Journal of Fundamental and Applied Sciences

Research Article

ISSN 1112-9867

Available online at http://www.jfas.info

EVALUATION OF ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF SPERGULARIA MARINA (L.) GRISEB EXTRACT

A. Miri[1], Z. Rashki Ghalehnoo[2] and E. Shaharaki [1,*]

[1]Department of Pharmacognosy, Faculty of Pharmacy, Zabol University of Medical Sciences, Zabol, Iran

[2]Department of Microbiology and Parasitology, Faculty of Medicine, Zabol University of Medical Sciences, Zabol, Iran

Published online: 10 June 2016

ABSTRACT

Spergularia marina (L.) Griseb is a species from *spergularia* genus (Caryophyllaceae). This genus has some medicinal effects such as antidiabetic, antioxidant and diuretic effect.

For many centuries people have been trying to alleviate and treat diseases with natural resources. Free radicals and oxidative agents cause disorders such as cardiovascular diseases, pulmonary diseases and rheumatoid arthritis. Synthetic antioxidants have toxic effects, so scientists are trying to find new antioxidants from natural resources such as plants.

Nowadays plants are known as an important resource for treating different diseases such as: common cold, cough, diarrhea, bronchiolitis, respiratory infections, UTI, and skin lesions, because of this reasons, a lot of plants have been studied to make new drugs. Due to increasing drug resistant in microorganisms, it is necessary to find new compounds with antimicrobial properties.

Author Correspondence, e-mail: el.shahraki@gmail.com

doi: http://dx.doi.org/10.4314/jfas.8vi2s.11

Journal of Fundamental and Applied Sciences is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License. Libraries Resource Directory. We are listed under Research Associations category.

In this work, firstly the total phenolic and flavonoid contents of Methanolic extract and Chloroform, Ethyl acetate and Water fractions from *Spergularia marina* (L.) Griseb were calculated, and then the antioxidant activites of all samples were determined using three methods, DPPH radical scavenging, Reducing power and Ferrous Chelating activity assay.

In this assays VitC, VitE, BHA, EDTA, rutin and gallic acid were used as reference compounds.

The antimicrobial activities of Methanolic extract from all part of plant determined against standard strains include: *Staphylococcus aureus*, *Escherchia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *Salmonella enterica*, *Klebsiella pneumoniae*, *Serratia Marssns* and *Candida albicans* by disc diffusion method.

In all antioxidant methods, Methanolic extract was showed the highest antioxidant activity and chloroform extract was the lowest antioxidant activity (aerial parts, seeds and all parts of plant). The results show methanolic extract of all parts of plant have any effect against above microorganisms.

KeyWords: *Spergularia marina*; caryophyllaceae; antioxidant; antimicrobial; DPPH; reducing power; ferrous chelating activit.

1. INTRODUCTION

Free Radicals and oxidant agents cause many diseases including cancer, cardiovascular disease such as atherosclerosis, Alzheimer's and memory loss, Parkinson's, multiple sclerosis, depression, glomerulonephritis, chronic renal failure, lung disease, cataracts and diseases related with retina, and rheumatoid arthritis. These compounds cause chemical spoilage of food, pharmaceutical and cosmetics materials (1) (2). The control of them is an important factor to prevent many diseases and material rotting.

Today the use of synthetic antioxidants is limited due to their side effects and health risks; food and pharmaceutical industries have been paid their attention to expand herbal and natural antioxidants.

Microorganisms are second important factors that cause some diseases and food spoilage (3).

In addition, antibiotic resistance increase day to day; because of it, finding the new anti-bacterial compounds is into consideration.

Spergularia is a cosmopolitan genus that grows in sub-tropical areas. Studies on some species of this genus represents anti diabetic, diuretic, high blood pressure, and cholesterol-lowering effects (4)(5).

The genus contains phytochemicals including Fatty acid, sterols, alkaloids, coumarins, Flavonoid, Steroids, saponins and Terpenoids (6).

Spergularia marina grows in desert (7), it contains β-sitosterol glycoside, tricin, dihydroferulic acid, vanillic acid, 4-hydroxybenzoic acid, 8-hydroxy cuminoic acid (8).

According to the best of our information, it has not done a study on the antioxidant effects of *Spergularia marina.* In this investigation, our team studied antioxidant and anti-bacterial activities of the methanolic crude extracts of aerial parts, and seeds and their fractions of Spergularia marina (L.) Griseb.

2. METHOD

2.1. Plant material

Spergularia marina (L.) Griseb was collected in the May 1391, Zabol, Iran. The voucher spacemanswas deposited at Ferdowsi University of Mashhad (No .29960).

Extraction was performed by maceration in methanol 85 at room temperature. The extracts were concentrated using rotary evaporator, and hold in dark and seal package in cold place.

The crud extracts were decanted to three fractions including water, chloroform and ethyl acetate.

2.2. Determination of Total Phenols and total flavonoid contents

Total phenols

The Total phenolic contents of each extract and its fractions were determined by the Folin-Ciocalteu method. Gallic acid was used as a standard (9, 10).

The amount of total phenol of each sample was expressed as gallic acid equivalents according to the standard charts (Figure1) (11).

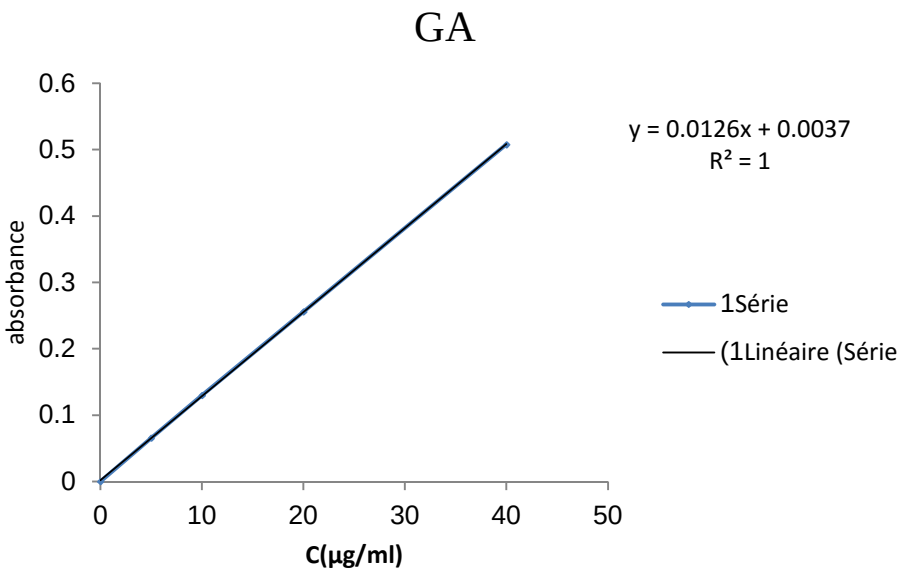

Fig.1. Absorbance standard curve vs Gallic acid concentration

Flavonoids

The content of flavonoid of each extract and its fractions were determined according to Lamaison. The method is based on the formation of flavonoids- aluminium complex, which has a maximum absorption at a wavelength of 430 nm(11). The routine was used as a standard (Figure 2).

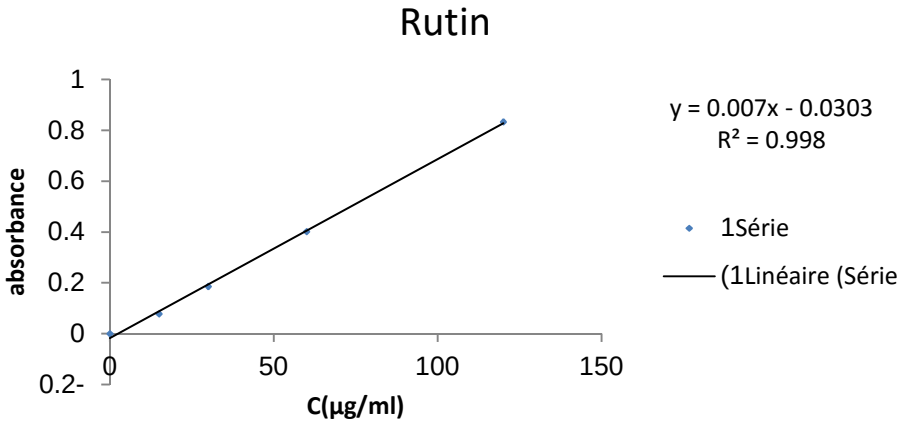

Fig.2. Absorbance standard curve vs rutin concentration

Flavonoids content was expressed in terms of routine equivalent (11).

Assessment of antioxidant activity

The antioxidant activity of aerial parts and seeds of S. marina were evaluated by various antioxidant assays, including DPPH free radical scavenging, metal chelating activity and reducing antioxidant power assay (12-15).

DPPH assay

DPPH assay is a valid, accurate, easy and affordable method with high repeatability, (16).

Radical scavenging activity was carried out according to the method described by (17). A DPPH radical is a free purple stable radical with central Nitrogen atom which can be measured by the absorbance at 517 nm after 30 min of reaction in darkness at 20 °C. Butylated Hydroxy Anisole (BHA) and Vitamin E were used as controls.

The results were expressed as IC50, that IC50 means concentration of samples giving 50% of inhibition (16).

2.3. Reducing power

The Reducing power experiment is based on , reduction of Iron III (Ferric) to Iron II (Ferro) as an index for potential of electron donation. In the method, measurement of antioxidant activity is based on light absorption at 700 nm. Increasing in absorption will expresses the increase of reducing power of samples (18).

Vit C And BHA were used as positive controls (12).

2.4. Metal chelating activity

The metal chelating activity of S.marina was estimated by the method (19).

Briefly the samples were added to a solution of FeCl2 , then ferrozine was added to above mixture, the violet-colored complex of ferrozine–Fe^{2+} was formed after 10 min in room temperature. Chelating agents prevent complex formation, so the intensity of violet colour decrease. Solution absorbance was measured at 562 nm. EDTA was used as standard (20).

2.5. Quality tests

The fixed foam formation method was used for identifying saponin. ; Liquorice root was used as a control (21).

Iron chloride was used as reagents for distinguishing of tannin and phenolic compounds; Oak extract was used as a control (22).

Magnesium was used as an indicator for **Flavonoids**.

The presence of flavonoids is determined in two minutes by appearance pale pink to cherry-red colour(23).

Dragandrof and Meyer reagent were used to identify the alkaloids. Berberis root was used as a control(24).

2.6. Anti-bacterial Activity

Disc diffusion method was used to evaluation of the antimicrobial effects of samples (25). Dimethyl sulfoxide as a negative control; gentamicin, Ceftriaxone, and amikacin were used as positive controls.

Standard Bacteria including *Bacillus subtilis(*6633*), Escherichia coli(*8739*), Serratia marcescens(*13880*), Streptococcus pneumonia(*PTCC*=1240), Klebsiella pneumonia(*10031*), Staphylococcus aureus(*6538*)* and *Pseudomonas* P. aeruginosa. *(*19433*)* were tested. Zone of growth inhibition was evaluated after 24 h of incubation (26).

2.7. Data analysis

Prism 5 was used for data analysis. The results were reported as mean ± SD and each test was repeated three times. The statistically significant difference was considered as 95% confidence interval.

3. RESULTS AND DISCUSSION

3.1. The efficiency of *Spergularia marina* (L.) Griseb

Total methanolic extracts of plant contain a wide range of polar and moderately non-polar compounds including alkaloids, sterols, triterpenes, flavonoids, carbohydrates and coumarins and etc (26).Chloroform, ethyl acetate, and water were used to provide fractions of methanolic extract. These solvents have different polarity. Chloroform phase contains mostly non-polar compounds such as carbide, sterols and chlorophyll oxidized derivatives.

The intermediate polar compounds such as flavonoids and phenols enter to ethyl acetate phase and Polar compounds including sugars, salts, saponins and glycoside get into the aqueous phase(27).

The efficiency of different parts of the plant has shown in the (Table 1).

Table 1. The amount of productivity of different parts of plant Spergularia marina (L.) Griseb

productivity / Extract Name	Plant aerial part	Plant seed
Total methanolic	11/24	7/1
Chloroform	5/93	4/21
water	5/28	2/84
Ethyl acetate	0/027	-

3.2. Results of qualitative test

In the Preliminary phytochemical tests, existence of tannins, saponins, flavonoids and alkaloids in the methanolic extract of *Spergularia marina* (L.) Griseb were qualitatively analysed. The results of these tests have presented in (Table 2).

Table 2. Preliminary phytochemical tests result of methanolic extract of Spergularia marina (L.) Griseb

Alkaloid	Flavonoid	Saponins	Tannins
+++	+++	+++	++++*

*average amount from – to +, - without compound, + a little positive, ++ Almost positive, +++ Significantly positive, ++++ Strongly positive

3.3. Microbial test results

The antimicrobial activity of methanolic extract of *Spergularia marina* (L.) Griseb were studied by disk diffusion method on eight types of bacteria, including *Staphylococcus aureus, Escherichia Coli, Bacillus Subtilis, Pseudomonas aeruginosa, Streptococcus pneumoniae, Salmonella enterica, Klebsiella pneumoniae, Serratia marcescens* and a fungus *C. albicans* (Table 3).

Table 3. Checking zone of growth inhibition in various microorganisms

Ceftriaxone disk) g(µ	Gentamicin disk) g(µ	Amikacin disk) g(µ	Methanolic)mg/ml(	solution		Blank	sample / concentration / Microorganisms
30	10	30	80	40	20	10	
-	-	30	0	0	0	0	0	*Staphylococcus aureus*
-	32	-	0	0	0	0	0	Escherichia coli
-	18	-	0	0	0	0	0	*Bacillus subtilis*
-	-	23	0	0	0	0	0	*Pseudomonas aeruginosa*
20	-	-	0	0	0	0	0	*Streptococcus pneumoniae*
-	-	-	0	0	0	0	0	*Salmonella enterica*
30	-	-	0	0	0	0	0	*Klebsiella pneumoniae*
-	22	-	0	0	0	0	0	Serratia marcescens
-	-	-	0	0	0	0	0	Candida_albicans

zone of growth in various microorganisms)mm(

According to the results obtained in the study, total methanolic extract of the plant was no halo formation.

3.4. Phenolic and flavonoid content

Phenolic and flavonoids are natural compounds that have great antioxidants effects.

phenolic compounds act as antioxidants by some mechanisms including free radicals scavenging, metal-chelating properties, the ability to regulate gene expression and co-antioxidant role (28) (29).The phenolic content of methanolic extract and its fractions of different parts of the plant are as follow:

The phenolic content of branches: methanol extract (53.47±0.18) > Water fraction (8.86±0.09) > Chloroform fraction (3.74±0.15) > Ethyl acetate (0.86±0.02)

The phenolic content of seed: methanol extract (19.83 ±1.01) > Water fraction (5.16 ± 0.03) > Chloroform fraction (1.85 ± 0.06).

Flavonoids are a class of phenolic compounds that have great antioxidant activity (30).

The amount of flavonoids in methanolic extract and its fractions is as fallow:

Flavonoid content of aerial parts: methanolic extract (12.33 ± 0.4) > Water fraction (6.92 ± 0.7) > Chloroform fraction (5.24 ± 0.05) > Ethyl acetate fraction (0.29 ± 0.02)

Flavonoid content of seed: methanol extract (7.14 ± 0.09) > Water fraction (3.23 ± 0.2) > Chloroform fraction (2.37 ± 0.08)

3.5. Antioxidant activity

Antioxidant activity of plant extracts cannot evaluate only by one way; this is due to the complex nature of the chemical compounds found in plants and their different antioxidants mechanisms. Therefore it is necessary that several common methods use to evaluate the antioxidant activity of the extracts (16) (31).

3.6. DPPH

Antioxidant activity of extracts of *Spergularia marina* (L.) Griseb and their fractions (extract of aerial parts of the plant (Figure 3) seed extract (Figure.4) and standards(Vit E And BHA) was investigated by DPPH method; IC_{50} , the concentration that causes 50% of radical scavenging activity, was used for results comparison (Table 4).

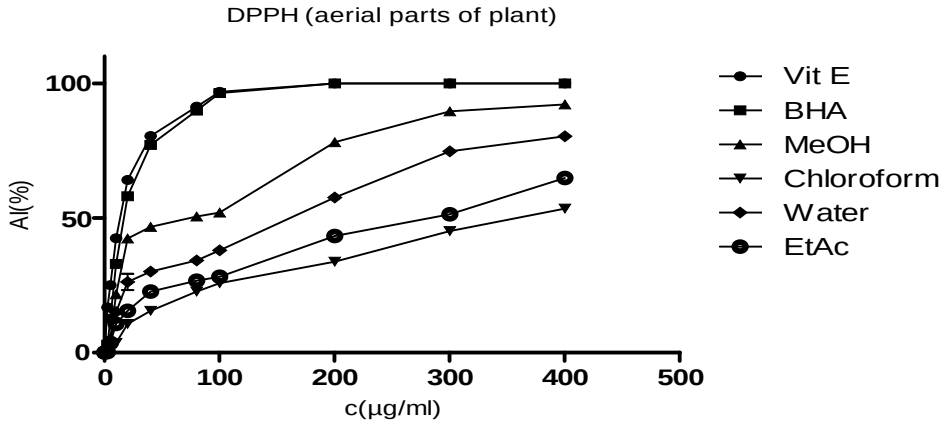

Fig.3. Index amount of the Antioxidant of Methanol extract and Athyl acetate, water and chloroform fractions of aerial part of *Spergularia marina* (L.) Griseb plant, Vitamin E and BHA in DPPH method

*Results are expressed in antioxidant index by measuring three time absorption at each concentration. The statistically significant difference was considered as 95% confidence interval.

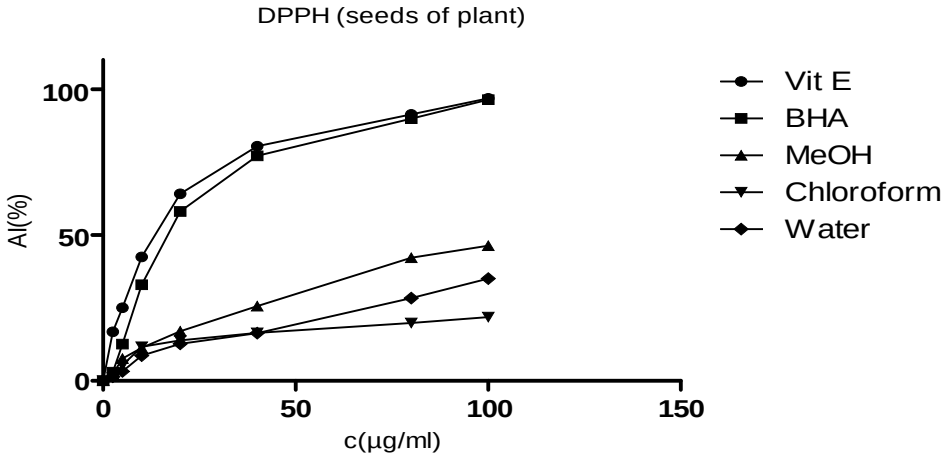

Fig.4. Index amount of the Antioxidant of Methanol extract and Athyl acetate, water and chloroform fractions of seeds part of *Spergularia marina* (L.) Griseb plant, Vitamin E and BHA in DPPH method

*Results are expressed in antioxidant index by measuring three time absorption at each concentration. The statistically significant difference was considered as 95% confidence interval.

Table 4. The results of calculation IC50 of the Methanol extract and fractions of Athyl acetate, water and chloroform of different parts of *Spergularia marina* (L.) Griseb plant, Vitamin E and BHA in DPPH method

IC_{50} (µg/ml)			
Plant seed	Plant aerial part	All parts of plant	Samples
4/12±0/05	4/12±0/05	±0/01** 4/12	Vitamin E
8/57±0/07	8/57±0/07	0/08±8/57	BHA
100/7±2/33	62/6±1/05	1/02 ±60/8	Methanol extract
366/8±5/50	346±3/62	5/34 ±337/9	Chloroform phase
t*	277/03±2/76	2/44±272/3	Athyl acetate phase
143/6±1/24	192/9±0/98	181/3±1/95	Water phase

*trace **Results are expressed as Mean ±SD by measuring three time absorption at each concentration. The statistically significant difference was considered as 95% confidence interval.

In order to investigate the relationship between total phenolic or flavonoids with antioxidant activity in DPPH method, The Linear regression graph between polyphenols or flavonoids and IC_{50} of methanoic extract and its fractions was drawn and the relationship between the phenols or flavonoids with antioxidant activity was expressed in terms of the correlation coefficient **(** R^2 **)** (Figure 5) (Figure 6). (32)(33).

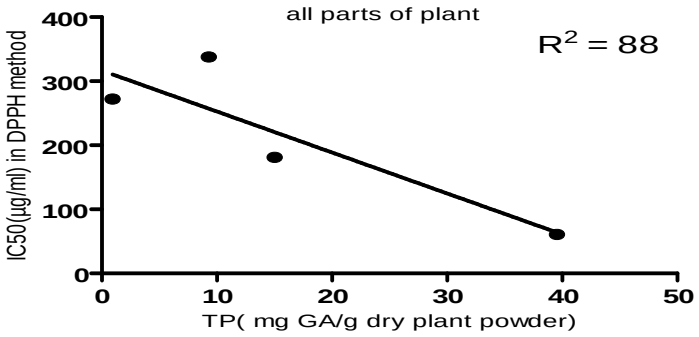

Fig.5. The relationship between the amount of total phenol in all parts of plant extract with index amount of antioxidant activity (IC 50) in the DPPH method

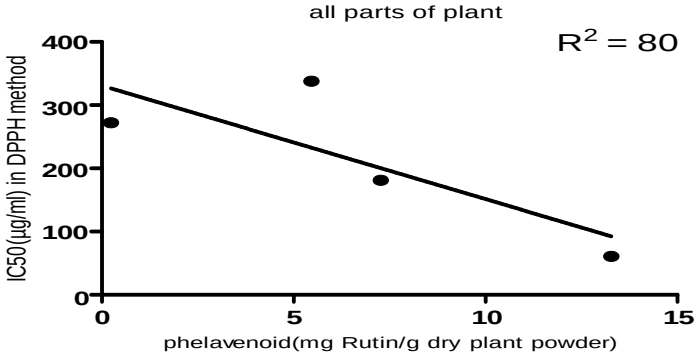

Fig.6. The relationship between the amount of phelavenoid in all parts of plant extract with index amount of antioxidant activity (IC 50) in the DPPH method

The result shows there is a strong relationship between total phenols obtained in different parts of the plant extracts and antioxidant activity (Based on IC_{50}).

The correlation coefficient between the total phenols of the methanolic extract of the aerial parts of plant and DPPH is 80 (R^2 = 80) which indicates 80 % of antioxidant activity of

methanolic extract is related to the total phenols and 20 percent of antioxidant activity is related to the other substances. Similarly, R^2 in the extracts of the seed is 75.

A strong relationship between flavonoids and antioxidant activity (Based on IC_{50}) was observed (R^2 = 79 for aerial parts of the plant, 75 for seeds).

Among phenolic compounds, flavonoids are stronger antioxidant compounds. They are strong hydroxyl and peroxide radicals deterrent (34).

The results obtained by other researchers have shown there is a strong relationship between the amount of phenolic and flavonoid compounds of plant extracts and their anti-radical activity; the higher phenolic in the extract cause higher antioxidant activity.

This finding confirms our results. the difference observed in the ethyl acetate extract, which unlike the low rate of phenolic compounds it has appropriate antioxidant activity, is attributed to the difference among the type of phenolic compounds in molecular weight, the availability of hydroxyl groups, and the sustainability of phenoxil radicals or existence of non-phenolic antioxidant compounds . Phenolic compounds with low molecular weight have accessible hydroxyl groups . In addition, phenolic compounds that donor their hydrogen become free fenoxyl radicals. The stability of these radicals can be affect to antioxidant capacity in phenolic compounds , because less stable fenoxyl radicals competes to DPPH in attracting hydrogen atoms, thus the percentage of trapping DPPH radicals reduce (35).

3.7. Reducing power activity

Antioxidant activity of polyphenols is mainly due to their oxidation-reduction properties therefore they act as reducing agents, hydrogen donors, singlet oxygen neutralizing and metal chelator(36).

In this study, reducing activity of different parts of the plant extracts was calculated based on mg Vit c E / g DW (Table 5) and mg BHA E / g DW (Table 6).

Table 5. Reducing activity of methanol extract and various fractions in terms of mg of Vit C in one gram dry plant powder

g dry plant powder /mg Vit C			Reducing activity / type of extract
Plant seed	Plant aerial part	All parts of plant	
40/65	71/34	73/23	methanol
8/38	17/42	19/93	water
-	0/09	0/1	Athyl acetate
1/68	2/5	6/98	chloroform

Table 6. Reducing activity of methanol extract and various fractions in terms of mg of BHA in one gram dry plant powder

g dry plant powder /mg BHA			Reducing activity / type of extract
Plant seed	Plant aerial part	All parts of plant	
53/43	91/61	93/59	methanol
13/49	26/93	28/1	water
-	0/14	0/1	Athyl acetate
9/26	13/19	17/67	chloroform

Total phenolic content (aerial parts and seeds) of the methanolic extract was more than the other fractions, in this case the methanolic extract showed the most reducing activity. Aqueous fraction has higher levels of phenolic compounds than the chloroform fraction,

In addition it showed a stronger reducing activity than the chloroform fraction.

Total phenols and flavonoids of chloroform extract are more than the ethyl acetate extract, also it showed a higher reducing activity.

The difference observed between the reducing power of extracts can be attributed to differences in the type of phenolic compounds that extracted by different solvents. In

addition, other substances that is soluble in polar solvent, extracts during the process of extraction. Since some of these compounds such as amino acids, proteins and sugars are electron donors, so a greater percentage of trivalent iron ions reduce by electron capture, thus the intensity of absorbance increases (37).

3.8. Ferrous chelating activity

The metal ions as pro-oxidants were chelate by polyphenolic compounds; therefore phenolics prevent the formation of free radicals resulting from the pro-oxidants (20).

Natural phenolic compounds including quercetin, rutin, catechin and caffeic acid act as a ferrous ion chelating agents (16).

Results were shown in Tabel (7).

Table 7. the existing amount of total phenols in methanol extraction and fractions of water, athyl acetate and chloroform of different part of *Spergularia marina* (L.) Griseb plant

g dry plant powder /mg GA			Amount of total phenols / type of extract
Plant seed	Plant aerial part	All parts of plant	
19/83±1/01**	35/47±0/18	39/53±0/1	Methanol
5/16±0/03	8/86±0/09	15±0/001	Water
t*	0/86±0/02	0/94±0/007	Athyl acetate
1/85±0/06	3/74±0/15	9/3±0/48	Chloroform

*trace **Results are expressed as Mean ±SD by measuring three time absorption at each concentration

The results obtained in this study compared with other researches in this area suggests that the amount of phenolic and flavonoid compounds, tannins and saponins have a direct connection with antioxidant activity .

Spergularia marina (L.) Griseb have relatively high amounts of phenolic and flavonoid, also it has tannins and saponins; the plant has suitable anti radical activity and medium chelating activity.

4. REFERENCES

[1] Pham-Huy, A.L., He, H. and Pham-Huy, C. Free radical,antioxidants in disease and health. Int. J. Biomed. Sci., 4, 2008, 89-96.

[2] Morrissey , A.P. and OBrien, MN.. Dietary antioxidants in health and disease . Int. Dairy J., 8, 1998, 463-472.

[3] Nostro A, Germano M, Angelo V, Marino A, Connatelli M. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. *Lett Appl Microbiol*; 2000. 30: 389-394.

[4] Vinholes, J., Grosso,C., Andrade,P. B., Gil-Izquierdo, A., Valentao, P. Guedes de Pinho, P., Ferreres, F. In vitro studies to assess the antidiabetic, anti-cholinesterase and antioxidant(2011).

[5] Jouad, H., Lemhadri, A., Maghrani, M., Zeggwagh, N. A., Eddouks, M. Cholesterol-lowering activity of the aqueous extract of *Spergularia purpurea* in normal and recent-onset diabetic rats. Journal of Ethnopharmacology 87(2003) .43–49.

[6] Sindhu S, Manorama S. Antioxidant Activities of *Polycarpaea corymbosa* Lam. (Caryophyllaceae) Using Various *In vitro* Assay Models. *the pharma innovation J*; 2013. 2: 77-95.

[7] Mozaffarian, and the first .chap .flvr Iran, pasture Forest Research Institute of Iran, Tehran, (1371), 1260

[8] The first volume, first edition, the capital of Tehran University Press 8. hero, or. Krvmvfyt of Iran (Plant Systematics). (1371), 309-291.

[9] Tawaha K, Alali FQ, Gharaibeh M, Mohammad M, El-Elimat T. Antioxidant activity and total phenolic content of selected Jordanian plant species. *Food Chem*; 2007. 104: 1372–1378.

[10] Rebiai A, Lanez T, Belfar M. Total polyphenol contents, radical scavenging and cyclic voltammetry of Algerian propolis. Int J Pharm Pharm Sci, 2014, 6(1), 395-400

[11] Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in Enzymology*; 2010. 299: 152–178.

[12] Boubekri C, Lanez T, Djouadi A. A comparative study on antioxidant activities and phenolic contents of five algerian eggplant cultivars. St. Cerc. St. CICBIA 2015, 16 (1), 29-46.

[13] Lanez T, Henni M. Antioxidant activity and superoxide anion radical interaction with 2-(ferrocenylmethylamino) benzonitrile and 3-(ferrocenylmethylamino) benzonitrile. J Iran Chem Soc. 2016, doi: 10.1007/s13738-016-0891-1

[14] Rebiai A, Lanez T, Belfar M. In vitro Evaluation of Antioxidant Capacity of Algerian Propolis by Spectrophotometrical and Electrochemical Assays. International Journal of Pharmacology 2011, 7(1), 113-118, doi: 10.3923/ijp.2011.113.118

[15] Khelef A, Lanez T. In vitro assays of the antioxidant activities of ferrocene derivatives bearing amine, amide or hydrazine groups. Der Pharma Chemica, 2015, 7(6),318-323

[16] Djeridane, A., Yousfi, M., Nadjemi, B., Boutassouna, D., Stocker, P. and Vidal, N. Antioxidant activity of some algerian medicinal plants extract containing phenolic compounds. Food Chem., 97,(2006). 654-660.

[17] Singh, R., Singh, S., Kumar, S. and Arora, S . Evaluation of antioxidant potential of ethyl acetate extract/fractions of *Acaciaauriculiformis*A. Cunn .Food Chem. Toxicol., 45, (2007).1216-1223.

[18] Miri, A. Monsef-Esfahani, Ha. Comparative chemical composition and antioxidant properties of the essential oils and aromatic water from Teucrium persicum Boiss. Iranian Journal of Pharmaceutical Research.11.2(2012).573-581.

[19] Jayaprakash GK, Singh RP, Sakariah KK. Antioxidant activity of grape seed extracts on per oxidation models *in vitro*. *J Agricultural Food Chem*; 2001. 55: 1018-1022.

[20] Dinis TCP , Madeir VMC, et al. Action of phenolic derivatives as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers.Arch.Biochem,Biophys 315. (1994).161-169.

[21] Liu, X., Zhao, M., Wang, J., Yang, B. and Jiang, Y . Antioxidant activity of methanolic extract of emblica fruit (*Phyllanthus emblica* L.) from six region in China . J. Food Compos. Anal., 21. (2008).219-228.

[22] Jansson AM, Scheffer J, Baeheim A. Antimicrobial activity of essential oils. *Plant Med*; 2006. 9: 395-398.

[23] S Majaw, Moirangthem J. Qualitative and Quantitative Analysis of *Clerodendron colebrookianum Walp*. Leaves and *Zingiber cassumunar* Roxb. Rhizomes. *Ethnobotanical Leaflets*. 2009;13: 578-89.

[24] Ashokkumar P, RaM K. Phytochemical Screenig and Antimicrobial Activity from Five Indian Medical Plants Aguinst Human Pathogens. *Journal of Scientific Research*. 2010;5(3): 157-62.

[25] The presence of alkaloids in plants: alkaloids reagents method compared to Brvmvkrzvl Gryn.mjlh School of Medicine, Tehran University of Medical Sciences, Volume 66, Number 4, July, 1387. p. 241-237.Rios JL, Recio MC, Villar A. Screening methods for natural products with antimicrobial activity. *J Ethnopharmacol*; 1988. 23: 127-149.

[26] Leite SP, Raphael J, Vieira C, Medeiros PL, Road P, Menezes-Lima VL, Xavier HS, Lima O. Antimicrobial activity of *Indigofera suffruticosa*. *Oxford J*; 2006. 3: 261-265.

[27] Markham, R.K. Techniques of flavonoid identification. London: Academic press.(1982) 45.

[28] Gulcin, I., Oktay, M., Kufrevioglu, I.Ö. and Aslan, A. Determination of antioxidant activity of *Lichen Cetriaria islandca* (L) Ach. J. Ethnopharmacol., 79. (2002).325-329.

[29] Neergheen, S.V., Bahorun., T., Jen, S.L. and Arouma, I.O. Bioefficacy of Mauritian endemic medicinal plant : Assessment of their phenolic contents and antioxidant potential. Pharm. Biol., 45,(2007). 9-17.

[30] Zhao, R. G., Xiang, J.Z., Ye, X.T., Yuan, J.Y. and Guo, X.Z. Antioxidant activities of *Salvia miltiorrhiza* and *Panax notoginseng*. Food Chem., 99, (2005).767-774.

[31] Pellegrini, N., Serafini, M., Colombi, B., Rio, D.D., Salvatore, S., Bianchi, M. and Brighenti, F. Total anioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different in vitro assay.J. Nutr., 133,(2003).2812-2819.

[32] Wangensteen H, Samuelsen AB, Malterud KE. Antioxidant activity in extracts from *Coriandrum sativum*. *Food Chem*; 2004. 88: 293–297.

[33] Ranilla LG, Kwon Y, Apostolidis E, Shetty K. Phenolic compounds, antioxidant activity and *in vitro* inhibitory potential against key enzymes relevant for hyperglycemia and hypertension of commonly used medicinal plants, herbs and spices in Latin America. *Bioresource Technology*; 2010. 101: 4676–4689.

[34] Shukla S, Mehta A, Bajpai VK, Shukla S. *In vitro* antioxidant activity and total phenolic content of ethanolic leaf extract of *Stevia rebaudiana* Bert. *Food Chemical Toxicol*; 2009. 47: 2338-2343.31-60.

[35] Kiselova, Y., Ivanova, D., Chervenkov, T., Gerova, D., Galunska, B. and Yankova, T. Correlation between the in vitro Antioxiant Activity and polyphenol content of aqueous extracts from Bulgarian herbs. Phytother. Res., 20, (2006).961-965.

[36] Chirinos R, Rogez H, Camposa D, Pedreschi R, Larondelle Y. Optimization of extraction conditions of antioxidant phenolic compounds from mashua (*Tropaeolum tuberosum* Ru´ız & Pav´on) tubers. *Separation and Purification Technology*; 2007. 55: 217–225.

How to cite this article:
Miri A, Rashki Ghalehnoo Z and Shaharaki E. Evaluation of antioxidant and antimicrobial activity of spergularia marina (L.) Griseb extract. J. Fundam. Appl. Sci., 2016, *8(2S), 501-517.*