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Study of the chemicals, phenols, flavonoids, and antioxidants content of the Syrian Arum *hygrophilum* Boiss. Plant

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Abstract
The Syrian flora is well-known for its richness and diversity, as it contains a large number of medicinal plant species. In Syria, there are a large number of varieties of the Syrian wild Arum plant. This moisture-loving type of Arum (Arum *hygrophilum),* is the most prevalent species in Syria, and it is considered as a distinctive type.
There is no scientific information available about the antioxidant properties of the Syrian wild Arum plant, nor about the content of phenols and flavonoids. Therefore, this work aims on estimating these properties was important and useful, especially to find a natural resource for antioxidants in Syria. Thus, the integrative antioxidant IA as the equivalent of ascorbic acid was determined using PLC photochemiluminescence for the first time.
By using PLC technique, it was possible to estimate IA in an accurate and easy way, and it was found that the highest antioxidant capacity of the Syrian wild Arum plant, estimated in the equivalent of ascorbic acid, which is found in the leaves of the Arum plant estimated around 81.96 nmol/g whereas 97.9 nmol/g in the roots. The antioxidant activity in this plant is attributed to the presence of flavonoids and phenols in this plant, including: isoorientin- Coumaric - Caffeic acid gallic acidvitexinferulic acid quercetin-3 O-rhamnoside- luteolin.
The purpose of using the medicinal plants in treatment of many health problems isconsidered as a return to nature, especially since synthetic and chemical medications have multiple side effects compared to these herbs.

Keywords: Photochemical fluorescence, ascorbic acid equivalent, antioxidant, Arum (*Arum hygrophilum)*

1. Introduction
Since old times, plants have been used for traditional medicine for the treatment of many health issues including minor health problem such as headache or stomachache or serious diseases such as cancer, obesity, and diabetes [1].
It has long-lobed leaves and reaches up to 5 feet in early autumn, followed by dark narrow flowers in early spring, and it is the variety used in this study, and there are species of Arum, including:
Arum palaestinum
Arum dioscoridis
Arum hygrophilum

All of these species are found in Syria, but the most widespread is what is known as the Syrian Arum or elephant ear is *Arum hygrophilum* [2].
Most of these types have been used in traditional and folk medicine, where they are collected, boiled, filtered and their extracts are prescribed for treatment of many health problems [3].
The Arum plant belongs to the Taro family of monocotyledons, common to the Eastern Arabia and Eastern Mediterranean, and its maximum biodiversity in the Mediterranean basin spreads in the mountainous, plain and desert lands. The arum plant is considered a poisonous plant if is consumed raw and uncooked [4].
Arum hygrophilum is a species of flowering plant in the family *Araceae*. It has a disjunct distribution, found in Eastern Mediterranean region specifically in Syria, Jordan, Lebanon, Morocco, and Cyprus and [5].
It is threatened by agricultural development and urbanization. The species is characterized by a white inner spathe, which has purple accents and the small spadix is a dark purple color [6].
In Syria, the traditional medicine has been using the edible Arum species, referred with the common Arabic name “Louf,” as a natural anticancer agent against colon cancer [7].

Syrian wild Arum plant (*Arum hygrophilum L*) possess many active compound in its leaves, stems and roots such as (coumarins - phenols - flavonoids - and antioxidants - and the active substances found in this plant). These compounds have therapeutic benefits for a great number of diseases. The compunds such as flavonoids and phenolic compounds have many biological effects such as protecting the liver, killing bacteria and preventing cancers [8]. The physiological advantages of phenolic compounds are owed to their antioxidant properties and ability to eradicate free radicals [9].
Previously, different flavonoids, such as quercetin, apigenin, vitexin, and isoorientin were isolated from *A. palaestinum* in a Jordanian study and their antimicrobial activities were established [10]. In a recent comparative study in *in vitro* and *in vivo* experiments, pancreatic lipase (PL) and dual α-amylase/α-glucosidase inhibitory potentials were demonstrated for the extracts of A. dioscorides and *A. palaestinum* as well as for some of their isolated compounds [9]. Earlier, Karahan *et al*. [11] reported free radical scavenging and ferric-reducing activities of ethanol, methanol, acetone, and water extracts of A. dioscoridis leaves. The study concluded that total phenolic and flavonoid contents were greatly influenced by the extraction medium. Also, a literature survey indicated that *A. hygrophillum* is the least evaluated Arum species, lacking on both phytochemical and biological evaluations. This study investigates the indigenous A. hygrophilumphytochemically and biologically. The *A. hygrophilum* crude aqueous extract (AE) modulation of the extrapancreatic digestive enzymes was examined *in vitro*. Additionally, acute *in vivo* effects were investigated. Antiproliferative potential of this species against colorectal cancer cell lines as well as possible pancreatic effects in β-cell line was evaluated. It is noteworthy that the discovery of some modern drugs was based on wild plants, mainly on extraction and isolation methods, for example the discovery of the anti-cancer drug Paclitaxel from TaxusBrev Folia (king horn, 1994).
In this research, an extract of the leaves of the Arum plant was studied, where it was extracted in different ways to cover and to know all the active compounds of the Arum plant, and then analyze the extract using the GC-MS and HPLC technology, and to know its chemical composition and its contents of important chemicals, and to determine the antioxidant effectiveness of the leaves and roots of this plant.
Despite the great nutritional and medicinal benefits of the Arum plant, there are no in-depth studies in Syria on the chemical content of this plant and there were no other studies to examinethe possibility of using this plant in pharmaceutical industry [12].Therefore, the purpose of this work was to study the chemical composition of the leaves and roots of the Arum plant and to identity the substances it contains, and the focus in this study on the Syrian Arum plant in terms of its effect as an antioxidant using the photochemical fluorescence method.
And the research dealing with its anti-cancer effects is very few [13], and therefore there are not many references with which we can compare the results of our research
In view of the absence of any study to estimate the antioxidant activity of the Syrian wild Arum (*A. hygrophilum*) plant, the aim of this study was to find an accurate method for the antioxidant compounds in the leaves of the Arum plant.

2. Materials and methods

2.1 Plant samples

Aerial parts of the flowering *A. hygrophilum* were collected from Damascus countryside, Syria and their taxonomic identity was established prior to the beginning of the study. The sample was dried at room temperature, isolated from light and moisture, to prevent spoilage of the active substances.

2.2 Apparatuses used

- UV booth with two lamps 254 nm and 365 nm
- Gas chromatograph with organic mass spectrometry GC-MS from Agilent Company with analytical program Chemstation and Willy-Nist research library.
- Rotary evaporator with Buchi. vacuum
- HPLC device from Agilent Company with dual pump, automatic injector and PDA detector with Chemstation analytical program
- Photochemical fluorescence measuring device for the determination of antioxidants, which is from the German company Analytik Jena
- Ultrasound device, Power Sonic model 405-
- Heraeus flaps, Megafuge 2.0R- A 550.
- Solvents and materials used: chloroform, hexane, methanol, glacial acetic acid from MERCK and hydrochloric acid from BDH:

2.3 Determination of coumarins

This process was carried out using the thin layer chromatography method: TLC. The methanol extract of Arum leaves is loaded onto a silica plate and placed in a transfer tank containing the mobile phase: toluene ether in a volume-to-volume ratio, saturated with 10% aqueous acetic acid, and then sprayed with a 10% methanol KOH solution. The luminescence of the plate was examined when exposed to UV rays at wavelength is 365 nm, and the appearance of a clear blue or yellow fluorescence indicates the presence of coumarins.

2.4 Extraction

Methanol extraction for identification of extracted compounds with GC-MS:
Extraction was done using a soxhlet device: 25 g of dried leaves powder was weighed and placed in an extraction tube, and then 150 ml of methanol was placed in the extraction flask.
The extraction process continued until the organic solvent in the extraction tube became colorless. Then the obtained extract was evaporated by rotary evaporator umtil dry in the presence of vacuum at 30 °C, then dissolved in 0.5 ml methanol with 0.5 ml dichlorometol, dried by placing a pinch of anhydrous sodium sulfate and stored at 4 °C in a dark container in preparation for analysis by GC technology -MS
Analysis by (GC-MS) Gas chromatography=mass spectrometry:
Using gas chromatography-connected mass spectrometry is an effective method for separating and detecting volatile organic compounds.Gas chromatograph with mass spectrometer (GC-MS) (Device Type: Agilent 5973)
The separation was carried out using an HP-5MS capillary column with the following specifications: length, 30 m, diameter 0.25 mm, thickness of the fixed phase 0.25 nm. The oven temperature program: from 60 degrees Celsius waiting (3 minutes) to 280 degrees Celsius at a rate of 3 degrees per minute.
Helium carrier gas at a flow rate of 0.9 ml/min.
Injector temperature 275°C
Detector temperature 275°C
The volume of the syringe is one microliterInjection model

without segmentationIonization energy is 70 electron volts
The compounds were identified by matching the mass spectra of the sample extract with those in the Wiley &NIST search libraries in the device software.

3. Results

The results showed the*A. hygrophilum*possess several compounds which they are biologically active.It represented 95.01% of the extract (Table 1). The most important compounds were phytol with a percentage of 26.7, and phytol acetate at a rate of 6.45 for these substances that have been identified as anti-inflammatory and anti-cancer properties, and a phytol compound that has activity against some cancer cells. 12.9, an essential fatty acid and hexadecanoic acid is a saturated fatty acid. These fatty acids are also a health supplement, while methyl ester derivatives are of biological importance.

Table 1: The compounds present in the leaves extract of *A. hygrophilum* analyzed by GC-MS technology

Retention Time	Compound name	Chemical formula	Molecular Weight	% Peak area
8.9	2cydopentin $C_5H_6O_2$e-1-one	$C_5H_6O_2$	82.1	4.7
12.2	valericaldhyde	$C_5H_{10}O_2$	86.13	1.5
23.3	p_vinylguaiacol	$C_9H_{10}O_2$	150.07	0.6
28.7	Fomanilide	C_7H_7NO	121.14	0.4
32.14	Borane	$C_7H_{18}BN$	127	3.9
37.4	Neophytadien	$C_{20}H_{38}$	278.52	0.7
39.2	Hexahydrofarnesyl acetone	$C_{18}H_{36}O$	268	4.18
43.5	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	12.9
47.3	Eloal	$C_{16}H_{22}O_4$	278.34	0.5
51.5	Linolenic acid, methyl ester	$C_{19}H_{32}O_2$	308.50	15.8
52.3	Phytol	$C_{20}H_{40}O$	296	26.7
52.9	Phytol acetate	$C_{22}H_{42}O_2$	338	6.45
53.6	Linolenic acid	$C_{18}H_{30}O_2$	278.43	2.4
54.8	Pluchidiol	$C_{13}H_{20}O_2$	208	1.5
55.9	Oleamide	$C_{18}H_{35}NO$	281.48	3.7
59.4	1_Ecosanol	$C_{20}H_{42}O$	298.56	4.8
62.1	1_Docosanol	$C_{20}H_{46}O$	326.61	3.1
63.8	Quercetin 7,30,40-tri-OCH3	$C_{181}H_{16}O_7$	344	2.7
65.3	α-Tocopherol	$C_{29}H_{50}O_2$	430.71	3.4

Determination of phenols and flavonoids using high-performance liquid chromatography with ultraviolet spectrophotometers.
High-Performance Liquid Chromatography and Uv/Visible Detector HPLC-DAD

- DAD HPLC device specifications - device type Agilent 1260
- ODS- C18 shaft, inner diameter mm 1.3
- Particle dimensions 3.5 um, shaft length mm 250Double automatic pump
- Ultraviolet detector with array system
- Flow rate 0.9 ml / min

The volume of the injected sample is 50 µl 100 g of dried-freeze leaves of *A. hygrophilum* were used after grinding them; this amount was soaked in about 200 ml 50/50 solution (ethanol / water) and placed in a dark flask in a water bath at 40 ° C for half an hour with stirring. After that, the filtrate was filtered and acidified with N HCl2 was extracted by ethyl acetate, then the ethyl acetate layer was separated and evaporated until dry by a rotary evaporator and then dissolved with a mobile phase liquid. The phenols and flavonoids extracted by HPLC technology were determined according to the following analytical conditions:

3.1 Dynamic phase

- The first phase: Acetic acid (0.5% v/v) water: A
- Phase II Acetonitrile: B

Gradient dynamic phase program, according to Table (2)

Table 2: The dynamic phase distribution ratios over time

Time	A	B
0 min	100%	0%
12 min	100%	0%
17 min	70%	30%
22 min	50%	50%
27 min	75%	25%
30 min	100%	0%

Table 3 has shown that *A. hygrophilum* contains high amounts of flavonoids and phenols. The highest amount was Virexin (135.12) followed by Leutolin (67.11), Quercetin-3- O-rhamnoside (55.87), Ferulic acid (49.54), Coumaric (47.15), Caffeic Acid (37.19), Gallic Acid (29.45), Catechin (6.42) and Falvones (4.7).

Table 3: Phenols and flavonoids identified by HPLC technology

Phenols flavonoids	Retention time	Concentration µg/ml
Gallic acid	5.21	29.45
Caffeic acid	7.14	37.19
Isoorientin	13.49	42.89
Vitexin	15.11	135.12
Ferulic acid	16.54	49.54
Quercetin-3-O-rhamnoside	17.31	55.87
Coumaric	18.96	47.15
Luteolin	19.84	67.12
Catechin	21.7	6.42
Flavones	23.9	4.7

It is clear from the results of the analysis that the leaves of the *A. hygrophilum*are rich in phenols and flavonoids, which have great medical and therapeutic benefits that make the Arum plant one of the most promising plants for medicinal uses.

3.2 Determination of the integral antioxidants

100 g of *A. hygrophilum* of leaves and 100 g of roots leaves were taken separately, after grinding them, they were soaked in about 200 ml of a 50/50 solution (ethanol / water) and placed in a dark flask in a water bath at 40 degrees Celsius for half an hour with constant stirring. Then the flask was placed in an Ultrasonic bath for 25 minutes, after that the liquid was filtered with a flow of one drop per second so that the liquid extract was obtained. Then the liquid extract sample was transferred to the rotary evaporator flask and evaporated at a temperature of less than 30 ºC until the yield is about 5 ml. Then 30 ml of an aqueous solution of 50% methanol was added, and after that it was put in the flask. The plant extract was in an Ultra sonic bath for two minutes, then it was evaporated the methanol in a rotary evaporator at a temperature of less than 30 ºC using vacuum. We thus obtained the aqueous extract placed in the refrigerator in preparation for its analysis. The method developed by Popov and Lewin [5] was used, and the total antioxidant capacity of water-soluble substances ACW was applied in this work. The antioxidant activity of all the components present in the leaves of A. sylvanus was measured. I purchased the necessary reagent kits (Analytic Yana Company – Germany). The ACW protocol was as follows:

The first three reagents were:

Reagent 1 (solvent), reagent 2 (aqueous buffer PH = 10.5), and reagent 3 (photosensitizer).

The working solution of reagent 3 (3-WS) is prepared by taking the solution of reagent 3 and diluting it with 750µl of reagent 2. The working solution of reagent 4 (4-WS) is prepared by taking the solution of reagent 4 and adding 490 µl of reagent 1 and mixing it with 10 µl of sulfuric acid with a concentration of 95-97% (Merck). The mixture was stirred for several seconds, 10 µl of the previous mixture was taken and diluted with 990 µl of reagent 1 to obtain a working solution 4 (4-WS). Table 4 shows all procedures and volumes used in the analysis.

Table 4: Volumes used in different measurements

Reagent	1	2	3-WS	4-WS	Sample
Control	1500µl	1000µl	25µl	0	0
Calibration	1500µl-x	1000µl	25µl	x	0
Measurements	1500µl-y	1000µl	25µl	0	y

x= 10, 15, 20µl, y= 10µl, WS (working solution)

ACW calibration and measurements were carried out according to the standard kit protocol as shown in the table, and the measurements were carried out with Photochem (R) device (Analytic Jena – Germany). The volumes used were prepared in microliters, and the measurements were repeated twice. A light emission curve was recorded in 240 seconds using an inhibitor as a parameter to estimate the antioxidant capacity, and the antioxidant capacity was determined by taking the integration given by the previous curve and expressed in mmol/liter ascorbic acid used as a standard to obtain the calibration curve.

Table (5) shows the water-soluble antioxidant capacity equivalent to the effectiveness expressed in nmol equivalent of ascorbic acid per gram of the studied product.

Table 5: The total antioxidant content of *A. hygrophilum* leaves and roots estimated by ascorbic acid equivalent (nmol/1g) using photochemical fluorescence technique

Measurements	*A. hygrophilum* extract	*A. hygrophilum* extract
First reading	78.9	98.3
Second reading	82.4	97.9
Third reading	84.6	97.5
Average	81.96	97.9

It is clear from Table 5 that the highest values of antioxidant capacity of Arum extract expressed as ascorbic acid are 97.9 (nmol/g) present in Arum roots extract.

4. Discussion

The current study represents the first comprehensive analysis of the leaves and roots of the Arum plant in Syria. The HPLC-DAD method described here also contributed to the sensitivity, selectivity and speed of analysis in identifying phenols and flavonoids, as the results obtained explained the past and current use of the Arum plant in folk medicine, and may also support The results we obtained from the uses of the Arum plant in health and nutrition as a functional food, The total phenol and flavonoid contents were high, which confirms the presence of a high content of antioxidants, and this may help confirm the validity of the use of Arum as a natural antioxidant in medical applications, taking into account its pharmaceutical and medical importance. The results of our study is similar to the results obtained in Jordan [14] in which the GC-MS analysisused in this study revealed the presence of Tert-butylhydroxy anisole (phenolic compound) in the water extractof Arum hygrophilumBoiss; that finding approves itsscavenging and antioxidant properties and goes with thereported results of Afifi *et al.* [15, 16] on the same plant.

5. Conclusion

Through the results of the research and their discussion, several points were concluded, the most important of which are:

- This research is the first to study the chemical content of the leaves and roots of the Arum plant spread in the wild in Syria
- We highlighted the content of the Arum plant from active substances such as phenols and antioxidants, and thus the Arum plant may turn from a burden plant on the national economy into a tributary plant.Phytochemical evaluation of Arum hygrophilum recovered flavonoids (luteolin, isoorientin and vitexin) and β-sitosterol. HPLC-MS analysis of its antioxidative ethanol extract further revealed the presence of caffeic-, ferulic-, gallic- and rosmarinic acids and quercetine-3-O-rhamnoside The natural products found in the Arum plant are good and important sources for developing new medications. Through the results we obtained, we see that they support the view that the leaves and roots of Arum plant are a promising source of natural antioxidants for their ability to stop oxidation and contain large amounts of flavonoids and phenols, and it was found that the average percentage of antioxidants (81.96nmol/g leaves) (97.9nmol/g roots)). They are high values of the antioxidant components due to the presence of phenols and flavonoids, while there are other chemical compounds such as isoorientin- Coumaric - Caffeic acid gallic acid.

6. Suggestions and recommendations

The results of this study have shown the importance of this plant as a source of active components which can be used in the pharmaceutical industries. More studies should be performed on this plant in order to explore its medical potential.

a) Analyzing, separating and isolating each component of the extract.
b) Test each of these separated and isolated components on some of the diseases mentioned in traditional medicine to confirm their effectiveness, and then chemically manufacturing them as medicines.
c) It's expected that this work will be an introduction to the exploration of this plant and its importance.

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