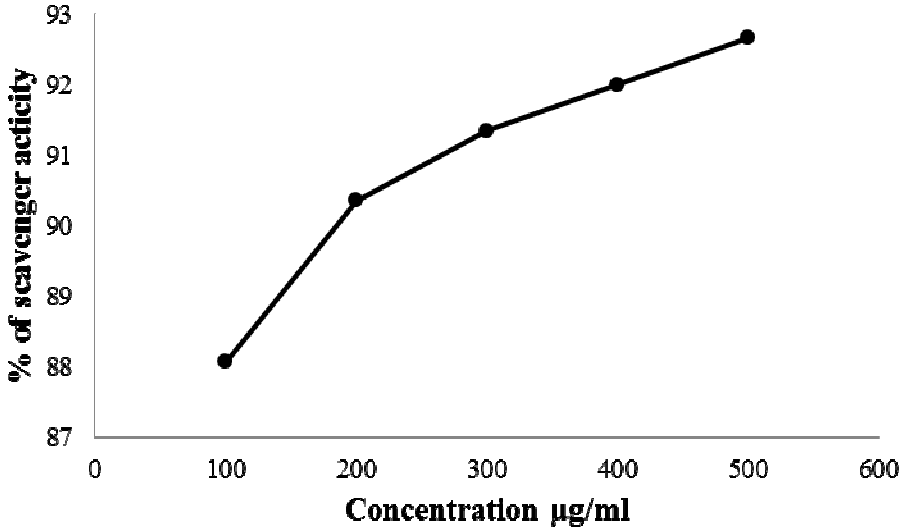
Table 1 : Phytochemical composition of aqueous and methanolic extracts of *C. longiflorus*

	Aqueous extract	Methanolic extract
Tannins	-	-
Resins	-	+
Coumarins	-	+
Saponins	+	-
Alkaloïds	+	-
Flavonoïds	+	-
Phenols	++	++
Terpenoïds	+	+
Volatile oils	++	-

The results obtained from the phytochemical screening presented in Table 1 show that *C. longiflorus* is rich in various secondary metabolites at different concentrations depending on the solvent used. Indeed, we note the presence of saponins, alkaloids, flavonoids and volatile oil in the aqueous extract while the MeOH extract is richer in resins and coumarins. Also, the phytochemical screening revealed the richness of the two extracts in phenols, terpenoids and their deficiency in tannins. Consequently, *C. longiflorus* by its richness in different secondary metabolites may have several medical importances such as analgesic due to the presence of alkaloids [16], antitumor especially aqueous extracts due to the presence of flavonoids [17] and antioxidant due to its richness in phenolic compounds [5].

Figure 1: Antioxidant activity of aqueous extract of *C. longiflorus*



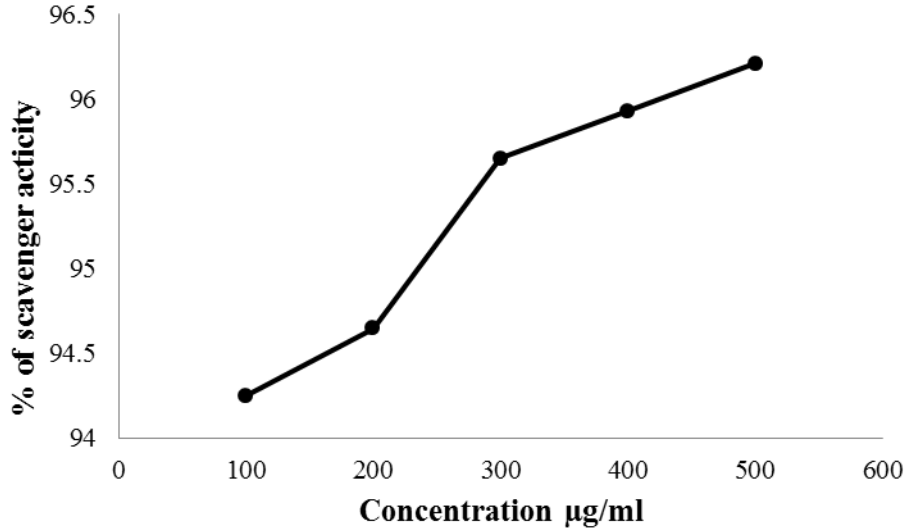
DPPH radical scavenging activity

A number of studies on flavonoids, polyphenols and tri-terpenoids have indicated that they have antioxidant potential and the ability to react as scavengers of free radicals. These phytoconstituents can exert multiple biological effects against tumors, heart disease and various diseases due to their radical activity. In our study, an assessment of the antioxidant activity of different extracts prepared from the plant *C. longiflorus* grown in Lebanon was performed *in vitro* by three different methods: DPPH, H_2O_2 and iron-ferrozine.

The Figure 1 shows an increase in the antioxidant activity of different concentrations of the aqueous extract of *C. longiflorus*. This increase was concentration-dependent and it reaches a percentage of 93 % at the concentration of 500 µg/ml.

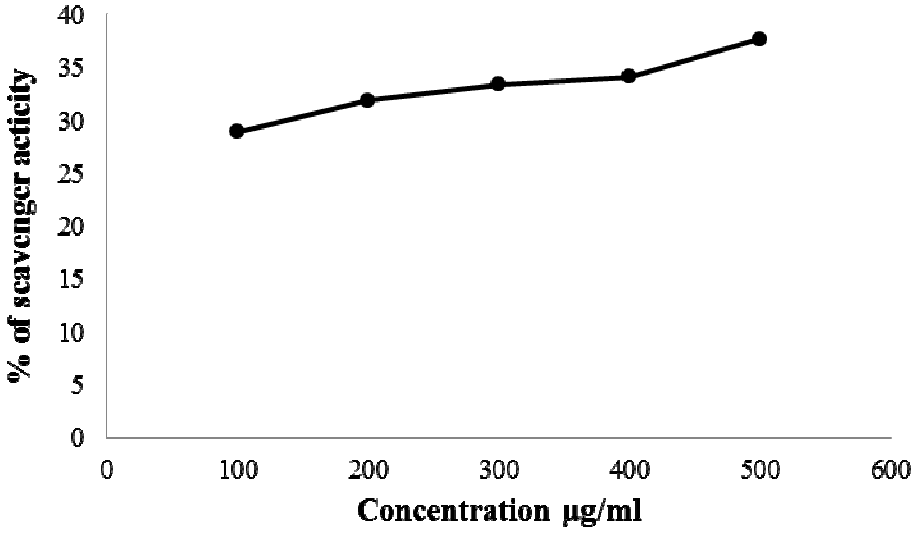
Figure 2 shows that the methanolic extract of *C. longiflorus* at different concentrations has exerted an antioxidant activity depending on the concentrations studied. This activity reached a percentage of 96.21 % at the concentration of 500 µg/ml.

Figure 2 : Antioxidant activity of methanolic extract of *C. longiflorus*



DPPH method is based on the disappearance of the purple color in the presence of antioxidant molecules that convert DPPH free radicals in a colorless gas through a reduction reaction resulting in a decrease in the absorbance at 517 nm. The DPPH test is known to give reliable results regarding the antioxidant capacity of tested solutions [18].

Figure 3 : Antioxidant activity of aqueous extract of *C. longiflorus*



Thus, the results obtained demonstrate the antioxidant activity of this Lebanese endemic plant which is consistent with the richness of the studied extracts in phenolic compounds detected by the phytochemical screening. Indeed, recent studies have shown that these compounds possess antioxidant activity and may help prevent certain diseases associated with oxidative stress such as cardiovascular diseases and diseases related to aging by neutralizing free radicals in the body [19].

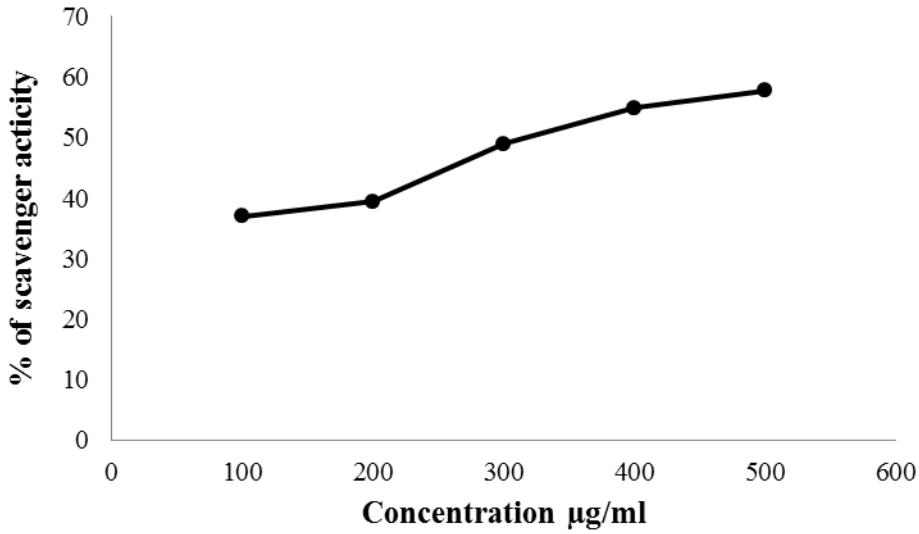
Ferrous ion chelating activity

To confirm the obtained results of antioxidant activity by DPPH method, we used the method Iron-Ferrosine.

Figure 3 shows that the different concentrations of the aqueous extract of *C. longiflorus* have exerted an antioxidant activity which reached 38 % at the concentration of 500 µg/ml. These results show that this same concentration has the ability to scavenge DPPH free radicals better than the Ferrosine.

Figure 4 shows the increase in the antioxidant activity of the methanolic extract of *C. longiflorus* with increasing concentrations. It reached 58 % at a concentration of 500 µg/ml.

Figure 4: Antioxidant activity of methanolic extract of *C. longiflorus*

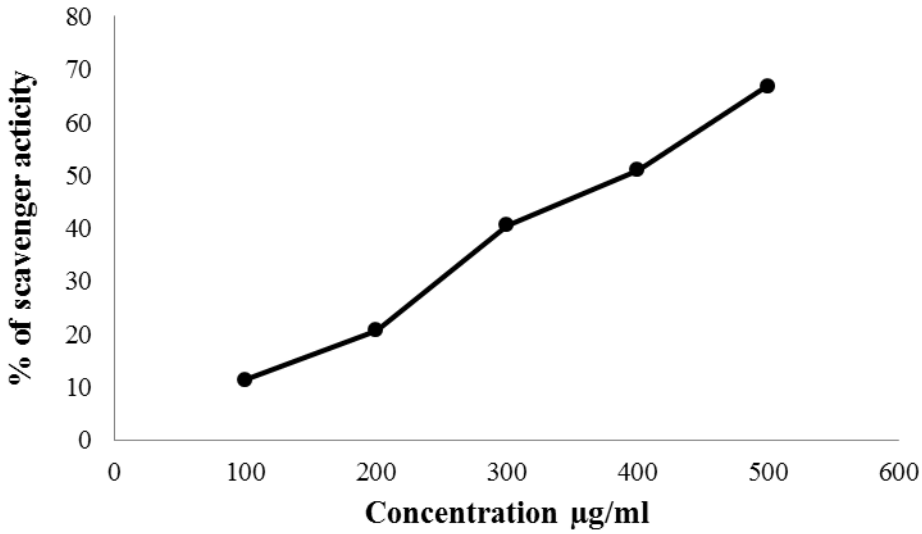


Iron-Ferrosine method is to measure the ability of the extract to chelate the metal. The present antioxidants inhibit the interaction between the metal and the lipids forming the ferrous ion with the insoluble metal complexes [20]. The results of the evaluation of the antioxidant activity by the method ferrosine confirmed the presence and importance of the antioxidant property of *C. longiflorus*.

H_2O_2 Radical Scavenging Activity

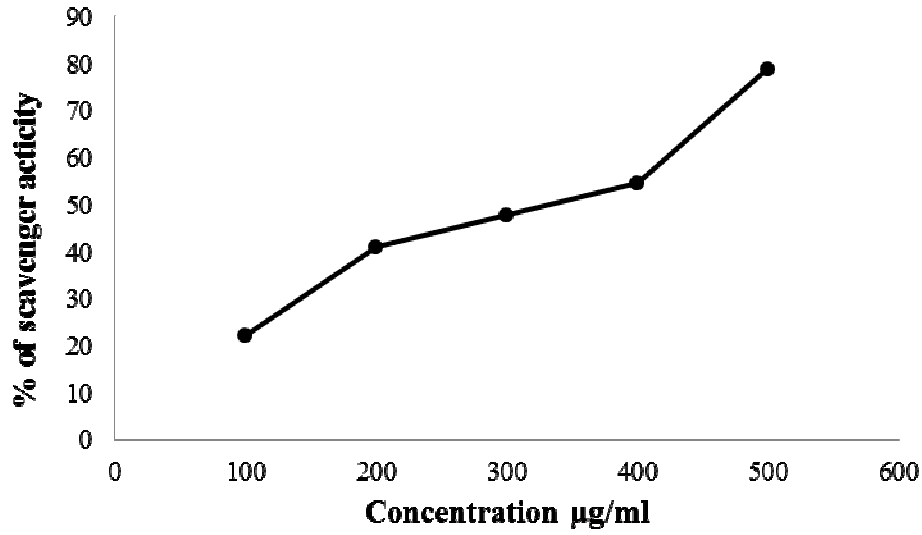
On the other hand and in order to confirm the antioxidant power of this plant the H_2O_2 test has been used. H_2O_2 is an important reactive oxygen species because of its ability to penetrate biological membranes. However, it may be toxic if converted to hydroxyl radical in the cell [21]. Scavenging of H_2O_2 by the plant extracts may be attributed to their phenolics, which donate electron to H_2O_2, thus reducing it to water. Our results demonstrated that aqueous extract from C. *longiflorus* possess high antioxidant activity reaching the 69 % at 500 µg/ml as shown in Figure 5.

Figure 5: Antioxidant activity of aqueous extract of *C. longiflorus*



On the other hand, Figure 8 shows an increase in the antioxidant activity of the methanolic extract of *C. longiflorus* with increasing concentration. It reaches the 79 % at the concentration of 500 µg/ml.

Figure 6: Antioxidant activity of methanolic extract of *C. longiflorus*



These results show that the studied plant has an antioxidant power and therefore it can be considered a good natural source that could be used in the treatment of diseases related to oxidative stress.

Determination of total phenol content

Figure 7 shows an elevated level of total phenol content at the concentration of 100 µg/ml. This level begins to decrease with the increase of the concentration to reach 0.66 mg/g cafeic acid which is equivalent to a concentration of 500 µg/ml.

Figure 7 : TPC of the methanolic extract of *C. longiflorus*

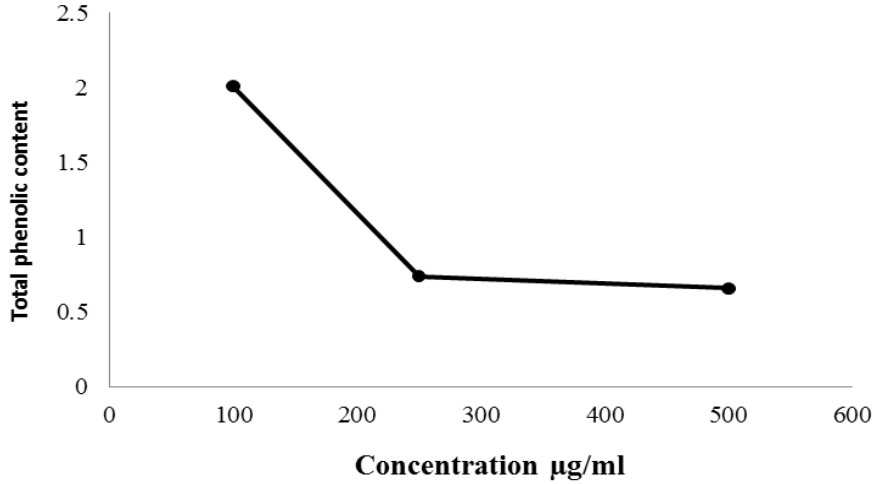
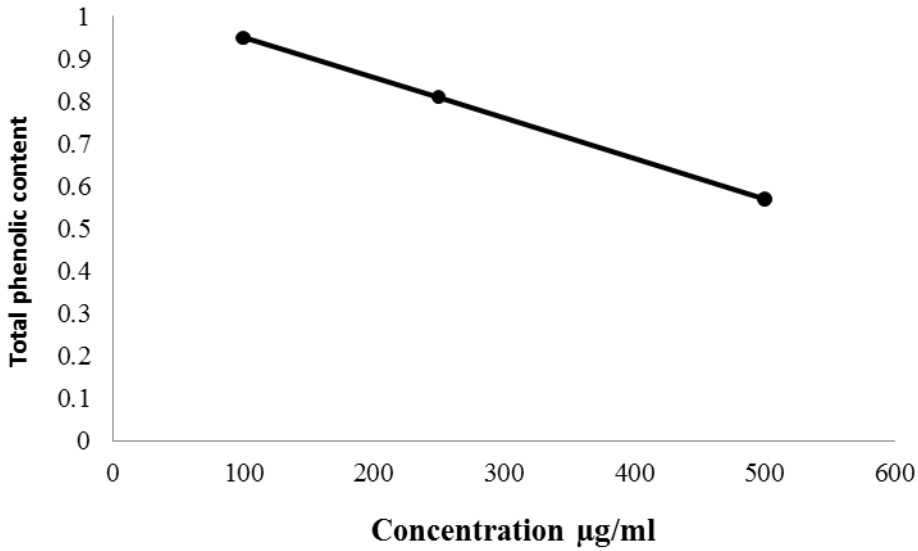


Figure 8 indicates that the amount of cafeic acid in methanolic extract of this plant is higher than in aqueous once. This amount was 0.95 mg/g at the concentration of 100 µg/ml and begun to decrease to become 0.57 mg/g at 500 µg/ml.

Figure 8: TPC of the aqueous extract of *C. longiflorus*



CONCLUSION

The results of our work demonstrated the importance of this Lebanese plant *Centranthus longiflorus* L. thus highlighting the possibility of its medical use of particularely its antioxidant activity due to its richness in different secondary metabolites, especially the polyphenol and therefore can be used in the prevention of several diseases associated with oxidative stress.

REFERENCES

[1] CS Yang; JM Landau; MT Huang; HL Newmark. *Ann Rev Nutr*, **2001**, 21, 381-406.
[2] PA Melendez; VA Capriles. *Phytomedicine*, **2006**, 13, 272-276.
[3] PN Diouf; T Stevanovic; A Cloutier. *Food Chem*, **2009**, 113[4], 897–902.
[4] H Rammal; R Soulimani. *Journal of Herbs, Spices & Medicinal Plants*, **2011**, 17[2], 154 - 168.
[5] H Rammal; J Bouayed; A Hijazi; M Ezzedine; R Soulimani. *J Nat Prod*, **2012**, 5, 54-59.
[6] H Farhan; H Rammal; A Hijazi; H Hamad; A Daher; M Reda; B Badran. *Asian J Pharm Clin Res*, **2012a**, 5[3], 234-238.
[7] H Farhan; F Malli; H Rammal; A Hijazi; H Hamad; A Bassal; N Ajouz; B Badran. **2012b**, 2[3], S1217-S1220.
[8] H Farhan; H Rammal; A Hijazi; H Hamad; B Badran. *Ann Biol Res*, **2012c,** 3[1], 149-156.

[9] H Farhan; H Rammal; A Hijazi; B Badran. *Int J Curr Pharm Res*, **2012d,** 4[1], 55-59.
[10] H Hong; G Liu. *Life Sci*, **2004,** 74, 2959–2973.
[11] V Katalinic; M Milos; T Kulisic; M Jukic. *Food Chem*, **2006**, 94, 550–57.
[12] JH Pak; T Kim; MJ Kim; JY Kim; H Choi; SA Kim; H Tchach. *Exp Eye Res*, **2006**, 82, 899-906.
[13] CC Wong; HB Li; KW Cheng; F Chen. *Food Chem*, **2006**, 97[4], 705–711.
[14] F Muanda. PhD Thesis, Paul Verlaine University (Metz, France, **2010**).
[15] A Chadegani; S Abdosamadi; N Fani; S Mohammadian. *Arch Toxicol*, **2009**, 83, 565-70.
[16] M Khader; N Bresgen; PM Eckl. *J Ethnopharmacol*, **2010,** 127, 319-324.
[17] C Kanadaswami; LT Lee, PPH Lee; JJ Hwang; FC Ke; YT Huang; MT Lee. *In vivo*, **2005,** 19:895-910.
[18] BS Jayashree; S Arora; KN Venugopala. *Asian J Chem*, **2008,** 20[1], 1-7.
[19] M Balunas; A Douglaskinghorn. *Life Sci*, **2005,** 78, 431-441.
[20] CL Hsu; W Chen; YM Weng; CY Tseng. *Food Chem*, **2003,** 83, 85-92.
[21] I Gulcin; M Oktay; E Kirecci; OI Kufrevioglu. *Food chem*, **2003,** 83, 371-382.