International Journal of Research in Pharmacy and Pharmaceutical Sciences
ISSN: 2455-698X; Impact Factor: RJIF 5.22
www.pharmacyjournal.in
Volume 1; Issue 3; July 2016; Page No. 36-38

Total Phenolic, Flavonoid Content and Radical Scavenging Activity of *Sambucus ebulus* L. from Iran

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Abstract
The total phenolic and flavonoids content and radical scavenging activity of ethanolic extract *Sambucus ebulus* herb that harvested from Ramsar heights (Javaherdeh), province of Mazandaran, Iran, were studied. This studied revealed that *Sambucus ebulus* ethanolic extract had 136 and 74.93 mg/l of phenolic a flavonoids in total, respectively. The radical scavenging activity was examined by $DPPH^{\cdot}$ method which shown the IC_{50} concentration of extract was acquired at 373.76 mg/ml.

Keywords: *Sambucus ebulus*, phenols, flavonoids, antioxidant activity

1. Introduction
Sambucus ebulus belong to Caprifoliaceous family, locally named as *Palem* or *Peilam* and grows wildly in the northern regions of Iran [1]. Flavonoids, steroids, tannins, glycosides, cardiac glycosides, caffeic acid derivatives, ebulitins and volatile substances of these species were previously reported [2]. This herb is used in Iranian folk medicine as remedy for some inflammatory cases such as, bee and nettle bites [3]. Aqueous extract from leaves of *Sambcus ebulus* were tested for antifeedant, growth regulation and ovicidal effects against the red floor beetle Tribolium confusum under laboratory condition. The results indicated a significant deterrence for feeding, and comparatively significant larval, pupal and adult weight decrease in treated vs. controlled insects [4]. In this study, the total phenolic and flavonoids content and radical scavenging activity as a symptom of anti-oxidant activity of *Sambucus ebulus* which grown in Javahrdeh (heights of Ramsar, Province of Mazandaran, Iran) are reported.

2. Material and methods
2.1 Chemicals and instruments
Rutin, aluminum chloride ($AlCl_3$), folin-ciocalteu, gallic acid were obtained from Merck (Germany) in analytical purity. 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$) was obtained from Sigma-Aldrich.
Varian Cary 300 UV/Vis spectrophotometer (USA) was used for all absorbance measurements.

2.2 Plant material
The aerial parts of *Sambucus ebulus* were collected from the Ramsar, Province of Mazandaran in December 2015. The plant material was identified in Forest and rangelands Institute of Iran. The plant tissues were washed with deionized water for removing dust and dried in shadow. The dried plant was powdered and kept in 4˚C.

2.3 Preparation of plant extract
Dried and powdered plant tissues were soxhlet extracted using ethanol for 48 h. the solution was filtered using No.1 wattman filter paper. The filtrate solution was stored at 4˚C until use.

2.4 Preparation of standard solution
0.5 of gallic acid was accurately weighed and dissolved in 10 ml ethanol and then diluted to 100 ml using deionized water. A series of 0, 50, 100, 150, 250 and 500 mg/l gallic acid were made by diluting of 0, 1, 2, 3, 5 and 10 ml of stock solution to 100 ml, respectively.
10 mg of rutin was dissolved in ethanol and diluted to 100 ml to making a 100 mg/l stock solution which use to obtain 5, 25, 50 and 100 mg/l standard solutions.

2.5 Determination of total phenolic content
Gallic acid was used as standard for analyzing of total content of phenolic compounds in *Sambucus ebulus* extract with folin-ciocalteu reagent. 50µl folin-ciocalteu reagent solution, 50 µl of 20% Na_2CO_3 solution was added to 10 µl of sample solution and diluted to 400 µl with deionized water. This solution was incubated in room temperature for 30 min. the absorbance of sample was measured in 765 nm with ethanol as blank. A calibration curve for gallic acid was drown at 765 nm using the standard solution after adding the reagent as describe above (figure 1) [5].

2.6 Determination of total flavonoid content
Determination of total flavonoid content was done using aluminum chloride method with a few changes in amount. For this purpose, 1 ml of sample solution was mixed with 1.5 ml 95% ethanol, 0.1 mi 10% $AlCl_3$, 0.1 ml NaOH and 28 ml deionized water. This solution was kept in dark place for 30 min and the absorbance was measured in 510 nm. The calibration curve was drown in 510 nm using the above procedure for rutin standard solutions (figure 2) [6].

2.7 Anti-Oxidant activity of *Sambucus ebulus* extract
Relatively stable organic radical $DPPH^{\cdot}$ has been widely used in the determination of the antioxidant activity of different plant extracts [7, 8]. This procedure is based on the reduction of $DPPH^{\cdot}$ solutions in the presence of plant extract. $DPPH^{\cdot}$ solutions show a strong absorption band at 517 nm appearing a deep violet color.
The ability of *Sambucus ebulus* extract to quench reactive species by hydrogen (H^+ ions) donation was measured

through DPPH radical scavenging activity assay. Activity was measured as relative decrease absorbance at 517nm as reaction between DPPH˙ and extract. Antioxidant activity was evaluated with %50 (IC_{50}). Briefly, a 2 ml of 0.1mM DPPH˙ methanol solution with 2 ml sample with 1, 5, 10, 50, 100, 200, 400, 600, 800 and 1000 mg/l concentration with shaking. After the solution was incubated for 30 min at 25˚ C in dark, the decrease in the absorbance at 517nm was measured. Control contained methanol instead of sample solution. The radical scavenging activity percentage (%RSA) was calculated by the blow equation.

%RSA= [(OD DPPH- OD sample)/OD DPPH] ×100

Results and Discussion

2.8 Total phenolic content

The table 1 and figure 1 show the absorbance amount of standards and calibration curve for determination of phenolic content in *Sambucus ebulus* extract after triple measurements.

Table 1: Concentration of standard gallic acid and their absorbance at 765 nm

Standard concentration (mg/l)	Absorbance
0	0.001
50	0.05
100	0.15
150	0.18
250	0.28
500	0.52

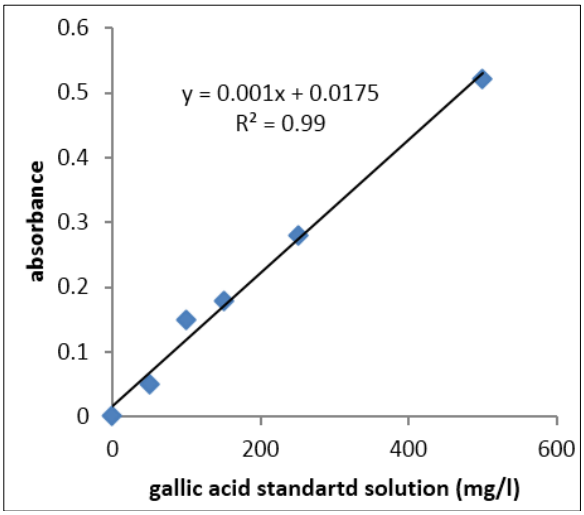

Fig 1: Calibration curve for standard gallic acid solution

Phenolic compounds are considered as one of classes which show the anti-oxidant activity of plant extracts. The amount of total phenolic constituents was determined using folin-ciocalteu method. Total phenolic content was expressed as gallic acid using the standard curve equation: Y=0.001X + 0.0175 (R^2=0.99) where Y is absorbance at 765 nm and X is total phenolic content. The total content of *Sambucus ebulus* was calculated 136 mg/l equivalent to absorbance 0.1535 for triple measurement.

2.9 Total flavonoid content

Table 2: Concentration of standard rutin and their absorbance at 510nm

Standard concentration (mg/l)	Absorbance
5	0.051
25	0.3552
50	0.6071
100	1.1163

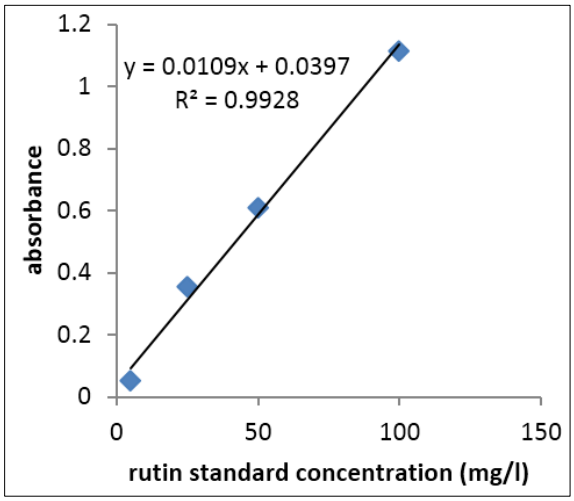

Fig 1: Calibration curve for standard rutin solution

Another anti-oxidant active substrates are flavonoids. The total content of flavonoids in *Sambucus ebulus* was calculated using the calibration curve equation (Y= 0.0109X +0.0397, with R^2 = 0.9928) after three time absorbance measurement as 74.93 mg/ml for mean absorbance of 0.8564

2.10 DPPH radical scavenging activity

DPPH˙ radical-scavenging activity percentage (%RSA) was reported in Table 3. Usually, higher total phenolic and flavonoids contents lead to better DPPH-scavenging activity [9, 10, and 11].

The values of DPPH˙ %RSA were presented in table 3 and the relation of extract concentration and %RSA was presented in figure 3.

Table 3: %RSA and absorbance of different concentration of *Sambucus ebulus* extract

Extract concentration (mg/ml)	absorbance	%RSA
DPPH˙	1.20	-
1	0.93	22.50
5	0.92	23.33
10	0.91	24.16
50	0.80	33.33
100	0.81	32.5
200	0.76	36.66
400	0.61	49.16
600	0.47	60.83
800	0.26	78.33
1000	0.01	99.16

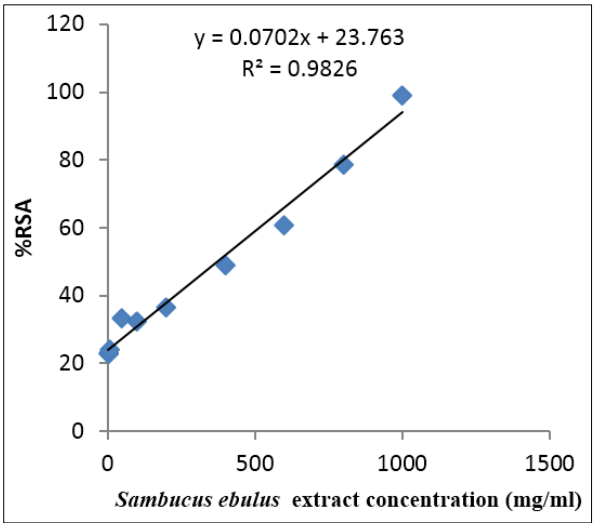

Fig 3: Relation of *Sambucus ebulus* extracts concentration and %RSA

As shown in figure 3, the IC_{50} concentration could be calculated using equation of curve (Y=0.0702x + 23.763) by replacing the amount of 50 instead of Y. *Sambucus ebulus* with high level of phenolic contents and highest amount of flavonoids showed the IC50 at 373.76 mg/ml.

3. Acknowledgement

Authors are grateful to Mr. Nima Mohammadi and Mrs. Nikoo ostovar (Science and Research Branch, Islamic Azad University) for their help in achieving UV-Vis spectrophotometer measurements and Dr. valliolah Mozaffarian (in Forest and rangelands Institute of Iran) in plant identification

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