

Anti-oxidant Activity of Methanol Extracts of *Arum maculatum* L. and *Physalis peruviana* L. Plants

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Abstract

Total flavonoid contents, reductive ability and radical scavenging activity were studied in the methanol extracts of *Arum maculatum* L. and *Physalis peruviana* L. The results revealed that *A. maculatum* extract had total flavonoids of $535.3 \pm 109.9 \mu\text{g/ml}$, which was significantly higher than the recorded flavonoids in *P. peruviana* extract ($352.0 \pm 12.7 \mu\text{g/ml}$). Assessment of reductive ability revealed that both extract were effective in such activity and concentration-dependent. The highest absorbance was found at the concentration 0.64 mg/ml of *A. maculatum* methanol extract (0.929 ± 0.006), which was significantly higher ($P \leq 0.05$) than the corresponding concentration of *P. peruviana* extract (0.850 ± 0.050) or trolox (0.278 ± 0.010), but the second extract also showed a significant increased absorbance compared to trolox. The results of DPPH radical scavenging activity confirmed those of reductive ability, and again the highest concentration (0.500 mg/ml) of both extracts (*A. maculatum* and *P. peruviana*) recorded the best radical scavenging activity (93.33 ± 0.58 and $95.33 \pm 2.52\%$, respectively), which was significantly higher than that of vitamin C ($64.67 \pm 5.03\%$). In conclusion, both extracts can be considered as important medicinal plants that have the potential of anti-oxidant and free-radical scavenging activities.

Keywords: *Arum maculatum* , *Physalis peruviana* , Flavonoids , Anti-oxidant.

Introduction

The human body has a complex system of natural enzymatic and non-enzymatic anti-oxidant defenses, which counteract the harmful effects of free radicals and other oxidants. Free radicals are responsible for causing a large number of diseases including cancer, cardiovascular disease, neural disorders, Alzheimer's disease, mild cognitive impairment, Parkinson's disease, aging and atherosclerosis [1]. Protection against free radicals can be enhanced by ample intake of dietary anti-oxidants, and there is a substantial evidence indicates that nutrients containing anti-oxidants and medicinal plants or their secondary metabolite are of a major importance in disease prevention; therefore, anti-oxidants are of a great benefit in improving the quality of life by preventing or postponing the onset of degenerative diseases due to oxidative stress [2]. The Oxidative stress results from an imbalance between excessive formation of reactive oxygen species (ROS) and/or reactive nitrogen species and limited anti-oxidant defenses [3]. One of the important anti-oxidants is flavonoids, which represent a range of polyphenolic compounds naturally occurring in plants [4]. Flavonoids are potentially involved in cardiovascular prevention mainly by decreasing oxidative stress and increasing NO bioavailability; therefore the estimation of flavonoid content in plant play important roles in providing protection against ROS [5]. Various *in vitro* and *in vivo* methods are used to investigate the anti-oxidant property of samples (diets or plant extracts). In the present investigation two *in vitro* evaluations (reductive ability and DPPH radical scavenging activity) were adopted. The former is based on the principle of increase in the absorbance of the reaction mixtures. Increase in the absorbance indicates an increase in the anti-oxidant activity. In this method, antioxidant compound forms a colored complex with potassium ferricyanide, trichloro acetic acid and ferric chloride. Increase in absorbance of the reaction mixture indicates the reducing power of the samples [6]. The second method employs DPPH molecule [1, 1-diphenyl-2-picrylhydrazyl (α,α -diphenyl- β -picrylhydrazyl)], which is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecule does not dimerize, as would be the case with most other free radicals. The delocalization of electron also gives rise to the deep violet color, characterized by an absorption band in ethanol solution. [7]. In this investigation, two plants (*Arum maculatum* and *Physalis peruviana*) were selected to assess their anti-oxidant activity because of their wide range of medicinal applications. *Arum maculatum* L. is a member of the family Araceae, and in north of Iraq is known as "Kardi". The plant is a rich source of important chemical constituents; for instance, alkaloids, saponins, glycosides and lectins [8]. Traditional and folk medicine revealed that *A. maculatum* is suggested to treat kidney stone disease, colitis, liver disease, rheumatic pains and hyperacidities. Furthermore, the plant substance *Ari Tubera* has been clinically demonstrated to have an anti-inflammatory activity in the intestinal and respiratory tract [9]. In addition, the plant has been reported to have anti-microbial activity, and the plant lectins have been shown to induce neutrophil migration *in vitro* [10]. *Physalis peruviana* L. is a member of the family Solanaceae, and in north of Iraq is known as "Gulewaje". It is eaten freshly and commonly used in folk medicine as anti-microbial, anti-pyretic and immune modulator agent. It is also suggested to treat malaria, cancer, leukemia, hepatitis, rheumatism, asthma, dermatitis and other diseases [11]. Phytochemical studies have isolated a number of compounds from *P. peruviana*, which confirmed its medical importance such as ticloidine, phygrine, withanolide and viscosalactone [12]. Thus different active agents have been extracted from the plant, with a variety of pharmacological activities, and anti-inflammatory and cytostatic activity being the most important. It is also an important source of vitamins A and C [13].

Material and Methods

The Plants

Arum maculatum (whole plant) and *Physalis peruviana* (fruits) were obtained from local markets in Erbil, and classified at the Herbarium of Biology Department (College of Education for Pure Sciences / Ibn Al-Haitham, University of Baghdad). Both plants were supplied as air-dried materials, and they were powdered using a coffee grinder.

Preparation of Plant Extract

Both plants were extracted with methanol as previously described [14]. Briefly, 50 grams of the plants powder were extracted with 80% methanol (250 ml) at 65°C for 3 hours using the soxhlet apparatus. The extracts solution were concentrated to dryness under reduced pressure in a rotary evaporator to yield dried crude extract, which was frozen at -20°C until use to prepare the required concentrations.

Determination of Total Flavonoids

Total flavonoids content was spectrophotometrically determined in the methanol extract as rutin (flavonoids standard) equivalent by aluminium chloride colorimetric method [15]. Briefly, the methanol extract (3.2 mg) was dissolved in 5 ml of 50% methanol, followed by addition of 1 ml of 5% (w/v) sodium nitrite solution. After 6 minutes, 1 ml of a 10% (w/v) aluminium chloride solution was added and the mixture was allowed to stand for a further 5 minutes before 10 ml of a 10% (w/v) NaOH solution was added. The mixture was made up to 50 ml with distilled water and mixed well. Then, the absorbance was measured at 450 nm with a spectrometer after 15 min. A similar procedure was applied to six concentrations (2.5, 5, 10, 20, 40 and 80 µg) of rutin in order to plot a standard curve.

Reductive Ability

The method described by Fua *et al.* [14] was adopted to evaluate the reductive ability, in which 1 ml of each concentration of the plant extract (0.02, 0.04, 0.08, 0.16, 0.32 and 0.64 mg/ml) was mixed with 1ml of 0.2M phosphate buffer (pH 6.6) and 1.5 ml of 1% potassium ferricyanide, and then incubated at 50°C for 20 minutes. After that, 1 ml of 10% trichloroacetic acid was added to the mixture to stop the reaction. The mixture was centrifuged for 10 minutes at 3000 rpm, and 2.5 ml of the supernatant was mixed with 2 ml of distilled water and 0.5 ml of freshly prepared 0.1% Ferric chloride. By then, the absorbance was measured at 700 nm. The same procedure was applied to trolox (vitamin E) solutions (standards). All tests were done in triplicates.

DPPH Radical Scavenging Activity

The anti-oxidant activity of plant methanol extract and standard (vitamin C) were assessed on the basis of the radical scavenging effect of the stable DPPH free radical [16]. An aliquot of 0.1 ml of the extract or standard (0.062, 0.125, 0.250 and 0.500 mg/ml) was added to 3.9 ml of DPPH solution in a test tube. After incubation at 37°C for 30 minutes, the absorbance of each solution was determined at 517 nm using spectrophotometer. All measurements were made in triplicates. The ability to scavenge DPPH radical was calculated by the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \left(1 - \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \right) \times 100$$

Statistical Analysis

Data are given as mean \pm standard deviation (S.D.), and differences between means were ANOVA (analysis of variance) followed by either LSD (least significant difference) of Duncan test. The analyses were carried out using the statistical package SPSS version 13.

Results and Discussion

Methanol extract of *A. maculatum* was found to have total flavonoids of 535.3 ± 109.9 $\mu\text{g/ml}$, which was significantly higher than the recorded flavonoids in *P. peruviana* extract (352.0 ± 12.7 $\mu\text{g/ml}$) (Table 1). However, in both cases, the extracts can be considered as rich sources of flavonoids. As demonstrated by phytochemical investigations, flavonoids belong to a very vast group of plant secondary metabolites with variable phenolic structures and can be found in fruits, vegetables, grains, bark, roots, stems and flowers, and have a broad spectrum of pharmacological activities including their potential role as anti-cancer agents which give them a significance therapeutic advantage [3,4,5]. In agreement with such scope, a positive correlation had been found between flavonoids-rich diet (from vegetables and fruits) and a lower risk of colon, prostate and breast cancers has been observed. It has also been evident, that a treatment with *A. maculatum* aqueous extract remarkably lowered the mitotic index in bone marrow cells of Swiss male mice at all exposure times, and in almost all concentrations tested compared to controls [17]. The authors justified their findings on the basis of high flavonoid contents acting in synergism with other constituents. In addition, flavonoids are known to inhibit lipid-peroxidation and platelet aggregation, and they exert these effects due to their anti-oxidant potential in scavenging free radicals [18]. Such potentials and high content of flavonoids encouraged further to assess the anti-oxidant activity of both extracts.

Assessment of reductive ability revealed that both extract were effective in such activity, in which it was concentration-dependent. The highest absorbance was observed at the concentration 0.64 mg/ml of *A. maculatum* methanol extract (0.929 ± 0.006), which was significantly higher ($P \leq 0.05$) than the corresponding concentration of *P. peruviana* extract (0.850 ± 0.050) or trolox (278 ± 0.010), but the second extract also showed a significant increased absorbance compared to trolox. However, in both extract, the increase was almost three-folds higher than that of trolox (positive control) (Table 2). The results of DPPH radical scavenging activity confirmed those of reductive ability, and again the highest concentration (0.500 mg/ml) of both extracts (*A. maculatum* and *P. peruviana*) recorded the best radical scavenging activity (93.33 ± 0.58 and $95.33 \pm 2.52\%$, respectively), which significantly higher than that of vitamin C ($64.67 \pm 5.03\%$) (Table 3).

The presented results suggest the antioxidant and free-radical scavenging activities of both extracts, which even exceeded the positive control values of trolox (vitamin E) and vitamin C. It is possible to explain that in the ground of high flavonoid contents, and as presented earlier, flavonoids of natural resources represent important anti-oxidant chemical constituents of plants [3,4,5]. With respect to *P. peruviana*, interest in the anti-oxidant properties of its fruits is also shared by recent investigations, and some of the medicinal properties of the plant have been associated with the anti-oxidant capacity of polyphenols [19, 20]. However, the anti-oxidant activity of *P. peruviana* has been suggested to be not a property of a single phytochemical compound, but due to the synergistic effect of different antioxidants existing in the fruits [21]. In a further investigation, hot aqueous and methanolic extracts *P. peruviana* were also evaluated for anti-oxidant activities, and the authors concluded that ethanol extract possessed good anti-oxidant activities, and the highest activity was observed in 95% ethanol extract, and in addition, this extract demonstrated a strong superoxide anion scavenging activity and xanthine oxidase inhibitory effect [12]; conclusions that are also favored by the present results. The *A. maculatum* plant is the less studied, but it showed better results than those obtained for *P. peruviana*, and the presented antioxidant property of the extract was higher. This is possibly might be associated with the higher concentration of flavonoids. The conclusion is that *A. maculatum* and *P. peruviana* are a rich source of flavonoids, which lend both plants to have important anti-oxidant and free-radical scavenging properties. However, the presented evidences were based on *in vitro* experiments,

and therefore *in vivo* evaluations are certainly required to explore the medicinal applications of the two plants especially in the fields of antioxidant and anti-inflammatory potentials.

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Table No.(1): Total flavonoid content in the methanol extract of *Arum maculatum* and *Physalis peruviana*.

Plant Methanol Extract	Mean \pm S.D. ($\mu\text{g}/\text{mg}$)	P \leq
<i>Arum maculatum</i>	535.3 \pm 109.9	0.05
<i>Physalis peruviana</i>	352.0 \pm 12.7	

TableNo.(2): Reductive ability of *Arum maculatum* and *Physalis peruviana* methanol extracts.

Plant Concentration (mg/ml)	Absorbance (Mean \pm S.D.)		
	Positive control (Trolox)	<i>Arum maculatum</i>	<i>Physalis peruviana</i>
0.02	^C 0.109 \pm 0.002 ^f	^A 0.504 \pm 0.014 ^f	^B 0.446 \pm 0.027 ^f
0.04	^C 0.117 \pm 0.002 ^{de}	^A 0.602 \pm 0.010 ^e	^B 0.491 \pm 0.022 ^e
0.08	^C 0.138 \pm 0.008 ^d	^A 0.655 \pm 0.024 ^d	^B 0.565 \pm 0.041 ^d
0.16	^B 0.164 \pm 0.015 ^c	^A 0.715 \pm 0.006 ^c	^A 0.704 \pm 0.013 ^c
0.32	^C 0.230 \pm 0.022 ^b	^A 0.846 \pm 0.006 ^b	^B 0.744 \pm 0.014 ^b
0.64	^C 0.278 \pm 0.010 ^a	^A 0.929 \pm 0.006 ^a	^B 0.850 \pm 0.050 ^a

Different upper case letters: Significant difference ($P \leq 0.05$) between means of columns.

Different lower case letters: Significant difference ($P \leq 0.05$) between means of rows.

Table No.(3): DPPH radical scavenging activity in *Arum maculatum* and *Physalis peruviana* methanol extract.

Plant Concentration (mg/ml)	Anti-oxidant activity (mean \pm S.D.; %)		
	Positive control (Trolox)	<i>Arum maculatum</i>	<i>Physalis peruviana</i>
0.062	^C 39.00 \pm 1.73 ^d	^A 72.33 \pm 0.58 ^d	^B 69.33 \pm 1.53 ^d
0.125	^B 46.00 \pm 4.58 ^{bc}	^A 80.00 \pm 1.00 ^c	^A 76.67 \pm 2.08 ^c
0.250	^B 50.33 \pm 6.81 ^b	^A 83.00 \pm 1.00 ^b	^A 88.33 \pm 2.08 ^b
0.500	^B 64.67 \pm 5.03 ^a	^A 93.33 \pm 0.58 ^a	^A 95.33 \pm 2.52 ^a

Different upper case letters: Significant difference ($P \leq 0.05$) between means of columns.

Different lower case letters: Significant difference ($P \leq 0.05$) between means of rows.

الفعالية المضادة للاكسدة للمستخلصات الكحولية لنباتي كاردي *Arum maculatum* L. وكولة وازه *Physalis peruviana* L.

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الخلاصة

درس المحتوى الفلافوني والقابلية الاختزالية وفعالية كنس الجذور الحرة في المستخلص الكحولي للنباتين كاردي *Arum maculatum* L. وكولة وازه *Physalis peruviana* L. أظهرت النتائج امتلاك مستخلص *A. maculatum* محتوى فلافوني كلي 109.9 ± 535.3 مايكروغرام/مل، بحيث كان مرتفع معنويا مما هو عليه في مستخلص *P. peruviana* (12.7 ± 352.0 مايكروغرام/مل). واتضح عند تقدير القابلية الاختزالية بأن كلا المستخلصين كانا مؤثرين في تلك الفعالية ومرتبطين على التركيز. لقد لوحظت أعلى امتصاصية في التركيز 0.64 ملغم/مل للمستخلص الكحولي لنبات *A. maculatum* (0.006 ± 0.929) والتي كانت مرتفعة معنويا ($p \leq 0.05$) مما هو عليه في مستخلص *P. peruviana* لنفس التركيز (0.050 ± 0.850) او عقار ترولكس (0.010 ± 0.278)، لكن كانت امتصاصية المستخلص الثاني هي الاخرى مرتفعة معنويا مقارنة بالترولكس. جاءت نتائج كنس الجذور الحرة لمادة DPPH معززة لتلك الملاحظة في القابلية الاختزالية، بحيث سجل أعلى تركيز (0.500 ملغرام/مل) لكلا المستخلصين (*P.* و *A. maculatum*) أفضل النتائج في كنس الجذور الحرة (0.58 ± 93.33 و $2.52 \pm 95.33\%$ ، على التوالي)، والتي كانت مرتفعة معنويا مما هو عليه في فيتامين C ($64.67 \pm 5.03\%$). يمكن الاستنتاج بأن كلا المستخلصين يعودان الى نباتين ذو اهمية طبية من ناحية القابلية الاختزالية وفعالية كنس الجذور الحرة.

الكلمات المفتاحية: *Anti-oxidant* , *Flavonoids* , *Physalis peruviana* , *Arum maculatum*.