Pharmaceutical Sciences, June 2016, 22, 120-125
doi: 10.15171/PS.2016.19
http://journals.tbzmed.ac.ir/PHARM

Research Article

Comparison of Phytoconstituents, Total Phenol Contents and Free Radical Scavenging Capacities between Four *Arum* Species from Jerusalem and Bethlehem

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Article Info

Article History:
Received: 10 December 2015
Accepted: 1 February 2016
ePublished: 30 June 2016

Keywords:
-Free radical
-Phenols
-*A. dioscoridis*
-*A. elongatum*
-*A. hygrophilum*
-*A. palaestinum*

ABSTRACT

Background: Palestine is a rich land with wild edible plants which used from the ancient times as food and medicine. Arum is a valuable genus of medicinal plants which is used in the ethnic medicine for treatment of cancer or consumed as integrated food. This work aimed to evaluate and compare the phytoconstituents, total phenols contents and free radical scavenging potential for *Arum dioscoridis*, *Arum elongatum*, *Arum hygrophilum* and *Arum palaestinum* a members of Palestinian flora.

Methods: Phytoconstituents screened by using standard analytical methods, total phenols determined by using Folin Ciocalteu's method and antioxidant activities were assessed by DPPH assay.

Results: The crude extracts of Arum plant studied species revealed the presence of several biologically active phytochemicals with the highest quantity of saponin, alkaloid, phenols and flavonoids. For A. dioscoridis, A. elongatum, A. hygrophilum and A. palaestinum free radical scavenging activities were 6.7±0.75µg/ml, 19.9±0.63µg/ml, 9.9±0.49µg/ml, and 6.9±0.62µg/ml respectively, while the IC50 for Trolox was 4.8±0.39µg/ml as well as the total phenols contents for these species were 60.07 ±0.12, 27.49 ±0.32, 41.75±0.12 and 53.17±0.22 (mg GAE/g extract), ±SD respectively.

Conclusion: The antioxidant activities in the studied Arum plant species showed a marked correlation with their total phenols contents. A. dioscoridis and A. palaestinum had the highest antioxidant activities with high contents of total phenols and they can be used as perfect choices for manufacturing of pharmaceutical, cosmeticeuticals and nutraceutical formulations.

Introduction

High daily consumption of plants which have free radical scavenging activity, has been proven to be associated with lower mortality rate and incidence of different degenerative illnesses such as cancer and chronic cardiovascular diseases.[1-3]

Pharmacological activities for phenols have been reported in many studies and these compounds are found in various plants parts especially in fruits, flowers and leaves.[4] They slowing oxidative degradation of lipophilicity thereby improving the nutritional and pharmaceutical values as well as the quality of foods and drugs,[5] thereby they played an important role in improving the healthy life style by protecting from cancer and coronary heart diseases.[6]

Edible wild plants have been very important in the Mediterranean Sea region and several studies have been established on the consumption of these plants as food and medicine during the past decade,[7] especially in Cyprus,[8] Greece,[9] Italy,[10,11] Spain,[12,13] Turkey,[14] France,[15] and in the other regions of Mediterranean area.[16]

Arum dioscoridis Sm., *Arum elongatum* Steven, *Arum hygrophilum* Boiss., *Arum palaestinum* Boiss. are perennial herbaceous plants, belonging to Araceae family, which native to Lebanon, Cyprus, Syria and Palestine. The leaves have light green, narrow and upright leaves. The flowers are light green spathes with purple edges in *A. hygrophilum*, spotted in *A. dioscoridis*, purplish red spathe in *A. elongatum* and purplish black in *A. palaestinum*[17] as seen in the Figure 1.

Palestine is a rich land with a broad diversity of flora and fauna also with a wide range of folkloric medicine and foods due to its old history and location. Fresh *Arum* leaves can be eaten just after cooking or roasting, which can be prepared by washing them well, cutting them in small pieces, salting and then the salted water decanted several times to get rid of the harmful calcium oxalates.

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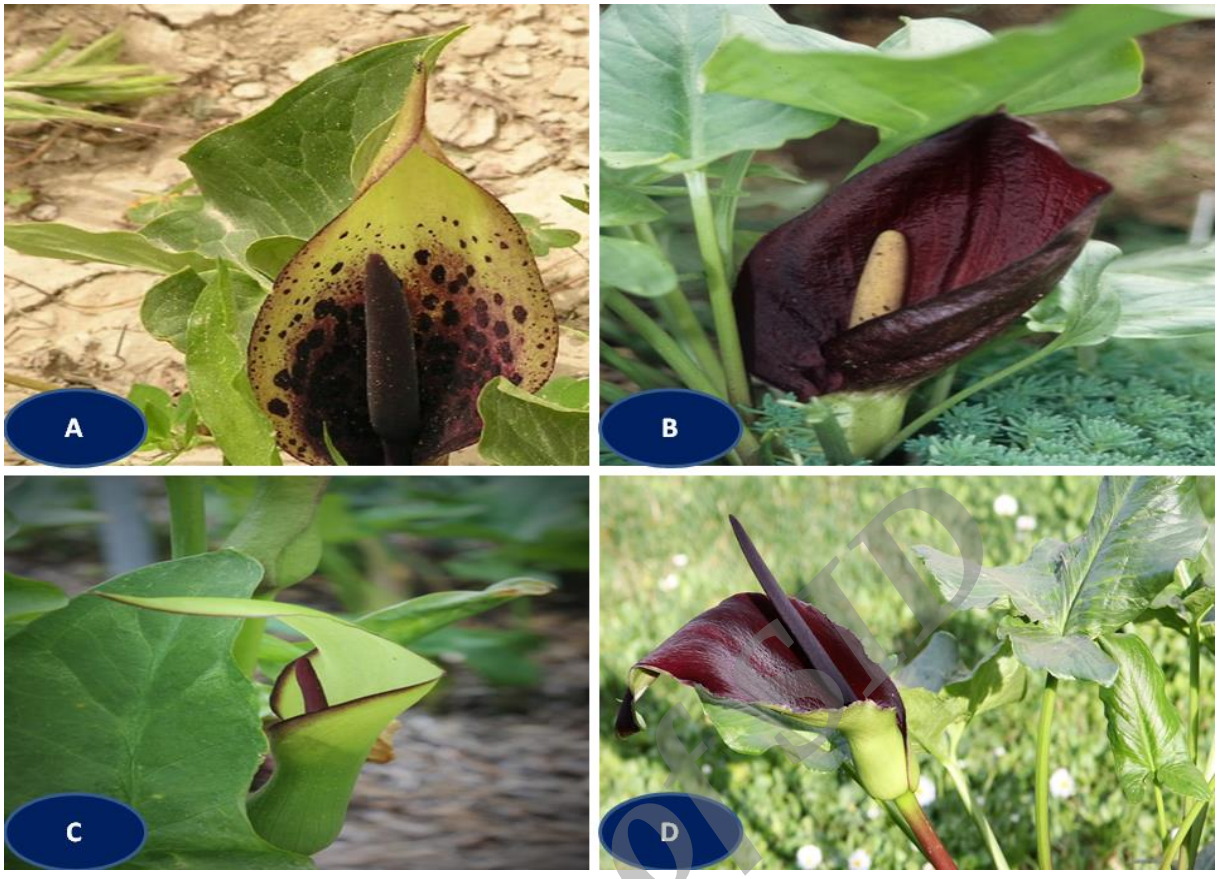

Figure 1. *Arum* plant species growing wildly in Palestine. **A:** *A. dioscoridis*, **B:** *A. elongatum*, **C:** *A. hygrophilum,* **D:** *A. palaestinum.*

Arum leaves used for treatment of cancer, cough, hemorrhoids, worms, constipation and urinary tract infections in the folk medicine in Palestine.[18-20]

Material and Methods

Collection and preparing plant materials

A. dioscoridis, *A. elongatum*, *A. hygrophilum*, *A. palaestinum* leaves were collected in April, 2015 from the Jerusalem and Bethlehem rural areas in the West Bank/ Palestine. The plants were botanically identified by pharmacognosist Dr. Nidal Jaradat. Voucher specimen were deposited in the Herbarium of the Pharmaceutical Chemistry and Technology Division (Laboratory of Pharmacognosy) and the voucher code were (Pharm-PCT-243) for *A. dioscoridis*, (Pharm-PCT-244) for *A.elongatum*, (Pharm-PCT-245) for *A. hygrophilum* and was (Pharm-PCT-246) for *A. palaestinum.*

The four sample leaves were washed and then dried in the shade at controlled temperature (25 ±2°C) and humidity (55 ±5 RH); this drying process took about three weeks until all the leaves became well dried. After that, they were powdered well using mechanical grinder and were placed into a well closed glass containers for further use.

Instrumentation

Shaker device (Memmert shaking incubator, Germany), rotary evaporator (Heidolph OB2000, VV2000, Germany), spectrophotometer (Jenway 7135, UK), grinder (Moulinex model, Uno, China), balance (Rad wag, AS 220/c/2, Poland), filter paper (Machrery-Nagel, MN 617 and Whatman no.1, USA).

Chemical reagents

The utilized reagents in this experiments included: Millon's reagent (Gadot, Haifa), Ninhydrain solution (Alfa Agar, England), Benedict's reagent (Gadot, Haifa), Molish's reagent, H_2SO_4, Iodine solution (Alfa-Aesar, England), NaOH (Gadot, Haifa), Chloroform, HCl (Sigma-Aldrich, Germany), Magnesium ribbon, Acetic acid (Frutarom LTD, Haifa), $FeCl_3$ (Riedeldehan, Germany), Methanol (Lobachemie, India), n-hexane (Frutarom LTD, Haifa), Trolox ((s)-(-)-6 hydroxy -2, 5, 7, 8-tetramethychroman- 2-carboxylic acid), (DPPH) 2, 2-Diphenyl-1-picrylhydrazyl, Folin-Ciocalteu's reagent and Gallic acid were purchased from Sigma Aldrich, Germany.

In all the experiments the used organic solvents were HPLC grade standard, except the extraction solvent (methanol) has technical quality.

Preparation of plant extracts for phytochemical analysis:

Aqueous and organic extracts of dehydrated leaves were carried out. Organic extraction was performed using Soxhlet extraction method, where a 20g of the dried powder uniformly packed into a thimble and then

extracted using 250 ml of methanol (polar protic solvent). The extraction processes was allowed to continue until the utilized organic solvent, in siphon tube of the extractor, became colorless. After that each of the obtained extracts was heated to 30-40ºC using water bath until complete evaporation of the used solvent. Generated dried crude extracts were stored 2-8ºC till use.

Aqueous extraction was performed in beaker by adding 200ml distilled water to 5g of each of the obtained plants powders. Each mixture was heated to 30-40ºC on hot plate with continuous stirring for 20 minutes. The heated mixtures were then filtered individually using Whatman filter paper. The filtrated were labeled and stored at 2-8ºC till use.[21]

Preparation of plants extracts for antioxidant experiments

About 10 g of the grounded four *Arum* plant species were soaked in 1 Liter of methanol (99%), placed in a shaker device at 100 rounds per minute for 72 hours at room temperature, then was stored in refrigerator for 4 days. After that, the extracts were then filtered using whatman filter paper no.1 and were concentrated under vacuum on a rotator evaporator. The crude extracts for two species were stored at 4 ºC in the refrigerator for further use.[22]

Qualitative phytochemical analysis

Preliminary qualitative phytochemical screening of primary and secondary metabolic compounds such as proteins, starch, phenols, cardiac glycosides, saponin glycosides, flavonoids, alkaloids, steroids, volatile oils, and tannins were carried out according to the standard common phytochemical methods described by Trease and Evans, 1983 [23] and Harborne, 1998.[24]

Determination of total phenols contents in the plant extracts

The concentration of phenols in the four *Arum* plant species (*A. dioscoridis*, *A. elongatum*, *A. hygrophilum*, *A. palaestinum* leaves) methanolic extracts were determined using spectrophotometric assay with some modifications.[25]

Gallic acid 1mg/ml stock solution was prepared by dissolving 0.1g Gallic acid up to 100ml methanol in a volumetric flask as a solvent, then from this stock solution a series of different concentrations (10, 20, 30, 50, 70 and 100µg/ml) were prepared .

The reaction mixture was prepared by mixing 0.5ml of each methanolic solution , 2.5ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5ml 7.5% $NaHCO_3$. Blank solution was concomitantly prepared**,** containing 0.5ml methanol, 2.5ml 10% Folin-Ciocalteu's reagent dissolved in water and 2.5ml of 7.5% of $NaHCO_3$. To construct standard calibration curve for Gallic acid the previous steps were repeated for each plant extract by preparing two dilutions for each extract.

The samples were thereafter incubated in a thermostat at 30°C for 90min. The absorbance was determined using spectrophotometer at λmax = 765nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained; then the content of phenols in extracts was expressed in terms of Gallic acid equivalent (mg of GA/g of extract).[26]

Antioxidant activity

Trolox standard and plant working solutions

Stock solutions of a concentration of 1mg/ml in methanol were firstly prepared for the four plants extracts and Trolox. The DPPH and Trolox assay was done according to the method of Brand-Williams et al. (1995) with some modifications.[27]

The working solutions of the following concentrations (1, 2, 3, 5, 7, 10, 20, 30, 40, 50, 80, 100µg/ml) were prepared by serial dilution with methanol from the stock solution.

Spectrophotometric measurements

DPPH was freshly prepared at a concentration of 0.002% w/v. The DPPH solution was mixed with methanol and the above prepared working concentration in a ration of 1:1:1 respectively. The spectrophotometer was zeroed using methanol as a blank solution. The first solution of the series concentration was DPPH with methanol only. The solutions were incubated in dark for 30 minute at room temperature before the absorbances were recorded at 517nm.[28]

Percentage of inhibition of DPPH activity

The percentage of free radical scavenging activity of the four *Arum* plants species and the Trolox standard were calculated using the following formula:

Percentage of inhibition of DPPH activity (%):

$(A-B)/A \times 100\%$ Eq.(1)

Where: A = optical density of the blank,

B = optical density of the sample.

The free radical scavenging activity half maximal inhibitory concentration (IC_{50}) for the four plants samples and the standard were calculated using BioDataFit program edition 1.02 (data fit for biologist).

Statistical evaluation and data processing

Statistical evaluations were carried out using the statistical software R (R, 2012). Simultaneous comparisons of means were performed using multiple contrast tests with corresponding custom contrast matrices. Differences between treatments were declared significant at $P < 0.05$ using the Tukey correction for multiple comparisons.[29]

Results and Discusion

Phytochemical screening

Phytochemical characteristics for the four *Arum* leaves species extracts are summarized in (Table 1).

Table 1. Phytochemical screening tests for aqueous and methanolic of the four *Arum* species.

Phytochemical compounds	*A. dioscoridis*		*A. elongatum*		*A. hygrophilum*		*A. palaestinum*	
	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol
Cardiac glycosides	-	-	-	-	-	-	-	-
Saponin glycoside	+++	-	+	-	+	-	++	-
Alkaloids	-	++	-	+	-	+	-	+++
Protein	-	-	-	-	-	-	-	-
Carbohydrates	+	-	+	-	+	-	+	-
Phenols	+	+++	+	+	+	+	+	++
Volatile oil	-	-	-	-	-	-	-	-
Tannin	+	-	+	-	+	-	+	-
Steroids	-	-	-	-	-	-	-	-
Flavonoid	+	+++	+	+	+	+	+	++

Where; (-) means the absence of the content, (+) means low contents, (++) means mild contents and (+++) means high contents.

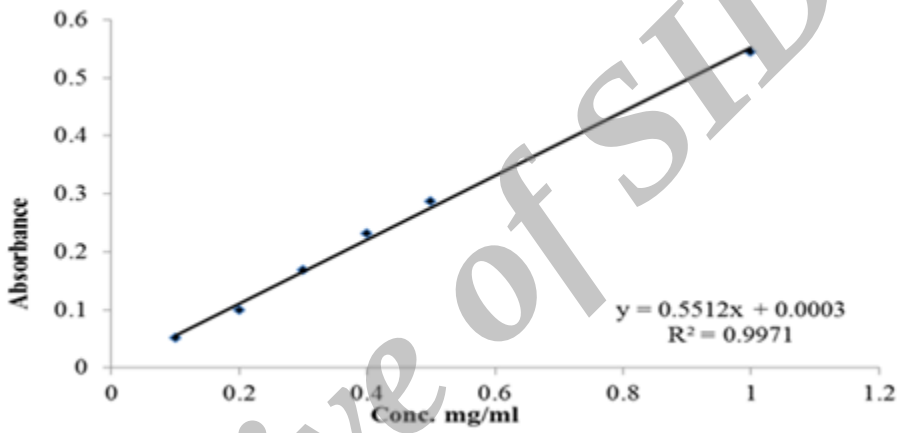

Figure 2. Standard calibration curve of different concentrations (mg/ml) of Gallic acid and their respective optical density at 765nm.

It is shown that phenols, flavonoids and alkaloids were found in the methanolic extracts, whereas, saponin glycosides, carbohydrates, phenols, tannins and flavonoids were found in the aqueous extracts while cardiac glycosides, proteins, volatile oils and steroids were missing in both methanolic and aqueous extracts.

Total phenols percentages

Standard calibration curve used for the determination of total phenols contents which was prepared using different concentrations of Gallic acid equivalent (mg of GAE/g) dry weights for all four *Arum* plant species extracts, and their respective optical density shown in Figure 2.

The absorbance of all samples was measured at 765 nm using UV-Vis. spectrophotometer after incubating at 30 °C for 1.5 hours.

The total phenols were expressed as mg/g Gallic acid equivalent (GAE) dry weight using the standard curve equation: $Y = 0.5512X + 0.0003$, $R^2=0.9971$ Eq.(2)

Where:

Y- Absorbance at 765 nm

X- Total phenol in the extracts.

The amount of total phenols varied widely in plant materials and ranged from 27.49 ±0.32 to 60.07 ±0.12mg GAE/g dry material (Table 2).

Among edible Arum plants, a low level of phenols was found in *Arum elongatum* and a moderate level in *A. hygrophilum* and *A. palaestinum*. However *A. dioscoridis* and contained relatively high amounts of phenols.

Table 2. Total Phenols in Plants Extracts.

Methanolic extract	Total Phenols Contents (mg GAE/g extract), ±SD
A. dioscoridis	60.07 ±0.12
A. elongatum	27.49 ±0.32
A. hygrophilum	41.75±0.12
A. palaestinum	53.17 ±0.22

Free radical scavenging activity using Trolox as standard equivalent

The free radical scavenging activity of the methanolic extract of *A. dioscoridis*, *A. elongatum*, *A. hygrophilum*, *A. palaestinum* has been tested by using DPPH radical method and Trolox as a reference standard. The concentrations ranged from 1-100µg/ml. The zero inhibition was considered for the solution which contained only DPPH without any plant extract. The results are showed in (Figure 3).

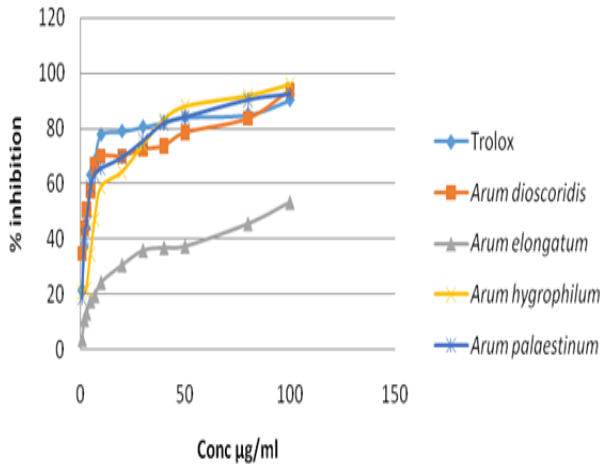

Figure 3. The antioxidant activity of different *Arum* species in comparison to Trolox.

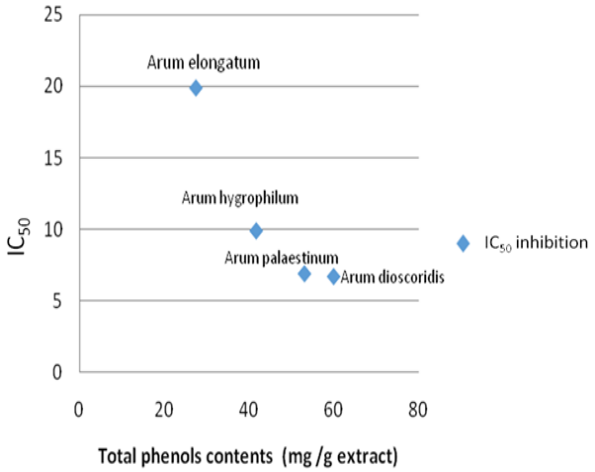

Figure 4. Correlation between the antioxidant activities of the *Arum* plant with its total phenolic contents.

Currently there is a rapid elevation of interest in a safety and available phyto-products as new sources of natural free radical scavengers which aimed to utilized them in cosmeceutical, pharmaceutical and nutraceutical formulation in order to replace the chemical free radical scavengers, which have been used as preservatives in food, cosmetics and as drugs.

The scientists have been approved that the chemical synthetic free radical scavengers have high probability risk on human health and also their high probable toxicity. Due to all that, there is an increasing in responsiveness among patients and consumers regarding food additive and their effectiveness and safety.[30]

These natural free radical scavengers have mainly phenolic character and they include various classes of phyto-chemicals including, flavonoids, saponins, glycosides, anthocyanins, tannins and many others.[17]

The total phenols content of methanolic extracts of the investigated plant species ranged from 27.49 ±0.32 to 60.07 ±0.12 mg GAE/g of dry weight, while the IC_{50} ranged from 6.7 ±0.75µg/ml to 19.9± 0.63µg/ml of dry weight in comparison with Trolox standard compound which has IC_{50} 4.8± 0.39µg/ml. All these results indicated and identified that there is positive linear correlation between free radical scavenging activity and the total phenols content for all the methanolic extracts. Figure 4 clearly demonstrate that positive correlation between the antioxidant activities of the *Arum* plant with its total phenolic contents, the graph show with the increasing of the total phenolic extract there is an increasing in the antioxidant activity represented by lower IC_{50}.

A. dioscoridis and *A. palaestinum* were the best sources for phyto-phenols and free radical scavenging compounds among the *Arum* plant species growing in Palestine.

Conclusion

It is traditionally renowned that, *Arum* leaves have been incorporated into holistic health systems as medicine and had been used in the same time as food due to their potentially beneficial therapeutic properties and high contents of dietary components. Most of the phyto-pharmacological studies have not focused on the comparison between these four species and which one can be the best medicine and food between them.

In conclusion, the studied four *Arum* plant species have potential antioxidant activity especially *A. dioscoridis* and *A. palaestinum* which can be used for manufacturing of cosmeceutical, pharmaceutical and nutraceutical formulations.

Acknowledgement

The authors would like to thank An-Najah National University for support.

Conflict of interests

The authors claim that there is no conflict of interest.

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