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Antimicrobial and Antioxidant Activities of *Porphyridium cruentum*

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Abstract

In the present study, it was aimed to cultivate and analyze the potential antimicrobial and antioxidant activity of *Porphyridium cruentum*. The biomass achieved from *P. cruentum* cultures was processed to obtain ethanol, n-hexane, methanol extracts and volatile components. Antimicrobial, antioxidant activities and total phenolic contents of the extracts were investigated by *in vitro* test methods. In addition, volatile compounds of *P. cruentum* were elucidated by GC/GC-MS. When compared to the other solvent extracts, a potential antimicrobial activity was observed only in the n-hexane extract. Besides, it showed the best activity against *Staphylococcus aureus* ATCC 6538P. Volatile parts were active only against *Pseudomonas aeruginosa* ATCC 27853. The highest amount of total phenolic compounds was found as 62.45±2.0 mg Gallic Acid Equivalent/g Dry Weight (mgGAE/gDW) in the n-hexane extract. Statistically, a remarkable correlation was established between radical scavenging capacities and the total phenolic contents of the ethanol extracts. The level of antioxidant capacities varied according to the test methods. Consequently, it was thought that *P. cruentum* could be considered as an important origin of natural compounds and a strong alternative to the other natural sources such as higher plants, because of potentially valuable antioxidant, antimicrobial constutients.

Keywords: *Porphyridium cruentum*, antimicrobial activity, antioxidant activity, volatile components.

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Özet

Porphyridium cruentum'un Antimikrobiyal ve Antioksidan Aktivitesi

Bu çalışmada, *Porphyridium cruentum*'un kültürünün yapılması, potansiyel antimikrobiyal ve antioksidan aktivitesinin analiz edilmesi amaçlandı. *P. cruentum* kültürlerinden elde edilen biyokütle, etanol, n-hekzan, metanol ekstreleri ve uçucu komponentlerin eldesi için işlemlerden geçirildi. Ekstrelerin antimikrobiyal, antioksidan aktiviteleri ve total fenolik içeriği *in vitro* test metodları ile araştırıldı. Ayrıca *P. cruentum*'un uçucu bileşenleri GC/GC-MS metodu ile aydınlatıldı. Diğer çözgen ekstreleri ile kıyaslandığında, sadece n-hekzan ekstresinde potansiyel antimikrobiyal aktivite gözlendi. Ayrıca, bu ekstre en iyi etkiyi *Staphylococcus aureus* ATCC 6538P'ye karşı gösterdi. Uçucu kısımları sadece *Pseudomonas aeruginosa* ATCC 27853'e karşı etkiliydi. Total fenolik bileşikler 62,45±2,0 mg Gallik Asit Eşdeğer/g Kuru Ağırlık (mgGAE/gKA) değeri ile en yüksek oranda n-hekzan ekstresinde bulundu. İstatistiksel olarak, etanol ekstresinin, radikal süpürme kapasitesi ve total fenolik içeriği arasında önemli bir korelasyon olduğu tespit edildi. Ekstrelerin antioksidan aktivite düzeyi test metoduna bağlı olarak farklılıklar gösterdi. Sonuç olarak, *P. cruentum*'un, potansiyel değerli antioksidan, antimikrobiyal içeriği ile doğal bileşiklerin önemli bir orijini ve yüksek bitkiler gibi diğer doğal kaynaklara güçlü bir alternatif olarak kabul edilebileceği düşünüldü.

Anahtar kelimeler: *Porphyridium cruentum*, antimikrobiyal aktivite, antioksidan aktivite, uçucu bileşenler.

1. Introduction

Algae are rich sources of proteins, lipids, carbohydrates, minerals and vitamins. They have various applications including food, cosmetics, pharmaceuticals and natural therapies [1-3]. Red algae (Rhodophyta) are of increasing interest because of valuable ingredients such as phycobiliproteins, sulfated exopolysaccharides, superoxide-dismutase and polyunsaturated fatty acids (PUFAs); arachidonic, eicosapentaenoic, docosahexaenoic acids [4, 5]. The red microalga genus *Porphyridium* also synthesizes nontoxic and rather stable pinkish-red colored phycobiliproteins, suitable for use in food and cosmetics [6]. Furthermore, the sulfated polysaccharide cell-wall of *Porphyridium* sp. exhibits considerable antiviral activity against Herpes simplex and Varicella zoster viruses [7]. *Porphyridium cruentum* (Rhodophyta, Porphyridiales) attracts commercial interest for the production of eicosapentaenoic acid as a food additive and other PUFAs for cosmetics [6]. In addition, sulfoglicolipidic fraction extracted from *P. cruentum* shows antioxidant, anti-inflammatory and anti-proliferative activities [8].

Recently, natural antioxidants in plants and seaweeds have been attracted more attention, mainly due to the possible health risk associated with synthetic antioxidants [3, 9-11]. Besides, many natural antioxidants, especially flavonoids, usually depend on their chemical structures, exhibit a wide range of biological effects, including antibacterial, antiviral, anti-inflammatory, antiallergic, antithrombotic and vasodilatory activities [11-13].

The present investigation aimed to determine the antimicrobial activity, antioxidant capacity, total phenolic content and volatile components of *P. cruentum* cultured in laboratory conditions.

2. Material and Methods

Cultivation and Extraction of *P. cruentum*

P. cruentum was obtained from Ege University, Microalgae Culture Collection, Izmir, Turkey (Ege-MACC-9) and cultivated in f/2 medium [14], at pH 7-7.5 and 22°C, under continuous illumination at 40 µmol photon m^{-2} s^{-1}. Cultures were monitored by growth curves which are obtained by counting of the increasing cell numbers by a Neubauer chamber, measuring of the amount of chlorophyl a, and dry weight of the cells. Algal cultures were harvested at the end of the stationary phase and freeze-dried. Volatile components were extracted by a Clevenger-type apparatus and separately solvent extracts were obtained by using ethanol, methanol and n-hexane in a Soxhlet extractor. The solvents were removed by a rotating evaporator and the extracts were kept at −20°C until testing [15].

GC and GC/MS analysis

The volatile components of *P. cruentum* were investigated by GC and GC/MS analysis. A HP 6890 gas chromatograph fitted with a FID and a 5 m x 0.2 mm HP-1 capillary column (0.33 µm coating) was utilized for the GC analysis. GC/MS analysis was conducted on a HP 5973 mass selective detector coupled with a HP 6890 gas chromatograph, equipped with an HP-1 capillary column. Each component was identified by comparison of mass spectra with literature data and by a comparison of their retention indices (RI) relative to a C8-C32 n-alkenes mixture [16]. A computerized search was performed using the Wiley 275 L. GC/MS library and the ARGEFAR GC/MS library, created with authentic samples.

Antimicrobial activitiy

The extracts of *P. cruentum* were tested by disc diffusion method [17, 18] against five Gram positive bacteria (*Bacillus cereus* ATCC 7064, *Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 6538P, *Staphylococcus epidermidis* ATCC 12228), four Gram negative bacteria (*Pseudomonas aeruginosa* ATCC 27853, *Enterobacter cloacae* ATCC 13047, *Escherichia coli* ATCC 29998, *Salmonella typhimurium* CCM 5445) and one yeast strain (*Candida albicans* ATCC 10239). The suspensions of the fresh cultures of test organisms were adjusted to a 0.5 Mc Farland Standard and inoculated on the surface of Mueller Hinton Agar medium for bacteria and Sabouraud Dextrose Agar medium for yeast. Stock solutions of solvent extracts and volatile components were prepared at 25 mg/mL and 0.3 mg/mL concentrations, respectively. Sterile paper discs (6 mm in diameter) were embedded with different dilutions of the stock solution of solvent extracts (1.50, 0.75 and 0.38 mg $disc^{-1}$) and volatile components (1.5 µg $disc^{-1}$). The saturated paper discs were placed on the inoculated medium, and then agar plates incubated at 35 °C for 24-48 h. The diameters of the growth inhibition zones were measured in millimeters. Gentamycin (30 µg $disc^{-1}$) and Nystatin (100 µg $disc^{-1}$) discs were used as positive controls. The tests were repeated three times and the means of the three results were calculated.

Antioxidant activity

Solvent extracts of *P. cruentum* were evaluated by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) [19] and Beta-carotene bleaching (BCB) [20] methods. Commercial antioxidants, butylated hydroxytoluene (BHT) and Vitamin E (Vit E) were used as positive controls. For the DPPH radical scavenging assay, a solution of 0.04% DPPH radical were prepared in methanol (w/v). The solvent extracts (1.5 mg/mL) were mixed with DPPH solution and incubated for 20 min in the dark, at

room temperature. The decrease in the absorbance at 515 nm was measured and the percent of inhibition was calculated. To the BCB test procedure, β-carotene solution (BCS) were added to the solvent extract solutions, then immediately measured the absorbance value at 470 nm by using a spectrophotometer. The mixture of the extract and BCS was placed in an agitating water bath at 50 °C. Alterations of the absorbance values dependent on the presence of antioxidant compounds were measured at 15 min intervals during 120 min, and percent of inhibition was calculated.

Total phenolic content (TPC)

TPC of *P. cruentum* was determined by using Folin-Ciocalteau reagent [10]. The reagent was added to the solvent extracts. The mixtures were allowed to react at room temperature for 5 min., and then 6% (w/v) sodium carbonate was added to the mixtures and agitated gently. After standing for 90 min, the absorbance values were read at 725 nm using a spectrophotometer and plotted against a gallic acid standard curve (R^2=99.41). Total phenolic compounds were calculated as mg Gallic Acid Equivalent/g Dry Weight (mgGAE/gDW).

Statistical analysis

The results were estimated statistically by ANOVA with LSD test and student's t test. P value of 0.05 or less was taken to indicate statistical significance. Each data value was obtained by making at least three independent measurements. Results are shown as mean ± SEM.

3. Results and Discussion

In recent years, biologically active a great number of algal extracts and extracellular products have been identified. Composition of useful products in algae is influenced by different factors affecting the growth such as temperature, light, algal species, and age of culture [9, 21-23]. In the present study a total of 12 components (63.01%), include many hydrocarbons and biologically active compounds such as carvacrol (2.61%) and caprylene (2.19%) were identified in the volatile components of *P. cruentum* on the basis of comparison with MS data base spectra and pure reference compounds (Table 1.).

Table 1. GC-MS analysis results of volatile components extracted from *P. cruentum*

Rt (min)	*Compound*	*Area (%)*
6.34	Cyclohexane, methyl	21.54
6.73	Heptane, 3-methyl	2.74
6.85	Cyclohexane, 1,2-dimethyl	1.98
7.31	Hexane, 2,3,4-trimethyl	3.69
7.53	2,4-Dimethyl-1-heptene	7.83
11.46	Decane, 2,4,6-trimethyl	3.28
11.96	Caprylene	2.19
12.37	Undecane, 5-methyl	2.81
16.07	Carvacrol	2.61
24.54	3-Ethyl-3-methylheptane	4.21
24.75	Eicosane, 10-methyl	7.83
26.34	n-Nonadecane	2.30
	TOTAL	63.01

Correspondingly, there are several examples in the previous literature about antimicrobial and antioxidant activities of various algae. Antibacterial and antifungal activities of the volatile components of *Spirulina platensis* [15] and antibacterial activities of the volatile components of *Jania rubens* [24] against both Gram negative and positive bacteria have been demonstrated clearly in the researches. The antibacterial and antifungal activity of the solvent extracts of *Chrococcus* sp., *Oscillatoria* sp., *Anabaena* sp., *Synechocystis aquatilis*, *Chlorella vulgaris* have been shown by Katircioglu et al. [23]. Similarly, strong antibacterial activities of dichloromethane and n-hexane extracts of *Oscillatoria* sp. against *S. aureus* have been declared in another study [25]. Considering the results of antimicrobial tests in this study, the n-hexane extract of *P. cruentum* showed a higher antibacterial activity against both Gram negative (at 1.5 mg / disc, between 6.5-12 mm zone diameter) and Gram positive bacteria (at 1.5 mg /disc, between 10-16 mm zone diameter) than the other extracts. It was clearly seen that *S. aureus* was the most susceptible (at 1.5 mg /disc, 16 mm zone diameter) bacterium to the n-hexane extract. However, volatile components of *P. cruentum* were active against only one Gram negative bacteria (at 1.5µg/disc, 9 mm zone diameter), *Pseudomonas aeruginosa*. None of the extracts demonstrated inhibitory activity against *C. albicans* (Table 2.).

Table 2. Antimicrobial activity of *P. cruentum* according to the agar disc diffusion test

	Zone diameters of inhibition (mm)											
	ME			***EE***			***HE***			***V***	***G***	***N***
G(+) Bacteria	a	b	c	a	b	c	a	b	c			
E.faecalis	-	-	-	-	-	-	10	8	-	-	17	nt
B.subtilis	-	-	-	-	-	-	-	-	-	-	22	nt
S.aureus	-	-	-	-	-	-	16	14	12	-	21	nt
B.cereus	-	-	-	-	-	-	-	-	-	-	17	nt
S.epidermidis	-	-	-	-	-	-	10	8	-	-	14	nt
G(-) Bacteria												
P.aeruginosa	-	-	-	-	-	-	6.5	-	-	9	21	nt
E.cloaceae	-	-	-	-	-	-	12	10	8	-	21	nt
E.coli	-	-	-	-	-	-	9	-	-	-	30	nt
S.typhimurium	-	-	-	-	-	-	-	-	-	-	18	nt
Yeast												
C.albicans	-	-	-	-	-	-	-	-	-	-	nt	30

ME: Methanol extract, **EE:** Ethanol extract, **HE:** n-Hexane extract, **a:** 1.5 mg /disc, **b:** 0.75 mg /disc, **c:** 0.38 mg /disc, **G:** Gram, **nt:** not tested, **V:** Volatile components (1.5 µg/disc), **G:** Gentamicin (30 µg/disc) , **N:** Nystatin (100 µg/disc)

Antioxidant capacity of ethanolic extracts of *P. cruentum*, *Phaeodactylum tricornutum* and *C. vulgaris* cultivated in Spain have been shown by BCB method and fine chemicals in the extracts have been analyzed by GC and GC/MS [26]. Similarly, antioxidant activity of sulphated polysaccharides of *Porphyridium* has been reported by Tannin-Spitz et al. [27]. Vijayavel et al. tested the antioxidant activity of the alcoholic extract of *C. vulgaris*, and it has been seen that the extract reduced the lipid peroxidation significantly [28]. Wu et al. have reported water extract of *Spirulina* was a stronger antioxidant than *Chlorella*, which was probably because of the higher content of antioxidative phenolic compounds [29]. However, a correlation between

total phenolic contents and antioxidant activity has not been detected in all studies related to natural sources, because antioxidant impact is caused by different types of natural chemicals in the extracts such as ascorbic acid, tocopherol and pigments [11, 30].

In this study, antioxidant potential of the solvent extracts of *P. cruentum* compared with each other and synthetic antioxidants, buthylated hydroxy toluene (BHT) and Vit E (Table 3.). The extracts generally exhibited less potent antioxidant effects compared to those commercial antioxidants. When considered the results, *P. cruentum* possesses varying degrees of antioxidative activity to the kind of test methods used. According to the DPPH assay, the best antioxidant effect was seen in the n-hexane extract (17.10±0.1 %) of *P. cruentum*, however, the best antioxidant capacity was found in methanol extract (45.19 ±8.8 %) according to the BCB method. Similarly, BHT and Vit E showed different antioxidant ability range depend on the type of the test methods. The quantities of the phenolics in solvent extracts of *P. cruentum* were shown in Table 3. The best rate of the TPC was found in the n-hexane extract (46.08±5.4 mgGAE /10gDW).

Table 3. Antioxidant activity and TPC of *P. cruentum* solvent extracts

Extracts (1.5 mg/mL)	Antioxidant activity (%)		TPC
	DPPH	BCB	(mgGAE/g DW)
Ethanol	8.61±0.4	15.50±5.0	9.81±0.4
n-Hexane	17.10±0.1	35.01±8.9	46.08±4.2
Methanol	12.03±0.5	45.19±8.8	34.22±5.4
BHT	62.24±4.2	91.35±0.8	-
Vit E	94.67±1.5	75.41±1.2	-

P <0.01 (mean±SD, n=3)

Statistically, the best correlation among TPC amounts, DPPH and BCB methods were observed in the ethanol extract (TPC-DPPH: 0.813, TPC-BCB: 0.966, DPPH-BCB: 0.908; P<0.01). While, an association was found almost none existing between TPC and results of the antioxidant tests for the n-hexane (TPC-DPPH: 0.459; P<0.05, TPC-BCB: 0.640; P<0.01), it was found a good correlation only between TPC and BCB for the methanol extract (TPC-BCB: 0.592; P<0.01) (Table 4).

Table 4. Correlation among the antioxidant activity test (DPPH, BCB) results and TPC

	Ethanol extract			n-Hexane extract			Methanol extract		
	DPPH	BCB	TPC	DPPH	BCB	TPC	DPPH	BCB	TPC
DPPH	-	0.908**	0.813**	-	0.885**	0.459*	-	0.832**	0.592**
BCB	0.908**	-	0.966**	0.885**	-	0.640**	0.832**	-	0.906**
TPC	0.813**	0.966**	-	0.459*	0.640**	-	0.592**	0.906**	-

** P < 0.01, * P < 0.05

4. Conclusions

The present study indicated that, the extracts of *P. cruentum* possess biologically active components that could be qualified as potentially valuable source of natural antioxidants and antimicrobials. The n-hexane extract had higher phenolic content as well as potent antimicrobial and antioxidant activity than the other extracts of *P. cruentum*. However, all the extracts showed less potent antioxidant and antimicrobial effects than the positive controls since the commercial antioxidants are pure chemicals. Indeed, it is necessary to analyze the original molecules responsible for the activity of the natural extracts. However, it should be considered that the extracts could be sometimes biologically more effective than pure molecules due to a synergistic interaction. Thus, new researches about this area should be encouraged and supported, because of their potential contributions to further studies on valuable natural sources.

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References

1. Apt KE, Behrens PW: Commercial Developments in Microalgal Biotechnology. Journal of Phycology 1999, 35: 215-226.
2. Ördög V, Stirk WA, Lenobel R, Bancirova M, Strnad M, Staden J, Szigeti J, Nemeth L: Screening microalgae for some potentially useful agricultural and pharmaceutical secondary metabolites. Journal of Applied Phycology 2004, 16: 309-314.
3. Spolaore P, Joannis-Cassan C, Duran E, Isambert A: Commercial applications of microalgae. Journal of Bioscience and Bioengineering 2006, 101: 87-96.
4. Vonshak A. Porphyridium. In: Borowitzka MA, Borowitzka LJ. (eds), Microalgal Biotechnology. Cambridge University Press; Cambridge, UK. 1988: pp 122-134 .
5. Ginzberg A, Cohen M, Sod-Moriah UA, Shany S, Rosenshtrauch A, Arad S(M): Chickens fed with biomass of the red microalga Porphyridium sp. have reduced blood cholesterol level and modified fatty acid composition in egg yolk. Journal of Applied Phycology 2000, 12: 325-330.
6. Dufosse' L, Galaup P, Yaron A, Arad S (M), Blanc PK, Murthy NC, Ravishankar GA: Microorganisms and microalgae as sources of pigments for food use: a scientific oddity or an industrial reality? Trends in Food Science and Technology 2005, 16: 389-406.
7. Huleihel M, Ishanu V, Tal J, Arad S(M): Antiviral Effect of Red Microalgal Polysaccharides on Herpes simplex and Varicella zoster Viruses. Journal of Applied Phycology 2001, 13: 127-134.
8. Bergé JP, Debiton E, Dumay J, Durand P, Barthomeuf CJ: In vitro anti-inflammatory and anti-proliferative activity of sulfolipids from the red alga Porphyridium cruentum. Agricultural Food Chemistry 2002, 50: 6227-6232 .
9. Siriwardhana N, Lee K-W, Jeon Y-J, Kim S-H, Haw J-W: Antioxidant activity of Hizikia fusiformis on reactive oxygen species scavenging and lipid peroxidation inhibition. Food Science and Technology International 2003, 9: 339-346.
10. Velioglu YS, Mazza G, Gao L, Oomah BD: Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. Journal of Agricultural and Food Chemistry 1998, 46: 4113-4117.

11. Li H-B, Cheng K-W, Wong C-C, Fan K-W, Chen F, Jiang Y: Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. Food Chemistry 2007, 102: 771-776.
12. Almajano MP, Carbo R, Lopez JA, Gordon MH: Antioxidant and antimicrobial activities of tea infusions. Food Chemistry 2008, 108: 55-63.
13. Sroka Z, Cisowski W: Hydrogen peroxide scavenging, antioxidant and antiradical activity of some phenolics acids. Food and Chemical Toxicology 2003, 41: 753-758.
14. Guillard RRL, Ryther JH: Studies of marine planktonic diatoms. I. Cyclotella nana hustedt and detonula confervacea cleve. Canadian Journal of Microbiology 1962, 8: 229-239.
15. Ozdemir G, Karabay NU, Conk Dalay M, Pazarbasi B: Antibacterial activity of volatile component and various extracts of Spirulina platensis. Phytotherapy Research 2004, 18: 754-757.
16. Adams RP: Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry. Allured Publishing Corp. Carol Stream, Illinois, USA, 1995.
17. Bradshaw LJ: Laboratory Microbiology. Saunders College Publishing; Emeritus California State University, Fullerton, New York, USA, 1992.
18. Collins CM, Lyne PM: Microbiological Methods. Butterworths and Co. Ltd., London, UK, 1989.
19. Brand-Williams W, Cuvelier ME, Berset C: Use of a Free Radical Method to Evaluate Antioxidant Activity. LWT-Food Science Technology 1995, 28: 25-30.
20. Ismail A, Hong TS: Antioxidant activity of selected commercial seaweeds. Malaysian Journal of Nutrition 2002, 8: 167-177.
21. Durmaz Y, Monteiro M, Bandarra N, Gökpınar Ş, Işık O: The effect of low temperature on fatty acid composition and tocopherols of the red microalga, Porphyridium cruentum. Journal of Applied Phycology 2007, 19: 223-227.
22. Kamenarska Z, Dimitrova-Konaklieva S, Stefanov K, Najdenski H, Tzvetkova I, Popov S: Comparative Study of The Volatile Compounds From Some Black Sea Brown Algae. Botanica Marina 2002, 45: 502-509.
23. Katırcıoglu H, Beyatlı Y, Aslım B, Yuksekdag Z, Atıcı T: Screening for Antimicrobial Agent Production of Some Microalgae in Freshwater, Internet Journal of Microbiology 2006, 2: 2.
24. Karabay-Yavasoglu NU, Sukatar A, Ozdemir G, Horzum Z: Antimicrobial Activity of Volatile Components and Various Extracts of the Red Alga Jania rubens. Phytotherapy Research 2007, 21: 153-156.
25. Kreitlow S, Mundt S, Lindequist U: Cyanobacteria-A Potencial Source of New Biologically Active Substances. Journal of Biotechnology 1999, 70: 61-63 .
26. Rodriguez-Garcia I, Guil-Guerrero JL: Evaluation of the Antioxidant Activity of Three Microalgal Species for Use as Dietary Supplements and in the Preservation of Foods. Food Chemistry 2008, 108: 1023- 1026.
27. Tannin-Spitz T, Bergman M, Moppes D, Grossman S, Arad S(M): Antioxidant Activity of the Polysaccharide of the Red Microalga Porphyridium sp., Journal of Applied Phycology 2005, 17: 215-222 .
28. Vijayavel K, Anbuselvam C, Balasubramanian MP: Antioxidant Effect of the Marine Algae Chlorella vulgaris Against Naphthalene-induced Oxidative Stress in the Albino Rats, Molecular Cell Biochemistry 2007, 303: 39-44.
29. Wu L-C, Annie HJ-A, Shieh C-H, Lu I-W: Antioxidant and Antiproliferative Activities of Spirulina and Chlorella Water Extracts, Journal of Agricultural Food Chemistry 2005, 53: 4207-4212.
30. Sengul M, Yıldız H, Gungor N, Cetin B, Eser Z, Ercisli S: Total phenolic content, antioxidant and antimicrobial activities of some medicinal plants. Pakistan Journal of Pharmaceutical Sciences 2009, 1: 102-106.