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Research Article

Comparison of the Total Phenol, Flavonoid Contents and Antioxidant Activity of Methanolic Roots Extracts of *Asphodelus microcarpus* and *Asphodeline lutea* Growing in Syria

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ABSTRACT

Medicinal plants are a source for a wide variety of natural antioxidants. In the study reported here, we have conducted a comparative study between two medicinal plants roots having the same geographic origin and growing in the same natural conditions. The present study is designed to evaluate the radical scavenging activity, total phenol content (TPC) and total flavonoid content (TFC) of the MeOH roots extracts of *Asphodelus microcarpus* and *Asphodeline lutea*. The antioxidant activity of the extracts was examined by DPPH method. Total phenol and total flavonoid quantities of the samples were determined spectrophotometrically using Folin-Ciocalteu and $AlCl_3$ reagents respectively. The roots in both plants exhibited the free radical scavenging property (IC_{50}= 0.30, 0.54 mg/ml) for *A. microcarpus* and *A. lutea*, respectively compared to that of the positive control BHT (IC_{50}= 0.017 mg/ml). As assumed, the amount of total phenolics was (17.90, 13.02 mg GAE/ g dry weight for *A. microcarpus* and *A. lutea* root extracts, respectively. Roots extract of *Asphodelus microcarpus* were the richest in total phenolic compounds and also it has been found to be rich in flavonoids (14.60 mg rutinoside/g dry weight). To our knowledge, this is the first report on the antioxidant activity of *A. microcarpus* and *A. lutea* root from Syrian origin and our findings suggest the possibility of using the roots as a novel source of natural antioxidant agent for the food and pharmaceutical industries.

Keywords: *Asphodelus microcarpus*, *Asphodeline lutae*, antioxidant activity, DPPH, Phenolic content; Flavonoids content.

INTRODUCTION

In developing countries, traditional medicine holds a great promise as a source of readily available effective and safe drugs to the people[1]. Phytochemical components in medicinal plants are of great importance in the manufacture of such drugs[2] and the affectivity of many available drugs is studied by many workers to test folklore medicinal plants for several pharmacological activities[3]. Epidemiological studies have shown that a diet rich in fruits and vegetables is associated with a decreased risk of cardiovascular diseases and certain cancers. These beneficial health effects have been attributed in part to the presence of phenolic compounds in dietary plants, which may exert their effects as a result of their antioxidant properties[4]. Free radicals and other reactive oxygen species are produced in the human body during various physiological and biochemical processes. Increase production of such free radicals can cause oxidative damage to biomolecules (e.g. lipids, proteins, DNA), eventually leading to many chronic diseases, such as cardiovascular diseases, cancer, atherosclerosis, diabetes, aging, and other degenerative diseases in humans[5]. Examples of these radicals include superoxide anions, hydroxyl, nitric oxide and hydrogen peroxide radicals. These radicals can be scavenged by the protective role of natural and synthetic antioxidant agents. Meanwhile, the ingestion of several synthetic antioxidants as has been reported toxic to man[6]. Therefore, naturally occurring nutritive and non-nutritive antioxidants have recently become a major area of scientific research due to safety and limitation of synthetic antioxidant usage[7,8]. Recently, many researchers have taken great natural antioxidants, especially phenolics and flavonoids, are safe; they protect the human body from free radicals and retard the progress of many chronic diseases as well as lipid oxidative rancidity in foods[9]. Numerous studies were carried out on plants with antioxidant properties[10-11]. However, there is still great interest in finding new antioxidants from natural sources.

Asphodelus microcarpus Salzm. et Vivi (*Asphodelaceae*) is a stout robust herb with roots of several spindle-shaped tubers, widely distributed over the coastal Mediterranean region[12]. It has been reported in the ethnobotanical literature for use as a diuretic, for otitis and toothache in Algeria[13], a wild food source in the Medieval Levant[14], and a skin emollient, lenitive and treatment for lung diseases in Sardinia[15]. Antioxidant and anticancer propertied were also reported[16]. Flower of *A microcarpus* used as emollient, lenitive and for lung diseases[17]. Lipids, carbohydrates, sterols, triterpenes, anthraquinones and arylcoumarins have been isolated from *A. microcarpus*[18]. The genus *Asphodeline* belongs to the *Asphodelaceae*

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family (until recently included in the family *Liliaceae*) and comprises of 14 species worldwide. It has fleshy roots and fragrant, starry flowers that are yellow in May to June. It grows up to 4 ft in well-drained soil. Its foliage is blue-green and grassy, with tall, narrow flower spikes[19]. Several *Asphodeline* species are consumed in salads; others have significant applications in traditional medicine[20]. The leaves of *Asphodeline* represent a good source of proteins of good nutritional value in addition to functional compounds, such as polyphenols and dietary fibre[21].

Asphodeline lutea (L.) Rchb. is a wild plant traditionally as food. The ancient Greeks roasted the roots like potatoes and ate them with salt and oil or mashed them with figs. In folkloric medicine, it used as an antispasmodic and diuretic[22]. Antioxidant properties of that plant were also reported, but its benefits of other medicinal properties are not well studied[23]. Syria is known for its wealth of plant species with medicinal properties, which have been used since early times. In fact, more than 3500 species belonging to 131 families have been found in Syria, hundreds of which may have medicinal and therapeutic significance. Throughout ancient times in Syria, as part of Bilad Alsham, and other lands in the region, humans used various natural materials as sources of medicines[24-27]. However, the subject of "comparison of the total phenol, flavonoid contents and antioxidant activity of methanolic roots extracts of *Asphodelus microcarpus* and *Asphodeline lutea* growing in syria" has received little attention in the literatures, and very little is known about these plants. Thus, to date, no articles devoted to them in Syria have been published. For these reasons, the objectives of this study were to investigate and comparison of the free radical scavenging activity of *A. microcarpus and A. lutea* methanolic *roots* extracts *growing in* Syria, because of the important roles of the total phenolics and total flavonoids as antioxidants, the amounts of total phenolics and flavonoids in the extracts were also determined.

MATERIALS AND METHODS

Chemicals

Methanol GR (Eurolab,UK), Gallic acid (Prolab,Spain), Folin-ciocalteu (Sohariab SL, Spain), anhydrous sodium carbonate (Pareac quimica sau medien, Spain), Methanol GR (Eurolab, UK), Folin-ciocalteu phenol reagent (Sigma-Aldrich, Switzerland), Sodium Carbonate anhydrous (PAREAC QUIMICA SAU, Spain), Gallic acid (Titan biotech LTD., India), Rutin (Extrasynthese Genay, France), Aluminum Chloride Hexahydrate (Scharalau Chemie, Spain), DPPH and BHT (Sigma-Aldrich, USA). Distilled deionized water (dd. H_2O. All other chemicals unless and otherwise mentioned were obtained from Research Laboratories in department of pharmacognosy, Faculty of Pharmacy, University of Aleppo, Syria

Plant Materials

Asphodelus and *Asphodeline* species were collected at flowering stage (May to July) from Maarrat al-Nu'man (Idlib governorate, Syria). Identification of plants was confirmed by Dr. Ahmad Jaddouh (faculty of Agriculture Engineering, Aleppo University, Syria). Roots were separated, washed and dried at room temperature. The dried parts were put in a plastic bags and stored until used.

Preparation of the methanolic extracts

To produce methanolic roots extracts, the air-dried samples (20 g) of the roots of *A. microcarpus* and *A. lutea* were extracted with 250 mL of methanol using by ultrasound-assisted extraction. The extracts concentrated under vacuum at 40°C by using a rotary evaporator, then they were stored at $^{+}4$◦C in dark until use. The extraction yields were (28.5%, 23.0%) for *A. microcarpus* and *A. lutea* respectively.

Determination of total phenol content (TPC)

Total contents of the phenolic compounds in the methanolic extracts were determined by the modified Folin-Ciocalteau assay[28]. Methanolic solution of the extract in the concentration of 1 mg/ml was used in the analysis. The reaction mixture was prepared by mixing 0.5 ml of methanolic solution of extract, 2.5 ml of 10% Folin-Ciocalteau's reagent dissolved in water and 2.5 ml 7.5% $NaHCO_3$. Blank was concomitantly prepared, containing 0.5 ml methanol. The samples were thereafter incubated in a thermostat at 45ºC for 45 min. The absorbance was determined using spectrophotometer at $^{\lambda}$max = 765 nm. The same procedure was repeated for the standard solution of gallic acid and the calibration line was constructed. Based on the measured absorbance, the concentration of phenolic was read (mg/ml) from the calibration line. Total phenolic contents in medicinal plants were expressed as mg gallic acid equivalents (GAE) per gram of dry weight of plant (mg GAE/g DW).

Determination Total Flavonoid Content (TFC)

The flavonoid content of the methanolic extracts was determined using a modified aluminum chloride colorimetric method[28], and used rutinoside as a standard. Formation of acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols in addition with aluminium chloride, which also forms acid labile complexes with the ortho-dihydroxyl groups in the A- or B-ring of flavonoids[29]. Various concentrations of standard rutin solution were used to make a standard calibration curve. The sample contained 1 ml of methanol solution of the extract in the concentration of 1 mg/ml and 1 ml of 2% $AlCl_3$ solution dissolved in methanol. After incubation of the samples at room temperature for 30 minutes, the absorbance of the samples was read at 415 nm against blank and the total flavonoid content was expressed as rutinoside equivalent per gram of plant's dry weight (mg RUE/g DW).

Antioxidant activity

DPPH radical scavenging activity

Quantitative measurements of radical scavenging assay were carried out according to the method described by Adedapo *et al*[30]. A solution of 0.135 mM DPPH in methanol was prepared and 1.0 ml of this solution was mixed with 1.0 ml of extract in methanol containing 0.02–0.1 mg of the extract. The reaction mixture left in the dark at room temperature for 30 min. The reduction of the DPPH free radical was measured by reading the absorbance at 517 nm by a spectrophotometer. The IC 50 value of the sample, which is the concentration of sample

required to inhibit 50% of the DPPH free radical, was calculated. Lower absorbance of the reaction mixture indicated higher free radical activity[31]. BHT was used as a standard and the same concentrations were prepared as the test solutions. Inhibition of DPPH free radical in percentage was calculated by the formula:

Percentage (%) of DPPH radical scavenging = $[(A_0 - A_1) /A_0 \times 100)$, where A_0 was the absorbance of the control, A1 was the absorbance of the plant species or standards.

RESULTS AND DISCUSSION

Substantial evidence has accumulated and indicated key roles for ROS and other oxidants in causing numerous disorders and diseases. As previously highlighted, the evidence has brought the attention of scientists to an appreciation of antioxidants for prevention and treatment of diseases and maintenance of human health[32-33].

Total Phenolic Content

Phenolics or polyphenols are secondary plant metabolites that are most commonly present in plants of high medicinal value. Phenolic compounds contribute to the antioxidant potential of plants by neutralizing free radicals and preventing decomposition of hydroperoxides into free radicals[34]. Hence, it is important to quantify phenolic derivatives and to assess its contribution to antioxidant activity. The levels of total phenols in the examined plant roots extracts reacted with the Folin-Ciocalteu's reagent were calculated according to the equation of calibration curve for gallic acid (Figure 1), ($y = 6.6104x + 0.0108$), $R_2 = 0.9939$). The total phenols were expressed as mg/g gallic acid equivalent. According to the results reported in Table 1, it was found that TPC were 17.90, 13.02 mg GAE/ g DW for *A. microcarpus* and *A. lutea* roots extracts, respectively. Root extract of *A. microcarpus* was the richest in total phenolic compounds. Comparatively, lower total phenolic content, was reported in the same species from other origins[35,36]. The difference in amounts of phenols is probably related to geographical and inviromental factors, processing methods and other intrinsic factors (genetic, extracting solvent) and extrinsic (environmental, handling and development stage)[37].which may play role in such a large variation. Also, the phenol content of a plant depends on a number of As well as the Folin-Ciocalteu's assay gives a crude estimate of the total phenolic compounds present in an extract/fraction. It is not specific to polyphenols, but many interfering compounds may react with the reagent, giving elevated apparent phenolic concentrations[38]. Moreover, various phenolic compounds respond differently in this assay, depending on the number of phenolic groups they have. TPC does not incorporate necessarily all.

Total flavonoids Content

Flavonoids are the most common and widely distributed group of plant phenolic compounds, characterized by a benzo-γ-pyrone structure. It is ubiquitous in fruits and vegetables[39]. Flavonoids are responsible for the radical scavenging effects of most medicinal plants through scavenging or chelating process *in vivo* as well as *in vitro*[40]. In this study, the concentrations of flavonoids was determined using aluminum chloride colorimetric method and calculated according to the equation of calibration curve for rutin (Figure 2), ($Y= 1.2537x+0.0134$, $R2= 0.9952$). The results reported in Table 1 showed (14.69, 7.63 mg RUE/g DW) for roots extracts of *A. microcarpus* and *A. lutea* respectively. Total flavonoid content was again found to be maximum in roots of *A microcarpus*. Also the results of this study are higher than reported in previous studies for the of *Asphodelus* and *Asphodeline* species[37,38]. Our results indicate that the higher antioxidant activity of the roots extract of the *A. microcarpus* higher than roots extract of *A. lutea* may be in correlation with the phenolic and flavonoid contents of the extracts.

Free-Radical-Scavenging Activity

The free radical scavenging activities of methanolic roots extracts of selected plants were evaluated through their ability to quench the synthetic DPPH radical and their activities were compared with that of the BHT (Figure 3), a standard compound. DPPH method has been used to examine antioxidative activity in complex biological systems because this assay is sensitive, requiring only small amount of samples and allows testing of both lipophilic and hydrophobic substances[41]. the results reported in Table 2 showed that roots of *A. microcarpus* displayed the higher total antioxidant activity with (IC50 =0.30 mg/ml) and comparing with *A. lutea* (IC50= 0.54 mg/ml. the DPPH radical scavenging activity of these extracts was lower than that BHT ((IC_{50}= 0.017 mg/ml). In this study, it is noted that the higher radical scavenging activities could be enlightened with the presence of higher phenols and flavonoids level in studied species, as in root extract of *A. microcarpus*, may be a reason for its higher DPPH-scavenging activity than that of *A. lutea*.

These results are in good accordance with literature, which showed that there is a positive correlation between phenolics content and radical-scavenging activity[42]. Accordingly can be regarded phenolics and flavonoids could be considered ones of the main responsible for the hydrogen donating properties of tested extracts. In addition to that polyphenols can contribute as metal ion chelators due to the presence of various hydroxyl groups. In addition to 3`,4`-dihydroxymoiety in the B ring, some flavonoids (flavonols) also have C-3 and C-5 OH groups and the 4-carbonyl group that can chelate metalions[43]. The literature data suggest that the radical scavenging activity of phenolic compounds is largely influenced by the number of hydroxyl groups on the aromatic ring, i.e. the bigger the number of hydroxyl groups, the larger the radical scavenging activity[44]. the root of *A. lutae* contain flavonoids such as catechin with two adjacent hydroxyl groups on phenolic ring B displays the highest antioxidant activity in a lipoprotein oxidation model. Also, Phenolic acids (hydroxycinnamic,hydroxybezoic acids) with the exception of *p*-coumaric acid, which does not possess an *o*-dihydroxy group, are good antioxidants. Caffeic acid with two adjacent hydroxyl groups on one ring exhibit lower antioxidant activity as compared to catechin[45]. On the other hand *A. microcarpus* root extract showed antioxidant activity, which could be attributed mainly to its high levels of total polyphenols and flavonoids. It is well known that polyphenols, and namely flavonoids, behave as

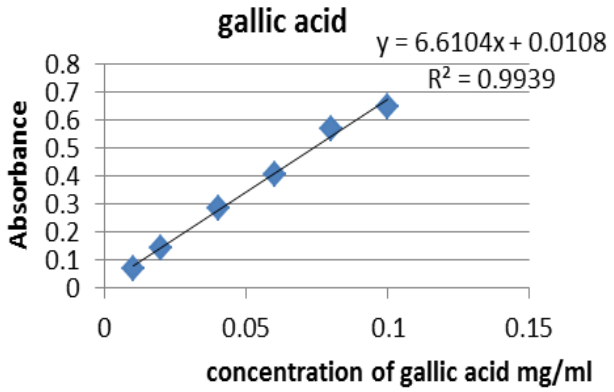

Figure 1: The calibration line for gallic acid.

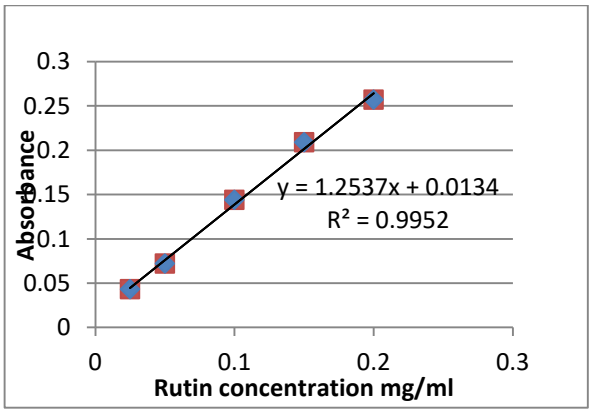

Figure 2: Calibration curve of Rutin.

Table 1: Yield extract, phenol and flavonoid content in roots extracts from *A.microcarpus* and *A. lutea*.

Root extract	Yield extract (%)	TPC (mg GAE g/ g of DW)	TFC (mg RUE/g of DW)
A. microcarpus	28.5%	17.90	14.69
A. lutea	23.0%	13.02	7.63

Table 2: IC50 value of roots extracts and positive control.

Plant	IC50 mg/ml
A. microcarpus	0.30
A. lutea	0.54
BHT	0.017

inhibitors of ROS generation and could be responsible for the antimelanogenic activity of plant extracts[35]. The major compound in the extract was the aglycone luteolin, a compound that in a previous study was reported to show whitening activity[35]. However, the polyphenolic compounds like flavonoids, tannins, phenolic acids anthraquinones and naphthalene derivatives in roots of *A. microcarpus* and *A. lutea*, contain various biological effects including antioxidant, and enzyme Inhibitory and antibacterial activities[18,23,42,46]. Furthermore, a dietary intake of phenolics has been associated with reduced risk

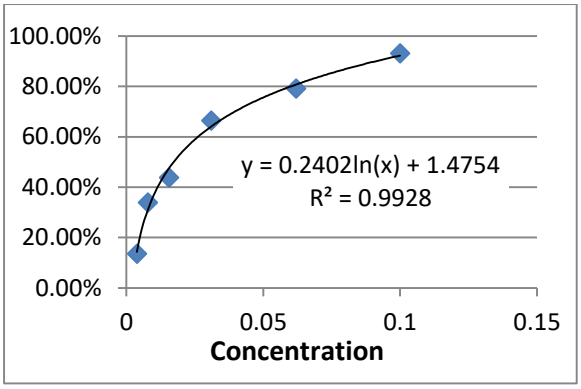

Figure 3: Scavenging activity of the standard BHT

of different diseases, such as cancer, cardiovascular disease, diabetes, or atherosclerosis, probably due to their potent antioxidant properties[47]. Thus, the estimated antioxidant activity of roots of plants under investigation can contribute to the benefits of this species.

CONCLUSION

In this work, the phenol and flavonoid contents of two Syrian medicinal plants and their related antioxidant activities are evaluated for the first time of this region, *A. microcarpus* and *A.lutea*. These species exhibited noticeable antioxidant activities, thus representing promising sources of plant based medicine. *A. microcarpus* root extract revealed higher levels of antioxidants and better antioxidant potential as compared with the other species. This investigation highlights *Asphodellaceae* species as a new source of bioactive compounds, functional food and their possible application as antioxidant agents. However, further studies are needed for understanding in vivo activities of *A. microcarpus* and *A. lutea.*

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