Research Article

Antioxidant Activity and Total Phenolic, Flavonoid Content of *Sarothamnus scoparius* L. Extracts Selected from Three Regions of Syria.

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Accepted on: 23-02-2016; **Finalized on:** 31-03-2016.

ABSTRACT

The objective of this study was to estimate antioxidant activity, total phenolic and flavonoid content of methanolic, chloroform and cyclohexane extracts of *Sarothamnus scoparius* L. arial parts selected from three regions of Syria (Jubata Al-Khashab, Al-Samraa and Al-Ghab). The antioxidant activity were measured by DPPH(2,2Diphenyl-1-Picrylhydrazyl) free radical scavenging assay, Total phenolic content was measured by Folin Ciocalteu reagent method and total flavonoid content was estimated by aluminum chloride colorimetric method. The results showed that all extracts have an important antioxidant activity and contained good levels of phenolic and flavonoid content. Methanol was the most effective solvent in this study. The Methanolic plant extract of Jubata Al-Khashab region presented the best antioxidant activity (IC_{50}=49.21 ± 0.58), and also showed the greatest level of phenolic (54.52 ± 0.80 mg GAE/g dry extract) and flavonoid (31.40 ± 0.16 mg QE pre g) content. From the results of the present study it is concluded that *S.scoparius* is a potential source of natural antioxidants and has a good antioxidant activity.

Keywords: *Sarothamnus scoparius* L., Antioxidant activity, Phenols, Flavonoids, DPPH assay, Scavenging activity.

INTRODUCTION

Oxidative stress is implicated in the pathogenesis of many chronic diseases such as cancer, arteriosclerosis, diabetes, gastritis, liver injury, cardiovascular diseases[1]. The oxidative stress is an imbalance between the production of free radicals and antioxidant potentiality therefore, antioxidants especially of natural sources have become the topic of interest in the past few years to protect the human body from dangerous disease caused by free radicals activity[2,3]. A great number of naturally occurring substances in several plants have been recognized to have potential antioxidant activity such as Phenols, Falavonoids and other active compounds[4]. Phenols and Flavonoids are capable effectively in scavenging the free radicals because of their phenolic hydroxyl groups[5].

Sarothamnus scoparius Linn. Belongs to the family Fabaceae, it is commonly known as *Scotch broom*, it is native to central southern Europe[6]. *S.scoparius* is claimed to have number of medicinal properties such as diuretic[7], antidiabetic, hepatoprotective[8], lithotriptic, hypnotic and sedative properties[9].

Chemical analysis of *S.scoparius* revealed the presence of flavones, flavonols, isoflavones, carotenoids and alkaloids[10,11]. It is presently known that Phenolic content and flavonoids derivatives including flavones, flavonols, isoflavones are strongly contribute for the antioxidant activities[12].

This study is aimed to evaluate the antioxidant activity and to estimate the total Phenolic and Flavonoid content of *S.scoparius* extracts, selected from three regions of Syria.

MATERIALS AND METHODS

Chemicals and Reagents

2,2-Diphenyl-1-Picrylhydrazyl (DPPH), Folin Ciocatteu Reagent (FCR), Gallic acid, Quercetrine, Aluminium chloride and potassium acetate were purchased from Sigma-Aldrish (St.louis USA). All chemicals used of analytical grade.

Plants Materials

A fresh *S.scoparius* samples were collected from three different regions in Syria (Jobata Al-khashab, Al-Ghab and Al-Samra), during May 2011 and were taxonomically identified and authenticated by Dr. Alkadi Imad, Department of Botany, Faculty of Science, Damascus University, Syria.

Extracts Preparation[13]

An aerial parts of *S.scoparius* were cleaned, dried under the shade and coarsely powdered, the powdered plant was extracted by solvents, taking from polar to non-polar solvents (methanol, Chloroform and Cyclohexane), the powdered samples was mixed with each solvent and stored at room temperature for 48 hours, each extract was concentrated in vacuo at 45° C then stored at 4° C for further use.

DPPH Free Radical Scavenging Assay[14,15]

The DPPH radical scavenging activity of plant extracts was determined by measuring the decrease in absorbance of methanolic solution of DPPH by applying the Modified Method of (Blois 1958).

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DPPH radical is purple in color and after reaction with hydrogen donor changes to yellow color. The hydrogen atoms and the electrons donation ability of extracts were measured by the bleaching of Purple-colored methanol solution of DPPH. 1ml of 0.55 µg/ml DPPH was added to 1ml of each different concentration of extracts and standard (ascorbic acid) (5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 µg/ml). The mixture was incubated at 37°C in a dark chamber for 30 min, and the absorbance of each concentration was measured at 517 nm, with spectrophotometer (SECOMAM-70ST0191), against a blank which consists of mixture of methanol and extract. The percentage inhibition of DPPH free radicals by the sample was calculated as:

$$\%\ Inhibition = \frac{A_{blank} - A_{sample}}{A_{blank}}$$

Antioxidant activity is measured by IC_{50} values, The lower the IC_{50} value reflects high antioxidant potential.

IC_{50} was obtained by linear regression analysis of curve plotting between different concentration and inhibition%.

Total Phenolic Content[16,17]

The total phenolic content was determined using FCR (Folin Ciocalteu reagent) method. The reaction mixture contained 100 µL of methanolic solution (1 mg/mL) of extract, 0.5 ml of FCR, 1.5 ml of 20% (w/v) sodium carbonate and 10 ml of distilled water. After 2h of reaction at ambient temperature, the absorbance was measured at 765 nm and used to calculate the phenolic content, using gallic acid as a standard. Then the total phenolic content was expressed as gallic acid equivalents (mg GAE/g dry extract).

Total Flavonoids Content[18]

Aluminum chloride colorimetric method was used to measure the total flavonoids content of each extract (methanol, chloroform and cyclohexane) of *S.scoparius*, 1ml of each plant extract was mixed separately with 3ml of methanol, 0.2 ml of 10% aluminum chloride, 0.2 ml of 1M potassium acetate and 5.6 ml of distilled water, after incubation for (30 min). The absorbance was read at 415 nm and the flavonoids contents was expressed in µg quercetrine equivalent (QE) per mg of dry extract.

Statistical Analysis

The measurements of total phenols, flavonoids and DPPH assay, were carried out for three replicates. All the results were expressed as mean ± SD (Standard Deviation). Linear regression analysis was used to calculate the IC_{50} values.

RESULTS AND DISCUSSION

DPPH Free Radical Scavenging Assay

In this study, methanol, chloroform and cyclohexane extracts of *S.scoparius* were investigated for their antioxidant activity using DPPH scavenging assay, DPPH is a stable free radical, it accepts an hydrogen radical to become a stable molecule[19], the reduction activity of DPPH radicals was measured by the decrease in its absorbance at 425 nm, which is caused by antioxidants[20].

IC_{50} values of DPPH radical scavenging assay are reported in Table 1, and the graphs of this assay of different extracts and regions are presented in Figures 1,2 and 3.

Results showed an important antioxidant power of *S.scoparius* extracts compared to the standard (ascorbic acid). The most effective solvent is methanol then chloroform then cyclohexane as an organic solvents.

The results showed that antioxidant activity of methanolic extract of Jubata Al-khashab region was superior to all samples tested, with an IC_{50} value of 49.2 µg/ml. and it is followed by that of Al-Samra (IC_{50} = 55.1 µg/ml), then Al-Ghab region (IC_{50} = 58 µg/ml).

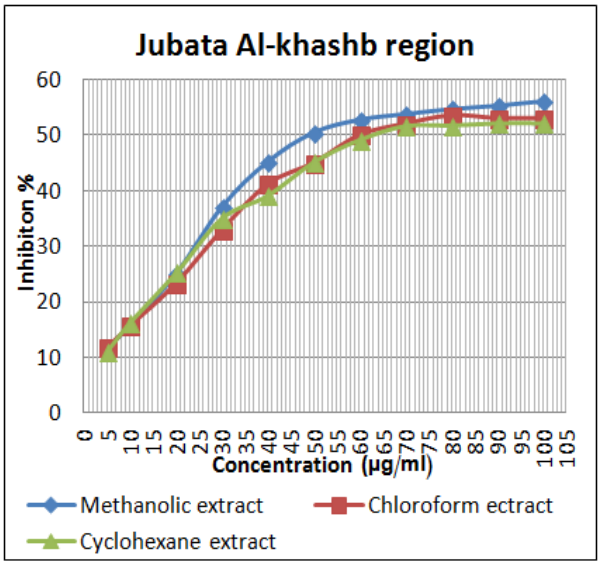

Figure 1: DPPH free radical scavenging assay of methanolic, chloroform and cyclohexane extracts of *S.scoparius* selected from Jubata Al-Khashab.

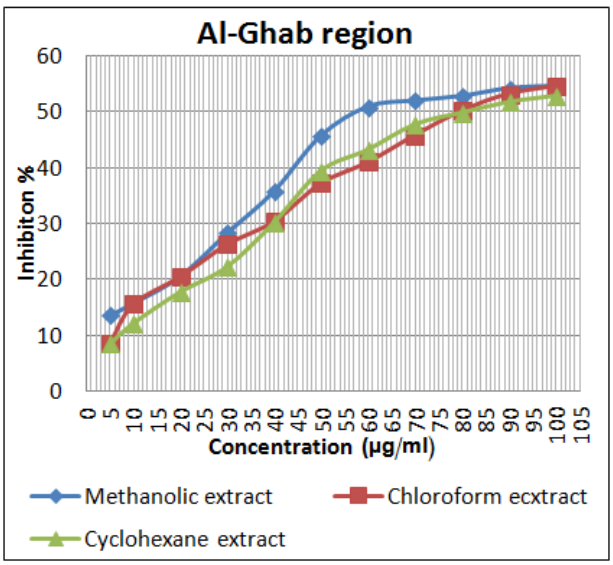

Figure 2: DPPH free radical scavenging assay of methanolic, chloroform and cyclohexane extracts of *S.scoparius* selected from Al-Ghab.

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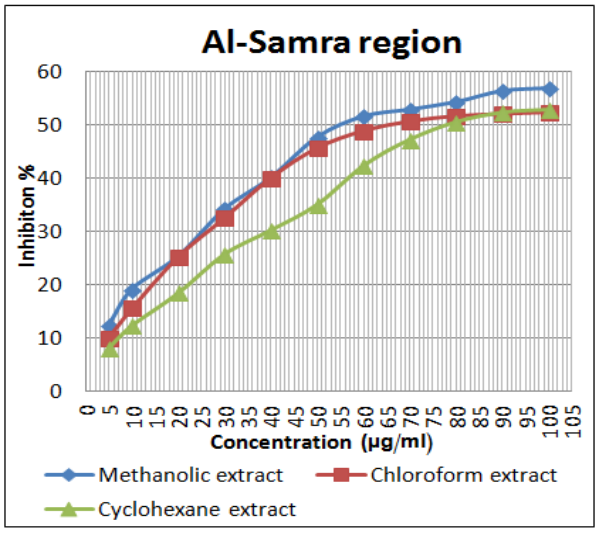

Figure 3: DPPH free radical scavenging assay of methanolic, chloroform and cyclohexane extracts of *S.scoparius* selected from Al-Samra.

Table1: IC_{50} values of *S.scoparius* extracts according to DPPH assay.

Region	Solvent	IC_{50} (µg/ml)
Jubata Al-Khashab	A	49.21 ± 0.58
	B	52.00 ± 0.35
	C	57.30 ± 0.23
Al-Samra	A	55.17 ± 0.84
	B	65.24 ± 0.17
	C	78.61 ± 0.28
Al-Ghab	A	58.10 ± 0.27
	B	79.39 ± 0.05
	C	81.22 ± 0.15

A:methanolic extract, B:chloroform extract, C: cyclohexane extract.

Total Phenolic Content

FCR (Folin Ciocalteu reagent) method was used for the determination of total Phenolic content of *S.scoparius* extracts. Total Phenol contents was expressed as gallic acid equivalent per gram dry weight of extract. The most effective solvent was Methanol. The highest level of total phenolic content was found in methanolic extract of Jubata Al-Khashab region (54.52 ± 0.80 mg GAE/g extract), then that of Al-Samra (54.12 ± 0.13 mg GAE/g extract), then Al-Ghab region (53.20+ 0.56 mg GAE/g extract), Table 2.

Total Flavonoids Contents

Aluminum chloride Cholorimetric method was used for the determination of flavonoids of *S.scoparius* extracts, Total Flavonoid contents was calculated as Quercetrin curve, and was expressed as Quercetrin Equivalent per gram dry weight of extract, the results were similar to phenols, we found the greatest total flavonoid content in methanolic extract of Jubata Al-Khashab region (31.40 ± 0.16 mg QE/g extract), then that of Al-Samra (29.78 ± 0.28 mg QE/g extract), then Al-Ghab region (29.20+ 0.54 mg QE/g extract), Table 2.

Table2: Total Flavonoid and Phenolic content of *S.scoparius* extracts.

Region	Solvent	mg GAE per g of dry extract	mg QE per g of dry extract
Jubata Al-Khashab	A	54.52 ± 0.80	31.40 ± 0.16
	B	54.13 ± 0.63	31.25 ± 0.07
	C	50.73 ± 0.20	30.84 ± 0.92
Al-Samra	A	54.12 ± 0.13	29.78 ± 0.28
	B	53.87 ± 1.06	28.66 ± 0.11
	C	49.24 ± 1.10	26.32 ± 1.15
Al-Ghab	A	53.20 ± 0.56	29.20 ± 0.45
	B	52.91 ± 0.41	28.96 ± 0.71
	C	47.43 ± 0.68	24.90 ± 0.85

A:methanolic extract, B:chloroform extract, C: cyclohexane extract.

CONCLUSION

S.scoparius exhibits good antioxidant activity, and good levels of total phenolic and flavonoid content.

Comparing the results of the three studied regions we found that antioxidant activity and total phenolic and flavonoid contents presented the descending order: *S.scoparius* extracts of Jubata Al-Khashab region followed by the extracts of Al-Samra, then Al-Ghab region. Methanol was the most effective solvent in this study followed by chloroform then cyclohexane.

From these results it is concluded that The plant *S.scoparius* is a good source of natural antioxidants and that might have benefits to health by reducing the free radicals levels in the body therefore, protecting the body from oxidative stress harm effects, However further studies and investigations are required to study the *in vivo* efficacy of these extracts, and to isolate and purify the active antioxidant compounds of it.

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Source of Support: Nil, **Conflict of Interest:** None.

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