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Phenolic components, antioxidant and antimicrobial activities of *Centranthus longiflorus* L.

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Abstract

A number of individual phenolic compounds and the in vitro biological activities of *Centranthus longiflorus* L. were analyzed in this study. Reverse phase-high performance liquid chromatography (RP-HPLC) revealed 16 phenolic constituents. Total phenolic compounds (TPC) and ferric reducing antioxidant power (FRAP) were used as determinants of antioxidant capacity. *Centranthus longiflorus* L. exhibited strong antioxidant activity and contained high levels of antioxidant compounds. Chlorogenic and caffeic acid were detected, but no gallic acid, proto-catechuic acid, proto-catechuic aldehyde, p-OH benzoic acid, vanillic acid, syringic acid, vanillin, syringaldehyde, p-coumaric acid, ferulic acid, sinapic acid, benzoic acid or rosmarinic acid.Among all tested microbial strains, only Mycobacterium smegmatis has exhibited sensitivity to both water and methanolic extract. The test results suggest that this plant may be of potential use in the prevention and treatment of various oxidative-stress related diseases.

Keywords: **A**ntioxidant, Antimicrobial, *Centranthus longiflorus* L., Flavonoid, Phenolics.

Introduction

Medicinal plants represent the most important natural source of drugs for traditional and folk medical systems, modern medicines, nutraceuticals, food supplements, pharmaceutical intermediates and chemical constituents of synthetic drugs. Pharmacological properties, such as antioxidant, antidiabetic, antibacterial, antiviral and antiulcer effects, of medicinal plants and their individual constituents have been reported in numerous studies (Aliyazicioglu *et al.*, 2013; Rammal *et al.*, 2013; Rammal *et al.*, 2012; Rammal *et al.*, 2011).

Oxidative stress is implicated in many acute and chronic diseases including cancer, cardiovascular disorders and neurodegenerative diseases. Maintaining the balance between antioxidation and oxidation is

essential in the preservation of a healthy biological system (Katalinic *et al.,* 2006; Hong *et al.,* 2004). Medicinal plants are also rich sources of natural antioxidants, such as phenolic acids, flavonoids and tannins, with very potent antioxidant activity (Rammal *et al.,* 2013; Wong *et al.,* 2006). They have been also reported to prevent and cure a number of infectious diseases (Boukraâ *et al.,* 2013).

We investigated the total phenolic contents, antioxidant, and antimicrobial activities of *Centranthus longiflorus* L., and also determined phenolic compounds by using RP-HPLC

Materials and Methods

Chemicals and Instrumentation

The phenolic standards were of HPLC grade.Gallic acid, protocathechuic acid, *p*-hydroxybenzoic acid, vanillic acid, caffeic acid, chlorogenic acid, syringic acid, gentisic acid, vanilline, protocateculaldehyde, rosemarinic acid, sinapic acid, syringaldehyde, *p*-coumaric acid, ferulic acid and benzoic acid as ISwere purchased from Sigma-Aldrich (Steinheim, Germany) and Merck (Darmstadt, Germany).

Methanol, acetic acid and acetonitrile were obtained from Merck (Darmstadt, Germany). Trolox (6-hydroxy–2,5,7,8-tetramethylchroman–2-carboxylic acid), TPTZ (2,4,6-tripyridyl-s-triazine), and Folin-Ciocalteu's phenol reagent were obtained from FlukaChemie GmbH (Buchs, Switzerland) and polytetrafluoroethylene membranes (porosity 0.45 µm) for extract filtration from Sartorius (Goettingen, Germany).

High performance liquid chromatography (HPLC) (Agilent 1100, DAD 1200 Agilent Technologies, Waldbronn, Germany) analysis of phenolic compounds was conducted using a reverse phase waters spherisorp ODS2-C18 column (4.6×250 mm, 5 µm), on a gradient program with a two-solvents system (A: 2% acetic acid in water; B: 0.5% acetic acid in acetonitrile: water [1:1]) at a constant solvent flow rate of 1.2 $mL.min^{-1}$. Injection volume was 20 µL. Signals were detected at 232, 246, 260, 272, 280, 290, 308 and 328 nm by DAD and at 280 nm by UV detection. Column temperature was maintained at room temperature, 25° C.

A Spectro UV-Vis Double PC–8 auto cell spectrophotometer (Labomed Inc., California, U.S.A.) was used in all absorbance measurements. All solutions were prepared using deionized water purified in an Elgacan® C114 Ultra Pure Water System Deioniser (The Elga Group, Buckinghamshire, England).

An IKA® RV 05 Basic (IKA®, Werke, USA) rotary evaporator system was used during evaporation, and a HeidolphPromax 2020 (Heidolph Instruments GmbH & Co., Schwabach, Germany) shaker during extraction. A HeidolphReax top vortex (Heidolph Instruments GmbH & Co., Schwabach, Germany) and Elma® Transsonic Digital ultra sonic water bath (Singen/Htw, Germany) were used during all dissolution procedures. A Hanna (HI 110 series) instruments microprocessor pH meter (Hanna Instruments, Inc., Rhode Island, U.S.A.) was employed in all pH measurements, and a Mettler Toledo (Mettler-Toledo GmbH., Gießen, Germany) scale was used for all weight measurements in grams.

Preparation of extracts for phenolics and antioxidant analysis

Centranthus longiflorus L. was gathered from Yusufeli, Artvin in spring between May and June, 2013, and biological authentication was carried out by Professor Kamil Coskuncelebi. The plant was divided into two parts asblossom and trunk. Approximately 5 - 10 g of fresh sample was extracted with 30 mL methanol in a flask attached to the condenser in a sonicator device at 60 ºC over 3 h. Ten milliliters was separated from each extract in order to determine antioxidant activities. The residual extracts of methanol were evaporated until dry and then concentrated in a rotary evaporator at 50 ºC. The crude extract was then dissolved in 10 mL distilled water, and liquid-liquid extractions were performed. Three consecutive extractions were performed with 5 mL diethyl ether and 5 mL ethyl acetate. The organic moiety was picked up in the same flask and evaporated to dryness under reduced pressure in a rotary evaporator at 40 °C. The residue was finally weighed and dissolved in methanol HPLC analysis.

Determination of Antioxidant Capacity

Total phenolic contents (TPC) were determined using the Folin-Ciocalteau procedure with gallic acid as standard. Briefly, 0.1 mL of various concentrations of gallic acid and methanolic samples (1 $mg.mL^{-1}$) were diluted with 5.0 mL distilled water. Next, 0.5 mL of 0.2 N Folin-Ciocalteureagent was added, and the contents were vortexed. After 3-min incubation, 1.5 mL of Na_2CO_3 (2%) solution was added.

After vortexing, the mixture was incubated with intermittent shaking for 2 h at 20 °C. Absorbance was measured at 760 nm at the end of the incubation period. TPC concentration was calculated as mg of gallic acid equivalents per gram of 100 g sample, using a standard graph.

Ferric-reducing/antioxidant power (FRAP) assay was used to determine antioxidant activity of the methanolic sample. The technique is based on the measurement of ferric reducing ability. FRAP assay was performed following the method described by Benzie and Straine (1996), with minor modifications. Working FRAP reagent was prepared as required by mixing 25 mL of 0.3 M acetate buffer at pH 3.6 with 2.5 mL of 10 mM 2,4,6-tripyridyl-S-triazine (TPTZ) solution in 40 mM HCl and 2.5 mL of 20 mM $FeCl_3.6H_2O$. Subsequently, 100 µL of sample was mixed with 3 mL of freshly prepared FRAP reagent. The reaction mixture was then incubated at 37 °C for 4 min. Absorbance was determined at 593 nm against a blank prepared using distilled water and incubated for 1 h rather than 4 min. A calibration curve was employed, using Trolox concentrations in the range of 100–1000 µM, r^2=0.97. For purposes of comparison, Trolox® was also tested under the same conditions as a standard antioxidant compound. FRAP values were expressed as µM Trolox equivalent of g sample.

Antimicrobial activity assessment

We obtained all micro-organisms from the Hifzissihha Institute of RefikSaydam (Ankara, Turkey). These were *Escherichia coli* ATCC 25922, *Yersinia pseudotuberculosis* ATCC 911, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Listeria monocytogenes* ATCC 43251, *Bacillus cereus* 702 ROMA, *Mycobacterium smegmatis* ATCC607, *Candida albicans* ATCC 60193 and *Saccharomyces cerevisiae* RSKK 251. For antimicrobial analysis, all plants were extracted with water and methanol. After filtered to homogenization, stock solutions were adjusted 1000 µg.mL^{-1}.

Agar-well diffusion method

The agar-well diffusion method (Perez *et al.*, 1990), as adapted previously (Ahmad *et al.*, 1998), was used for susceptibility screening. Each bacterium was suspended in Mueller-Hinton (MH) broth (Difco, Detroit, MI). Yeast-like fungi were suspended in Yeast extracts broth. Micro-organisms were subsequently diluted to approximately 10^6 colony forming units (cfus) per ml. Potato Dextrose Agar (PDA) (Difco, Detriot, MI) was used for yeast-like fungi and Brain heart infusion agar (BHIA) for *M. smegmatis* (Woods *et al.*, 2003). These were first "flood-inoculated" onto the surface of MH and PD agars and subsequently dried. Wells measuring 5 mm in diameter were cut from the agar with the help of a sterile cork-borer. 50 µL of extract substances were then placed into the wells. The plates were incubated for 18-24 h at 35 °C. *M. smegmatis* was incubated for 3 to 5 days on BHIA plates at 35 °C. Antimicrobial activity was calculated by measuring the zone of inhibition against the test organism. Ampicillin (10 µg), streptomycin (10 µg) and fluconazole (5 µg) were standard drugs. All three solvent (water and methanol) was used as solvent control.

Results and Discussion

Total antioxidant capacity

Total antioxidant capacities of the methanolic extracts obtained from samples were calculated by means of the FRAP test. These methods are based on electron transfer and are regarded as accurate indicators of total antioxidant power, since total reducing power is defined as the sum of the reducing powers of the individual compounds contained in a particular sample (Tezcan *et al.*, 2011). Total antioxidant activity of samples is given in Table1.

Table - 1. Antioxidant activities of *Centranthus longiflorus* L.

Parameter	TPC (mg GAE/100 g DW)	FRAP (µmol Trolox/100 g DW)	DPPH radical scavenging activity (SC_{50}: mg.mL^{-1})
Blossom	391 ± 0.022	114 ± 0.019	0.94 ± 0.006
Trunk	502 ± 0.038	156 ± 0.021	1.30 ± 0.009

All results are given as mean ± SD (standard deviation). Standard antioxidants used were BHT (10 µg mL^{-1}, 0.01 ± 0.25).

The free radical scavenging effect of *Centranthus longiflorus* L. extracts was determined using the DPPH test with BHT as a reference. Maximum DPPH radical scavenging power was exhibited in the plant methanolic extract. This may be due to significant levels of variation in the polyphenols in the samples, as with their scavenging capacities. It may be speculated that the phenolic compounds behaved as free radical scavengers on the basis of their hydrogen-donating property (Molyneux, 2004). The methanolic extract possessed hydroxyl radical scavenging properties acting as donor for hydrogen atoms or electrons in the DPPH test. This enhanced iron autoxidation, while significantly reducing the accessibility of iron to oxygen molecules by oxidizing ferrous ion to a ferric state, this then inhibited hydroxyl radical production (Yoshino *et al.,* 1998). These findings suggest that the antioxidant activity of the methanolic extract is related to the high level of phenolic compounds.

Aqueous and methanolic extracts from *Centranthus longiflorus* grown in Lebanon have been studied for their scavenger activity using three different *in vitro* tests, DPPH, H_2O_2 and iron chelating (Rammal *et al.,* 2013). The phytochemical screening results identified flavonoids, phenols, essential oils, alkaloids and terpenoids. The DPPH test showed an antioxidant potential as high as 80%. The H_2O_2 test gave a figure of 70%. The iron-chelate test showed an antioxidant activity as high as 50% (Rammal *et al.,* 2013).

These results show the important antioxidant activity of *Centranthus longiflorus* L. due to its numerous different secondary metabolites, polyphenol in particular, and its potential use to prevent several oxidative stress-related diseases such as cardiovascular and aging-associated diseases by neutralizing free radicals in the body (Balunas *et al.,* 2005).

Total phenolic compounds (TPC)

TPC was determined in comparison with standard gallic acid. TPC of the methanolic samples was calculated at 37±0.034 mg GAE.100 g^{-1} dry weight (DW) of aqueous *Centranthus longiflorus* L. extract using the method described by Folin-Ciocalteu (Table 1). Rammal *et al*. (2013) reported TPCs at a level of 0.95 $mg.g^{-1}$ at a concentration of 100 $\mu g.mL^{-1}$ which decreased then to 0.57 $mg.g^{-1}$at 500 $\mu g.mL^{-1}$.

Plants compounds are used to obtain active natural products with numerous diverse structures and biological characteristics. Phenolic compounds are common in both edible and non-edible plants. Previous studies have reported that such compounds exhibit multiple biological effects, including antioxidant activity (Yoshino *et al.,* 1998). A correlation between antioxidant activity and phenolic content has been shown in previous studies (Nagai *et al.,* 2003; Yang *et al.,* 2002). A close association has been established between the efficacy of natural antioxidants and the chemical components and structures of active extract components. The antioxidant activity of an extract cannot therefore be explained in terms of its phenolic content unless characterization is also performed (Heinonen *et al.,* 1998). Elevated antioxidant activity may also be associated with non-phenolic compounds soluble in different solvents.

Identification of phenolic compounds using RP-HPLC

Various biologically active elements in plants protect them from a number of physical and chemical threats, including diseases, parasites and bacteria (Kolayli *et al.,* 2010; Aliyazicioglu *et al.,* 2013). Their phenolic constituents mean that they may also exhibit biologically active properties. Samples will inevitably contain a range of different phenolic compounds, meaning that individual measurement is problematic. We measured only 16 phenolic substances using HPLC. RP-HPLC was used to analyze 16 phenolic acids, gallic, proto-catechuic, gentisic, *p*-hydroxybenzoic, chlorogenic, vanillic, caffeic, syringic, *p*-coumaric, ferulic, sinapic, benzoic, rosmarinic, vanillin, syringaldehydeand *proto*-catechuic aldehyde. The RP-HPLC chromatograms obtained from the standard phenolic compounds are shown in Fig. 1. Comparison of the individual phenolic compounds contents identified chlorogenicacid as the main phenolic component in both section of blossom and trunk of *Centranthuslongiflorus* L. Besides chlorogenic acid, caffeic acid was identified from blossom parts of *Centranthus longiflorus* L., but no gallic, proto-catechuic, *p*-hydroxybenzoic, gallic, gentisic, vanillic, caffeic, syringic, *p*-coumaric, ferulic, rosmarinic, vanillin, syringaldehydeor *proto*-catechuic aldehyde. (Fig. 2 & 3 and Table 2 & 3).

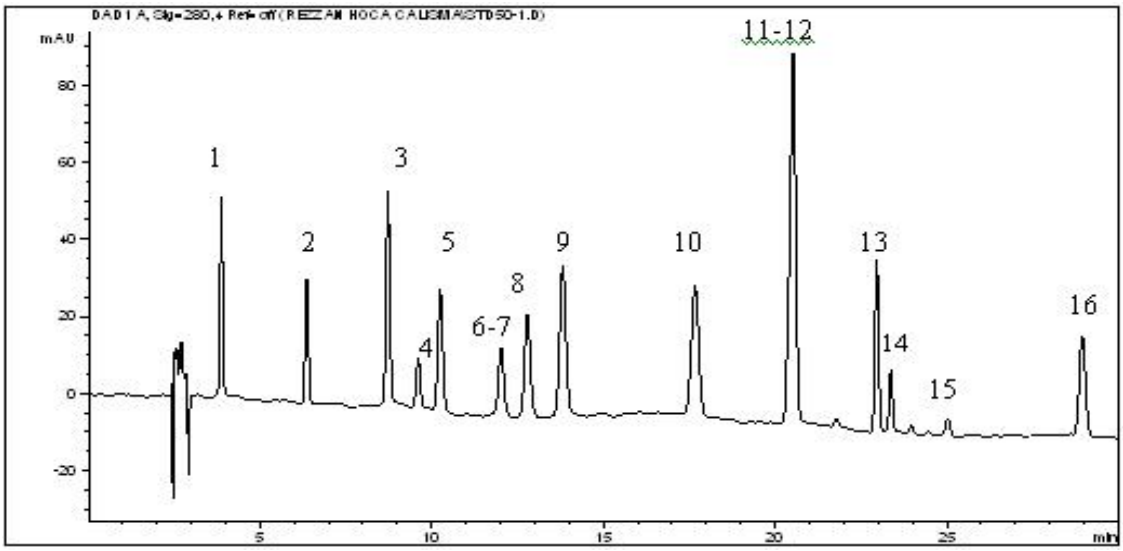

Figure-1. RP-HPLC chromatogram of phenolic standards (50 µM) searched in *Centranthus longiflorus* L. samples detected at 280 nm by DAD. Waters spherisorp ODS2 -C18 column (4.6×250 mm, 5 µm), gradient eluent acetic acid/acetonitrile/water, flow rate 1.2 mL/min. Peak identification: (1) gallic acid, (2) *proto*-catechuic acid, (3) *proto*-catechuic aldehyde, (4) gentisic acid, (5) chlorogenic acid, (6) *p*-OH benzoic acid, (7) vanillic acid (8) caffeic acid, (9) syringicacid, (10) vanillin, (11) syring aldehyde, (12) *p*-coumaric acid, (13) ferulic acid, (14) sinapic acid, (15) benzoic acid, (16) rosmarinic acid.

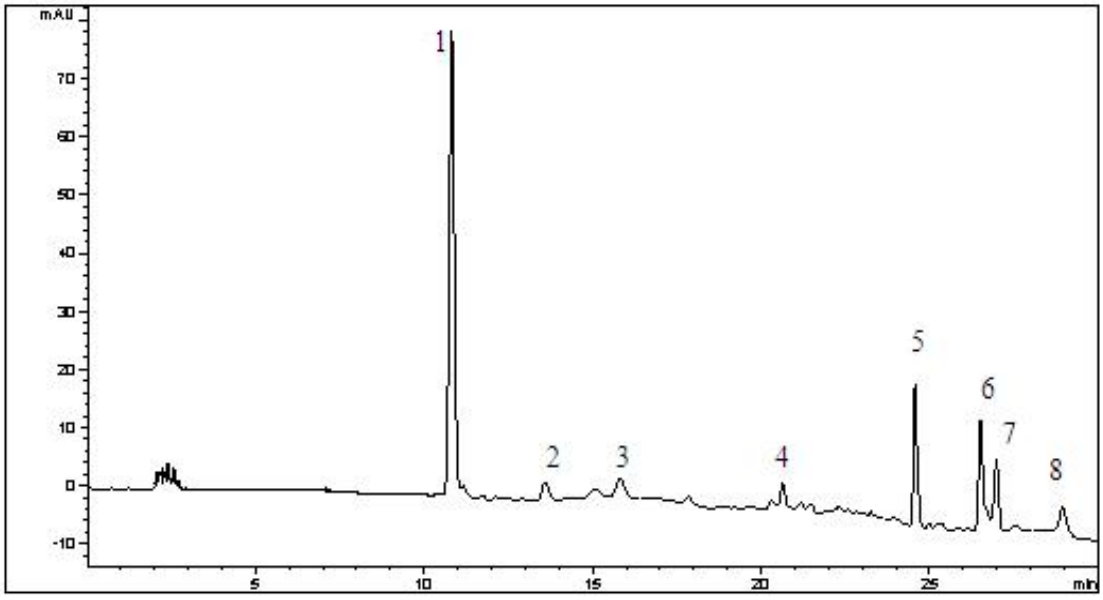

Figure-2. RP-HPLC-DAD chromatogram of blossom parts of *Centranthus longiflorus* L.

Peak identification: (1) chlorogenic acid, (2) caffeic acid, (3) unknown, (4) unknown (5) unknown, (6) unknown, (7) unknown, (8) unknown.

Table - 2. Retention times and peak areas of phenolic component peaks of blossom parts of *Centranthus longiflorus* L.

Peak number	Phenolic acid name	Retention time (RT)	Peak area
1	Chlorogenic acid	10.816	826
2	Caffeic acid	13.596	44.5
3	Unknown	15.813	63.5
4	Unknown	20.636	53.5
5	Unknown	24.563	189.4
6	Unknown	26.51	184.4
7	Unknown	26.976	117.5
8	Unknown	28.941	72.8

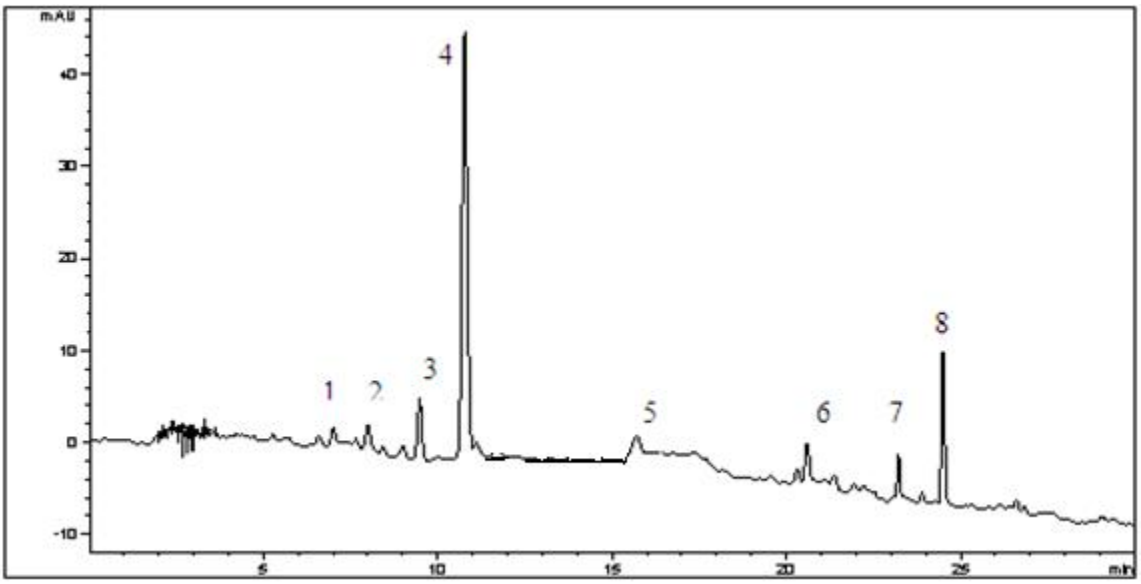

Figure - 3. RP-HPLC DAD chromatogram of stem parts of *Centranthus longiflorus* L.
Peak identification: (1) unknown, (2) unknown, (3) unknown, (4) chlorogenic acid, (5) unknown, (6) unknown, (7) unknown, (8) unknown.

Table - 3. Retention times and peak areas of phenolic component peaks of trunk parts of *Centranthus longiflorus* L.

Peak number	Phenolic acid name	Retention time (RT)	Peak area
1	Unknown	7.006	14.9
2	Unknown	7.996	27.4
3	Unknown	9.48	56.8
4	Chlorogenic acid	10.77	479.3
5	Unknown	15.7	32.1
6	Unknown	20.597	36.6
7	Unknown	23.215	26.9
8	Unknown	24.495	106

Antimicrobial activity

Disc diffusion was used to test methanolic and aqueous extracts for antimicrobial activity against eight bacteria and two yeast strains. The results were interpreted in terms of diameter of the inhibition zone and are summarized in Table 4. Activity against *Mycobacterium smegmatis* and *M. tuberculosis* by extracts of South African medicinal plants was reported in another study (Mativandlela *et al.,* 2008). The ethanol and aqueous extract of *Centranthus longiflorus* L. was most effective against *M. smegmatis* ATCC607.

Table - 4. Results of antimicrobial screening of the extracts determined by the agar diffusion method.

No	Stock extract (µg mL^{-1})	Zone of inhibition (mm)								
		Ec	Yp	Pa	Sa	Ef	Bc	Ms	Ca	Sc
P1	10.000	-	-	-	-	-	-	62.5	-	-
P2	10.000	-	-	-	-	-	-	31.3	-	-
Amp.		10	18	>128	35	10	15			
Str.								4		
Flu									<8	<8

P1: aqueous extract, P2: methanolic extract, Ec*: Escherichia coli* ATCC 25922, Yp: *Yersinia pseudotuberculosis* ATCC 911, Pa: *Pseudomonas aeruginosa* ATCC 43288, Sa: *Staphylococcus aureus* ATCC 25923, Ef: *Enterococcus faecalis* ATCC 29212, Bc: *Bacillus cereus* 702 Roma, Ms: *Mycobacterium smegmatis* ATCC607, Ca: *Candida albicans* ATCC 60193, Sc: *Saccharomyces cerevisiae* RSKK 251, Amp.: Ampicillin, Str.: Streptomycin (—): Flu.: Fluconazole, (—): no activity.

Conclusion

The results of studied parameters clearly indicate that the extracts of *Centranthus longiflorus* L. have rich phenolic compositions, antioxidant and antimicrobial activities that with potential for use as raw material by the pharmaceutical, and food industries.

Abbreviations Used

FRAP, ferric-reducing/antioxidant power; DW, dry weight; GAE, gallic acid equivalents; RP-HPLC, reverse phase-high performance liquid chromatography; IS, internal standard; Trolox®, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; TPTZ, 2,4,6-tripyridyl-s –triazine; BHT, butylatedhydroxytoluene; DPPH, 2,2-diphenyl-1-picrylhydrazyl.

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