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

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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.

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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 activities 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 activity.

**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 antibacterial 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 specimen was 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 crude 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).
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. The routine was used as a standard (Figure 2).

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

<table>
<thead>
<tr>
<th>Extract Name</th>
<th>Plant seed</th>
<th>Plant aerial part</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total methanolic</td>
<td>7/1</td>
<td>11/24</td>
</tr>
<tr>
<td>Chloroform</td>
<td>4/21</td>
<td>5/93</td>
</tr>
<tr>
<td>Water</td>
<td>2/84</td>
<td>5/28</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>-</td>
<td>0/027</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Alkaloid</th>
<th>Flavonoid</th>
<th>Saponins</th>
<th>Tannins</th>
</tr>
</thead>
<tbody>
<tr>
<td>++++</td>
<td>+++</td>
<td>+++</td>
<td>+++*</td>
</tr>
</tbody>
</table>

*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

<table>
<thead>
<tr>
<th>Ceftriaxone disk g(µ)</th>
<th>Gentamicin disk g(µ)</th>
<th>Amikacin disk g(µ)</th>
<th>Methanolic solution g(µ)/ml</th>
<th>Blank</th>
<th>Microorganisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>10</td>
<td>30</td>
<td>80</td>
<td>40</td>
<td>20</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>-</td>
<td>32</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>-</td>
<td>18</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>23</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>30</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>-</td>
<td>22</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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).

![DPPH](attachment://DPPH.png)

**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.

**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

<table>
<thead>
<tr>
<th>Samples</th>
<th>Plant seed</th>
<th>Plant aerial part</th>
<th>All parts of plant</th>
<th>IC50 (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin E</td>
<td>4/12±0/05</td>
<td>4/12±0/05</td>
<td>±0/01 4/12</td>
<td>4/12±0/05</td>
</tr>
<tr>
<td>BHA</td>
<td>8/57±0/07</td>
<td>8/57±0/07</td>
<td>0/08 8/57</td>
<td>8/57±0/07</td>
</tr>
<tr>
<td>Methanol extract</td>
<td>100/7±2/33</td>
<td>62/6±1/05</td>
<td>1/02 60/8</td>
<td>100/7±2/33</td>
</tr>
<tr>
<td>Athyl acetate phase</td>
<td>t°</td>
<td>277/03±2/76</td>
<td>2/44 272/3</td>
<td>t°</td>
</tr>
<tr>
<td>Water phase</td>
<td>143/6±1/24</td>
<td>192/9±0/98</td>
<td>181/3±1/95</td>
<td>143/6±1/24</td>
</tr>
</tbody>
</table>

*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 IC₅₀ 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 (R²) (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 IC₅₀).

The correlation coefficient between the total phenols of the methanolic extract of the aerial parts of plant and DPPH is 80 (R² = 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

<table>
<thead>
<tr>
<th>Plant seed</th>
<th>Plant part</th>
<th>Aerial part</th>
<th>All parts of plant</th>
<th>Type of extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>40/65</td>
<td>71/34</td>
<td>73/23</td>
<td>methanol</td>
<td></td>
</tr>
<tr>
<td>8/38</td>
<td>17/42</td>
<td>19/93</td>
<td>water</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>0/09</td>
<td>0/1</td>
<td>Athyl acetate</td>
<td></td>
</tr>
<tr>
<td>1/68</td>
<td>2/5</td>
<td>6/98</td>
<td>chloroform</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Plant seed</th>
<th>Plant part</th>
<th>Aerial part</th>
<th>All parts of plant</th>
<th>Type of extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>53/43</td>
<td>91/61</td>
<td>93/59</td>
<td>methanol</td>
<td></td>
</tr>
<tr>
<td>13/49</td>
<td>26/93</td>
<td>28/1</td>
<td>water</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>0/14</td>
<td>0/1</td>
<td>Athyl acetate</td>
<td></td>
</tr>
<tr>
<td>9/26</td>
<td>13/19</td>
<td>17/67</td>
<td>chloroform</td>
<td></td>
</tr>
</tbody>
</table>

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, ethyl acetate and chloroform of different part of *Spergularia marina* (L.) Griseb plant

<table>
<thead>
<tr>
<th>g dry plant powder /mg GA</th>
<th>Amount of total phenols</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant seed</td>
<td>Plant aerial</td>
</tr>
<tr>
<td>19/83±1/01**</td>
<td>35/47±0/18</td>
</tr>
<tr>
<td>5/16±0/03</td>
<td>8/86±0/09</td>
</tr>
<tr>
<td>trace</td>
<td>0/86±0/02</td>
</tr>
<tr>
<td>1/85±0/06</td>
<td>3/74±0/15</td>
</tr>
</tbody>
</table>

*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.

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