

## Preliminary Study and Phytochemical Screening of *Arum dioscorides* Sibth. in Syria

Ayoubi Ahmad Mohieddin<sup>1\*</sup>, Al-Kurdi Khalil<sup>1</sup>, Kattah Abdullah<sup>1</sup>, Trefi Saleh<sup>2</sup>

<sup>1</sup>Department of chemistry, Faculty of Science, Aleppo University, Syria

<sup>2</sup>Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Aleppo University, Syria

Received: 11<sup>th</sup> Dec, 16; Revised: 6<sup>th</sup> Feb, 17; Accepted: 14<sup>th</sup> Feb, 17; Available Online: 25<sup>th</sup> February, 2017

### ABSTRACT

Raphides (calcium oxalate needles) were detected in both of *Arum dioscorides* Sibth. et Sm. leaves and rhizomes. The extraction method of the leaves was optimized, in order to choose the most suitable solvent and method. The best solvent was the following mixture: ethanol, water, hexane, chloroform, acetone 38:16:25:9:12 v/v respectively. In addition, the second best solvent was aqueous ethanol 65%. While for the extraction method itself, sonication aided extraction at room temperature gave significantly better yields than maceration for 72 hours. Moreover, and the suitable time for sonication-aided extraction is 20 minutes. Because there was no significant yield increase through time prolongation after 20 min. Qualitative phytochemical screening of both the leaves and rhizomes was carried out indicating the presence of these phytochemicals in both of leaves and rhizomes: alkaloids, amino acids, phenolic acids, carbohydrates, coumarines, flavonoids, lignans, saponins, sterols, tannins, triterpenoids, pro anthocyanidin, catchins and traces of cyanogenic glycosides. While it has shown the absence of anthranoids and cardiac glycosides.

**Keywords:** *Arum dioscorides*, “extraction method”, “phytochemical screening”, raphides “calcium oxalate”.

### INTRODUCTION

Araceae Family is a large family of monocotyledons, which is mainly a tropical family, with approximately 4000 species counted, including 117 genera. The genus *Arum* is composed of 28 or 29 species<sup>1,2,3</sup>, distributed in Europe, North Africa, Middle East and Central Asia<sup>2</sup>. *A. dioscoridis* Sibth. et Sm. is the most spread specie in Syria<sup>4</sup>. It has many local names as (alloof, alloof al-mobakka-a, samm al-hayya, alloof al-mobarkash, and as-sahm). Its flowers smell like a mixture of dung, rotten fruits and faeces, because of its contents of skatole, volatile amines, phenols and esters<sup>5</sup>. While according to Urru<sup>6</sup>, *A. dioscoridis* flowers emit alkenes, 2-heptanone and p-cresol only. Leaves and rhizomes of many plants of the Araceae family are used as vegetables such as *Arum italicum* in Catalonia<sup>7</sup> and southern Croatia<sup>8</sup>, *Arum maculatum* in the Czech Republic, Bulgaria and Turkey<sup>9,10,11</sup>, *Xanthosoma sagittifolium* Schott in Brazil, Africa, and Asia<sup>12</sup>, *Stylochiton warnecke* Engl. in Senegal<sup>13</sup>. The aboriginals in Odisha collect different types of wild tubers especially of genus *Amorphophallus* and store them for off agriculture seasons, food scarcity and medicinal purposes. The major wild tuber crops are belongs to family Araceae<sup>14</sup>. Beside to *Colocasia esculenta* (L.) Schott (its old name is *Arum colocasia*) which is eaten cooked like potato; it is sweet, with a remarkable flavor, according to the study of 17th century in Brazil<sup>15</sup>. Moreover, it is considered as one of the basic food crops in many countries<sup>16</sup>. Seven species of Araceae are used in China<sup>17</sup>. In addition, many plants of the Araceae are used to treat

some disease. So, the leaves of *Arum italicum* Mill. are used topically as anti-warts in Italy<sup>18</sup>, the leaves of *Xanthosoma sagittifolium* exhibits cytotoxic effects against the immortalized line of human T-lymphocytic and myelogenous leukemia cells inducing apoptosis<sup>12</sup>. *Eminium spiculatum* (Blume) Kuntze is used in Jordan as an anticancer agent with antiproliferative activity comparable to that of cisplatin for T47D cell line<sup>19</sup>. Ethanol extract of *C. esculenta* significantly decreased blood glucose level in diabetic<sup>20</sup>. The ability of inhibiting the enzyme AChE, increases the levels of the neurotransmitter acetylcholine in the brain, thus improving cholinergic functions in patients with Alzheimer's disease<sup>21</sup>. Alkylresorcinols and polyhydroxy alkaloids was discovered in aroids<sup>22</sup>, beside to a new kind of Phenyl-terminated fatty acids in seeds of various aroids<sup>23</sup>.

### *Arum* Uses and phytochemistry

El-Desouky et al have isolated a new pyrazine bioactive alkaloid from *Arum palaestinum* Boiss and they called it piperazirum<sup>24</sup>. *A. dioscoridis* Sibth. et Sm.. was reported to have ethnopharmacological anticancer uses<sup>25</sup>, and according to Jaradat et al that both of *A. dioscoridis*, and *A. palaestinum* were two of three most commonly used medicinal plants for the treatment of cancer in Palestine<sup>26</sup>. However, to the best of our knowledge, there was no detailed scientific research studies about its phytochemical constituents, its antiproliferative cytotoxicity or other pharmacological effects according to Afifi-Yazar et al<sup>27</sup> and so about *A. hygrophilum* Boiss. which was reported to have fungicidal activity according to Khalil et al<sup>28</sup>. While

\*Author for Correspondence: ayoubi2ahmad@gmail.com

*A. palaestinum* Boiss. was reported to have dose-dependent suppression in the proliferation of breast carcinoma cells and lymphoblastic leukemia cells by its ethyl acetate fraction<sup>29</sup>. Moreover, a comprehensive metabolite profiling of *A. palaestinum* leaves using liquid chromatography–mass spectrometry was done by Abu-Reidah et al<sup>30</sup>, beside to the study of the cytotoxic activity and phytochemical analysis of *A. palaestinum* by Farid et al<sup>31</sup>. In addition to Fatima conclusion that the anticancer activity of the extracts of *Typhonium flagelliforme* (which is used in Malaysia to treat cancer) in vitro are more potent than its major individual constituents such as pheophorbides and hence its compounds had a synergistic effect on antiproliferation of cancerous cells<sup>32</sup>.

A characteristic of species from the Araceae family is the typically irritating and toxic property of their exudates, which is mainly due to the abundance of calcium oxalate raphides, and possibly necrotizing proteinaceous toxins, which makes it necessary to steep the plants well to eliminate the oxalate<sup>33</sup>. The hypothesized roles of Calcium Oxalate formation are supporting tissue structure, regulating excess tissue Calcium and as a defense mechanism against herbivores. In higher plants, their distribution like their morphology follows species-specific patterns, indicating regulation over the sites and modes of their accumulation<sup>34</sup>.

The range of morphologies of Calcium Oxalate crystals is very large and can include block-like rhombohedral or prismatic crystals, found as single or multiple crystals per cell, bundles of needle-shaped raphide crystals, masses of small angular crystals, and multifaceted conglomerate crystals called druses<sup>35</sup>.

To our knowledge, no phytochemical investigation has been carried out on the extracts of *A. dioscoridis* to date, which motivated us starting studying this plant.

## MATERIALS AND METHODS

### Chemicals and Equipment

#### Chemicals

##### From Merck

Acetone, Aluminum Chloride, Ferric Chloride, Bismuth nitrate, n-Butanol, Chloroform, Ethanol, Ethyl Acetate, Folin-Ciocalteu Reagent, Gallic Acid, Hexane, Mercuric Chloride, Methanol GR, Picric Acid, Sodium Carbonate Anhydrous, Sodium Hydrogen Carbonate, Sulphuric Acid, Vanillin Aldehyde Diphenylboryloxyethylamine (NP Reagent).

##### From BDH

Ammonia 25%, Boric Acid, Calcium Chloride Anhydrous, Hydrochloric Acid 32%, Formic Acid 85%, Iodine, Potassium Iodide, Potassium Hydroxide, n-Propanol, Ninhydrin, Borax,  $\alpha$  Naphthol, Copper Sulphate, Potassium Sodium Tartrate, Sodium Hydroxide, Anisaldehyde, Dinitrobenzoic Acid, Dichloromethane, n-Heptane, Magnesium Turnings, Phosphoric Acid 85%, Ethyl Methyl Ketone, Diethyl Ether,  $\beta$ -Carotene, Antimony Trichloride, Acetic-Anhydride, Lead Acetate, Gelatin, Amyl Alcohol, iso-Butanol.

##### From Surechem

Acetic Acid Glacial, Toluene.

### Equipments

Ultrasonic Bath (Hwashin· Power Sonic 405), Centrifuge (Heraeus Megafuge 2.0R), Rotary evaporator (Heidolph Laborata 4000, Germany), UV-Vis spectrophotometer (Jasco, V-650, Japan), UV Detecting Chamber 254 nm, and 365 nm lamps. Drying Oven Albeshr model DR 280 SI. Ultrapure TM water purification system (Lotun Co., Ltd., Taipei, Taiwan). Microscope (KRÜSS, MBL-2000T, Germany).

### Plant Material

Fresh leaves of *A. dioscorides* Sibth. et Sm. were collected in April and rhizomes in July 2013 from wild plants growing in Froulok region in the range (36° 03' 24"-36° 04' 75" N and 35° 42' 13"-35° 44' 52" E), near Lattaqya city northwest of Syria). The plant materials were authenticated by an expert at the Faculty of Agriculture - Aleppo University, Syria. They were washed, heated at 80 °C for 10 min and 60 °C for 30 min to deactivate enzymes, which may break down the available phytochemicals. Then they were dried in an oven with good ventilation at 35 °C, powdered using mechanical grinder and kept in airtight glass container until use.

### Microscopic Method

Anatomical crosssections were conducted in a leaf and a rhizome, they were put on a slide, covered, examined under light microscope to examine the raphides. While both of the plant leaves and rhizomes were dried and grinded to fine powder, sieved and examined under light microscope, for the examination of single calcium oxalate needles.

### Extraction Method

According to both of Andersen et al and Zhao et al: Extraction by two-phase system such as hexane with methanol-water or acetone-aqueous ammonium sulfate, combined with sonication gave better yields<sup>36,37</sup>. Therefore, a new two-phase system mixture was suggested and tried for extraction. The fundamental of this idea depends upon that the hexane phase dissolves the non-polar compounds, while the aqueous alcohol phase dissolves the polar compounds.

1 gr of the plant leaves powder was extracted using 10 ml of each solvent (Hexane, Chloroform, Acetone, Ethanol, Ethanol 65% or solvent mixture: Ethanol, Water, Hexane, Chloroform, Acetone 38:16:25:9:12 respectively) by either maceration for 72 hours at room temperature with intermittent agitation, or sonication for 10, 20, 30, 40 minutes at room temperature. After that, the mixture was centrifuged for ten minutes at 5000 cycle /minute. Moreover, the residual leave powder was re-extracted twice applying the same procedure. Finally, the combined extracts were evaporated under reduced pressure in a rotary evaporator at 40 °C until the residual material became viscose, then the viscose residues were put into a petri dish in a desiccator containing dry silica gel under reduced pressure until fixed weight. Every extraction process was repeated three times, and the extraction yields, their averages and standard deviations were calculated depending upon MS Excell 2013.

### Phytochemical Screening

All experiments were carried out as mentioned here in accordance with the fundamentals of the reference bibliographic methods with minor modifications to decrease the chemicals used or to make them applicable to all extracts.

#### *Alkaloids*

20 ml of the plant extract was evaporated to thick syrup, and subjected to partition between 4 ml 0.1N aqueous Sulphuric Acid and 5 ml Chloroform. Then the aqueous phase was made alkaline with ammonia, and alkaloids were extracted using 5 ml chloroform followed by re-extracting them with 2 ml 0.1N aqueous Sulphuric Acid. Finally, the acid layer was divided into four test tubes; 0.25 ml of each of the following reagents was added: Dragendorff's, Mayer's, Hager's and Wagner's, a precipitate of the following colors orange, cream, yellow and brown respectively is considered positive. At least two of them must give a positive result to assure the presence of Alkaloids<sup>38,39,40</sup>.

#### *Phenolic Acids*

2D-TLC silica gel 60 technique was employed with the mobile phases of n-hexane-ethyl acetate-glacial acetic acid (31:14:5) respectively, in one direction and after drying with chloroform-methanol-formic acid (44:3.5:2.5) in the second direction. Phenolic Acids were detected by spraying with 1% methanolic diphenylboryloxyethylamine and then 5% polyethylene glycol 4000, they appeared as blue fluorescent bands at 365 nm UV<sup>39</sup>.

#### *Amino Acids*

The extracts were chromatographed using TLC silica gel 60 and the mobile phase: n-butanol:n-propanol:glacial acetic acid:water (3:1:1:1, v/v respectively). The TLC was dipped in ninhydrin 0.2% in acetone containing acetic acid 3%, then heated for 10 min at 110 °C, giving the usual purple colour<sup>39</sup>.

#### *Antraquinones*

##### *Borntrager Reaction*

About 0.1 g of the dry plant extract was boiled with 6 ml of 1% HCl and filtered. The filtrate was shaken with 1 ml toluene and the toluene layer was separated. 1 ml of 10% ammonia was added to the toluene layer with good stirring and the colour of the alkaline phase was observed. Formation of pink or red colour of the water layer indicates the presence of anthraquinones<sup>41</sup>.

##### *Shouteten Reaction*

About 100 mg of the dry plant extract was dissolved in 100 ml boiling water and filtered. The filtrate was shaken with 1 g talc and filtered. 10 ml of this filtrate was diluted to 100 ml by water, with adding 0.025 g borax. Formation of yellow colour with yellowish green fluorescence at 365 nm UV indicates the presence of anthraquinones<sup>42</sup>.

##### *TLC Tests*

Separation of anthraquinone mixtures was done using ethyl acetate- methanol- water (100: 16.5: 3.5) as a mobile phase on silica gel plates. Anthraquinones were detected by their visible and UV colors, and by spraying with 10% methanolic KOH, their original yellow and yellow-brown colors changed to red, violet, green or purple besides to their fluorescence at 365 nm UV<sup>43</sup>.

#### *Carbohydrates*

##### *Molisch's Test*

Small amount of every dry extract was dissolved in a little quantity of methanol, a few drops of Molisch reagent (1% methanolic alpha-naphthol) was added with stirring and concentrated sulphuric acid was added into the sides of the test tube to form a layer. A violet ring indicates the presence of carbohydrates<sup>44</sup>.

##### *Fehling's Test*

Small amount of every dry extract was heated with equal amount of both of Fehling's A and B solutions in boiling water. Formation of Cu<sub>2</sub>O brick red precipitate indicates the presence of reducing sugars<sup>44</sup>.

##### *TLC*

Carbohydrates were chromatographed in ethyl acetate: n-propanol: acetic acid: H<sub>2</sub>O (4:2:2:1 v/v) and detected by Anisaldehyde-H<sub>2</sub>SO<sub>4</sub>: a fresh solution of 0.5 ml of anisaldehyde was dissolved in 9 mL EtOH, with adding 0.5 ml H<sub>2</sub>SO<sub>4</sub> and 0.1 mL of acetic acid. The TLC plates ought to be evenly sprayed or quickly dipped into the solution, dried, and heated at 100 °C for 5-15 min, giving colors between green to blue or gray<sup>39</sup>.

##### *Cardiac Glycosides*

About 100 mg of every extract was dissolved in 10 ml aqueous methanol 50% and boiled five minutes with 1% lead acetate, filtered then extracted three times with 4 ml chloroform, the chloroform layer was separated and evaporated on a water bath till dryness<sup>42</sup>.

##### *Keller killiani's Test*

About 100 mg of every dry extract was dissolved in 1 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayered with 1 ml of concentrated sulphuric acid. A brown ring is obtained at the interface, blue or green colour of the above layer indicates the presence of a deoxy sugar characteristic of cardenolides. A pink colour appears below the brown ring from the reaction of the concentrated sulphuric acid with the steroidal skeleton of the cardiac glycosides<sup>42</sup>.

##### *Kedde's Test*

About 10 mg of every extract was dissolved in 1 ml 2,4 dinitrobenzoic acid 1% in methanol, 0.5 ml of 2 N sodium hydroxide was added. A violet colour indicates the presence of cardenolides<sup>42</sup>.

##### *Baljet's Test*

About 10 mg of extract was dissolved in 1 ml saturated picric acid aqueous solution. 0.5 ml of 2 N sodium hydroxide was added. An orange colour indicates the presence of cardenolides<sup>42</sup>.

##### *TLC Test*

Separation of cardiac glycosides mixtures was done using the mobile phase ethyl acetate- methanol- water (100: 13.5 :10) on silica gel plates. They were detected by spraying with methanolic dinitrobenzoic acid 1%. With 1 N sodium hydroxide. A violet spots indicate the presence of cardenolides<sup>42</sup>.

##### *Coumarins*

##### *UV Test*

About 10 mg of every extract was evaporated to dryness in a test tube and the residue was dissolved in hot distilled water, cooled and divided into two test portions, one was

the reference. To the second test tube, 0.5 ml of 10 % ammonia was added. The occurrence of an intense fluorescence under UV light ( $\lambda$  365 nm) is a positive test for the presence of coumarins and derivatives<sup>41</sup>.

#### *TLC Tests*

Optimal selectivity for investigated compounds was obtained on silica layers using: ethyl acetate:dichloromethane:n-heptane 40:30:30 v/v respectively as a mobile phase. It made complete separation of coumarins<sup>39</sup>. It was treated with 1 N NaOH solution and examined at 365 nm UV light, a yellow or blue fluorescence indicates the presence of coumarins<sup>41</sup>.

#### *Cyanogenic Glycosides*

Picrate papers were prepared by dipping a filter paper in saturated aqueous picric acid neutralized with NaHCO<sub>3</sub>. For the dry extracts, put about 50 mg of the dry extracts into a tube, add 2 ml 3M H<sub>3</sub>PO<sub>4</sub>, the tube should be left at 40°C for 24-48 h, and then examined for any non-enzymic release of HCN<sup>43,45,46,47</sup>.

#### *Flavonoids*

##### *Aluminum Chloride Test*

3 ml of every extract was mixed with 1 ml 5% methanolic aluminum chloride in a test tube. Formation of yellow colour indicates the presence of Flavonoids<sup>41,42</sup>.

##### *Shinoda Test*

Few magnesium turnings was added to 3 ml of the methanolic solution of the dry extract and concentrated hydrochloric acid dropwise, pink, crimson red or occasionally green to blue colour appeared after a few minutes<sup>42,48</sup>.

##### *Alkaline Reagent Test*

To the test solution, a few drops of dilute sodium hydroxide solution is added, intense yellow colour is formed which turn to colourless on addition of few drops of dilute acid indicate the presence of flavonoids<sup>42,48</sup>.

#### *TLC Tests*

TLC separation of flavonoid aglycones is performed on silica gel plates with the following mobile phase consisting of toluene-ethyl acetate-formic acid-water (1:9:2.5:2, v/v respectively). While the flavonoid glycosides require more polar phase such as the widespread one: ethyl acetate-ethyl methyl ketone-formic acid-glacial acetic acid-water, 50:30:7:3:10. NP (natural products reagent) /PEG 4000 produces an intense fluorescence under 365 nm UV light so it is used for the location of the spots giving colors in the range: orange-yellow-green<sup>36,39</sup>.

#### *Lignans*

##### *2-D TLC Autographic*

About 100 mg of the dry plant extract is loaded on silica using the following mobile phase n-hexane-diethyl ether (7:3) in the first direction and chloroform-diethyl ether (9:1) in the second direction enabled a complete separation of lignans. The developed TLC plates were sprayed with a 0.02% solution of  $\beta$ -carotene in CH<sub>2</sub>Cl<sub>2</sub> and placed under natural light until discoloration of the background. All lignans appear as yellow spots against white background<sup>39</sup>.

#### *Saponins*

##### *Froth Test*

Small amount of the dry extracts was dissolved in 5 ml of distilled boiling water in a test tube, and shaken

vigorously; a stable persistent froth indicates the presence of saponins<sup>41,42,43</sup>.

##### *Emulsion Test*

The solution of froth test was mixed with 3 drops of olive oil and shaken vigorously; the formation of emulsion indicates the presence of saponins<sup>41,49</sup>.

##### *Haemolysis Test*

Small amount of the dry extracts was dissolved in 0.2 ml normal salt serum. After that 0.2 ml of blood (diluted in salt serum and buffered at pH=7,4) was added, mixed well and compared with control<sup>42,48</sup>.

#### *Steroids*

##### *TLC*

Separation was improved by two dimensional TLC using the following mobile phases. Hexane-diethylether (7:3 v/v) and benzene-diethylether (9:1 v/v) which were used for the separation of sterol subclasses and their conjugates in various sample matrices. Visualization of TLC spots was carried out with SbCl<sub>3</sub> and heating then exposure to UV light<sup>39</sup>.

##### *Salkowski Test*

A small quantity of the different dry extracts was dissolved in 2 ml chloroform then 2 ml concentrated H<sub>2</sub>SO<sub>4</sub> was added. The solution was shaken well. If the extract contains steroids the chloroform layer would turn pinkish, red and acid layer would show greenish yellow fluorescence<sup>50</sup>.

##### *Liebermann' Burchard Reaction*

A small quantity of the different dry extracts was dissolved in 0.5 ml dry chloroform in a test-tube and 0.1 ml acetic-anhydride and 0.5 ml acetic acid was added. A few drops of concentrated sulphuric acid were added along the side of the test-tube with cooling. Development of bluish colour in the chloroform layer immediately turning to violet and finally to green indicates the presence of sterols. However, the formation of deep pink colour in the chloroform layer indicated the presence of triterpenes<sup>48</sup>.

#### *Tannins*

##### *Ferric chloride Test*

About 0.05 g of every dry extracts was dissolved in 10 ml ethanol and filtrated into a test-tube, and two drops ethanolic ferric chloride 1% was added. Development of dark blue green or violet colour or precipitate indicates the presence of tannins<sup>42</sup>.

##### *Lead acetate Test*

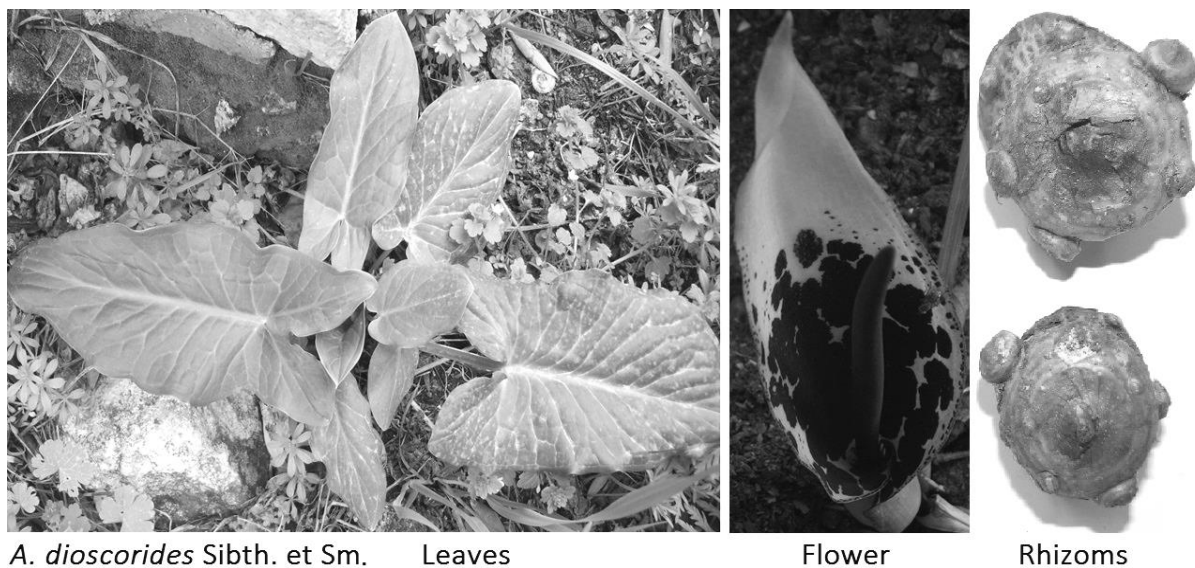
About 0.05 g of the different dry extracts was dissolved in 10 ml dd. water and filtered into a test-tube, its pH must be controlled into the range 6-8; and three drops of 9.5% lead acetate solution was added. Development of white or brown precipitate indicates the presence of tannins<sup>42</sup>.

##### *Gelatin Test*

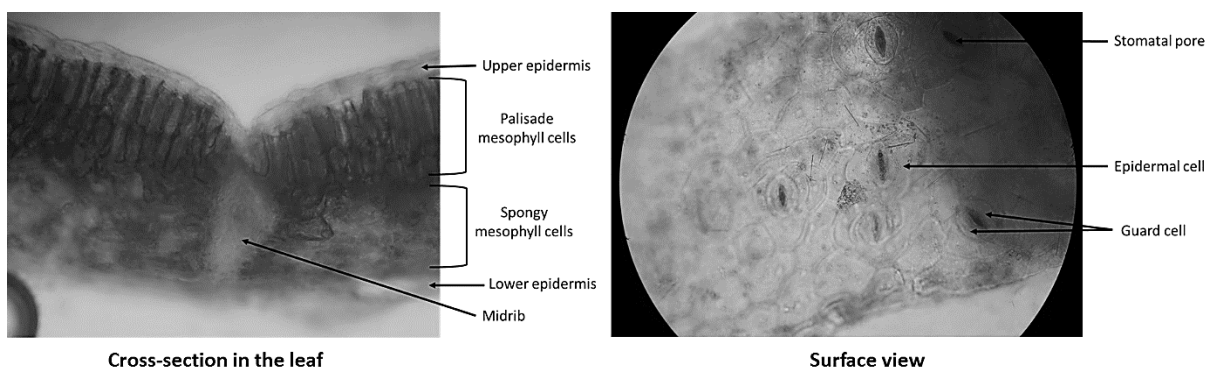
About 0.05 g of the different dry extracts was dissolved in 10 ml dd. water and filtered into a test-tube, its pH must be controlled into the range 6-8; and 2 ml of 0.5% gelatin solution was added. Development of white precipitate or suspension indicates the presence of tannins<sup>42,51</sup>.

##### *Anthocyanidin*

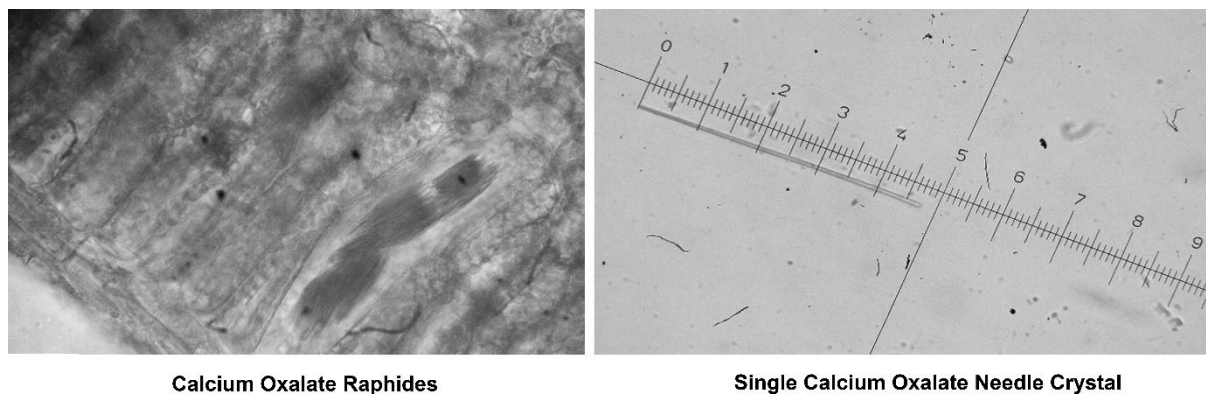
The dry extract was heated in 2 M HCl in a test tube and filtered. The cooled extract was twice washed with ethyl acetate to remove flavones, the remaining aqueous layer



*A. dioscoridis* Sibth. et Sm. Leaves Flower Rhizoms  
 Figure 1: *A. dioscoridis* Sibth. et Sm.. leaves, flower and rhizomes.



Cross-section in the leaf Surface view  
 Figure 2: *A. dioscoridis* Sibth. et Sm.. cross-section in the leaf, and a surface view.



Calcium Oxalate Raphides Single Calcium Oxalate Needle Crystal  
 Figure 3: Raphides and single calcium oxalate needles of *A. dioscoridis* Sibth. et Sm..

was heated 3 min at 80°C to remove ethyl acetate traces. The pigment was then extracted by a small volume of amyl alcohol. Which was pipetted off and concentrated to dryness on a boiling water bath<sup>43</sup>.

**TLC**

The residue was dissolved in 2-4 drops of methanolic HCl and chromatographed on paper in Forestal mobile phase (concentrated HCl-HOAc-H<sub>2</sub>O 3:30:10)<sup>43</sup>.

**Visible Spectra**

Anthocyanidins was further identified by measuring their visible spectra. About 0.05 g of the different dry extracts

was dissolved in 10 ml 1 % methanolic HCl and filtered into a test-tube, scanned in the range (250-700 nm) using methanolic HCl as a blank. If  $\lambda_{max1}$  is in the range 465-560 nm and  $\lambda_{max2}$  is in the range 270- 280 nm, and by adding alkali dropwise the colour changes from red to blue the pigment must be of anthocyanidin nature<sup>43,52</sup>.

**Proanthocyanidin and Catchins**

**TLC**

The dry extracts was dissolved in a little amount of methanol and chromatographed on paper in iso-BuOH-HOAc-H<sub>2</sub>O (14: 1 :5) mobile phase. They were detected

Table 1: The average yield percentages w/w% of *A. dioscorides* Sibth. et Sm.. leaves with STDEV.P calculated by MS Excel 2013 for triplets.

Solvent Used		Hexane		Chloroform		Acetone		Ethanol		Ethanol 65%		Mixture	
Extraction Method	Yield %	±	Yield %	±	Yield %	±	Yield %	±	Yield %	±	Yield %	±	
Maceration	0.663	0.048	1.17	0.06	4.49	0.17	6.29	0.11	9.36	0.11	10.87	0.15	
For 3 days													
With Sonication	10 min	0.764	0.057	1.28	0.07	4.81	0.15	7.05	0.15	10.46	0.13	12.47	0.12
	20 min	0.786	0.07	1.41	0.07	5.05	0.18	7.12	0.16	10.62	0.13	12.72	0.10
	30 min	0.777	0.061	1.41	0.09	4.98	0.16	7.12	0.17	10.61	0.15	12.74	0.09
	40 min	0.795	0.059	1.43	0.07	5.01	0.17	7.15	0.10	10.64	0.16	12.73	0.11

by vanillin HCl (4g vanillin with 75 ml methanol and 25 ml concentrated HCl) then warming. Red or purplish red spots are produced immediately by proanthocyanidin or catchins<sup>52</sup>.

## RESULTS AND DISCUSSION

### Microscopic Examination

At first a cross-section in the leaf was done, from which the upper epidermis, Palisade mesophyll cells, Spongy mesophyll cells, Lower epidermis and Midrib were appeared. While a surface view showed the stomatal pores, epidermal and guard cells in Figure 2.

From Figure 3: The abundance of raphides in thin sectors of *A. dioscorides* Sibth. was so significant, and so was the abundance of single calcium oxalate needle crystals in the dry powder of its leaves and rhizomes, their length was in the range 2-5.5 micrometer.

### Extraction Yields

It was clear for all solvents used (Hexane, Chloroform, Acetone, Ethanol, Ethanol 65% or solvent mixture: Ethanol, Water, Hexane, Chloroform, Acetone 38:16:25:9:12 respectively) that extraction by maceration for 72 hours gave less yields than sonication assisted extraction. Moreover, increasing the sonication time from 10 to 20 minutes significantly increased the yields, while increasing the sonication time for 30, 40 minutes had no significant effect. So it may be recommended to perform the extraction by sonication instead of maceration and for only 20 minutes, to avoid the possibility of phytochemicals dissociation. In addition, the more polar solvent the more yield it gave, except for the mixture, which gave the highest yield because of its ability to dissolve the different compounds according to their polarities in the suitable separate layers of the solvent mixture, but determining the composition of the ideal solvent mixture for extraction needs more experiments. The extraction yields are shown in the Table 1.

### Phytochemical Screening Results

The alkaloids were found in all of the *A. dioscorides* Sibth. et Sm.. extracts of both leaves and rhizomes except the hexane extracts, while all the others even the alkaline aqueous solutions contain alkaloids. In addition, that assured the presence of two types of alkaloids, the first one dissolves in water in alkaline solution in accordance with Dring et al. [22] who mentioned the occurrence of polyhydroxy alkaloids, which are soluble in water and alcohols, but they do not dissolve in chloroform or low polar organic solvents. While the second type of alkaloids

is the common one, which dissolves in water in acidic solutions, but it does not dissolve in alkaline solutions, moreover its base form (in alkaline solutions) dissolves well in less polar organic solvents.

Amino acids were detected in small amounts in the ethanol, in good amount in aqueous ethanol, while they were absent in all the others, and that was logical because of their high polarity which made them more soluble in water than aqueous methanol, but not soluble in less polar organic solvents. All extracts gave negative results for anthraquinones and cardiac glycosides, which assure that the plant is free from them. ethanol extracts showed traces of carbohydrate, while aqueous ethanol showed abundance of them, but the other extracts did not show their presence. All of the extracts of *A. dioscorides* Sibth. et Sm. contained coumarins, which indicated the presence of simple coumarins (they dissolve only in less polar organic solvents) and hydroxycoumarins that dissolve only in more polar solvents. In addition, all of the extracts contained steroids and triterpenes, which indicated the presence of free steroids and triterpenes (they dissolve in none polar and chloro organic solvents) and their glycosides which dissolved only in more polar solvents. Polar Cyanogenic Glycosides were found in ethanol and ethanol 65% extracts, as they dissolve only in polar solvents, while the other extracts were free, which indicated the presence of Cyanogenic Glycosides in both of the leaves and rhizomes in traces amounts only.

All extracts (except the Hexane extract) contained Flavonoids, which indicated the presence of acetylated or methylated Flavonoids as traces or moderate amounts, because of their solubility in chloroform and acetone, beside to the presence of hydroxylated Flavonoids (free and glycosides) as abundant amounts because of their solubility in ethanol and aqueous ethanol. The polar and relatively polar extracts contained the following phytochemicals as moderate amounts tannins, proanthocyanidins, catchins and lignans. In addition, they contained the saponins and phenolic acids as abundant amounts. The Anthocyanidins were found in the ethanol and aqueous ethanol extracts as traces in the leaves, while in the rhizomes their result was unclear, because of the Anthocyanidins positive charge, they could not be dissolved in low polarity solvents. The screening results were summarized in Table 2.

## CONCLUSIONS

The results of this study of *Arum Dioscorides* Sibth. et

Table 2: Phytochemical screening results of *A. dioscorides* Sibth. et Sm.. leaves and rhizomes.

The Extract		Hexane		Chloroform		Acetone		Ethanol		Ethanol 65%	
part used		leaves	rhizomes	leaves	rhizomes	leaves	rhizomes	leaves	rhizomes	leaves	rhizomes
Alkaloids	Dragendorff's	-	-	++	+	++	++	+++	+++	+++	+++
	Mayer's	-	-	+	±	+	+	++	++	+++	+++
	Hager's	-	-	+	+	+	+	+	+	+++	+++
	Wagner's	-	-	-	-	-	-	+	+	++	++
Amino acids		-	-	-	-	-	-	+	+	++	++
Anthraquinones	Borntrager's	-	-	-	-	-	-	-	-	-	±
	Shouteten's	-	-	-	-	-	-	-	-	-	±
	TLC	-	-	-	-	-	-	-	-	-	-
Carbohydrates	Molisch's	-	-	-	-	-	-	+	+	++	+++
	Fehling's	-	-	-	-	+	+	+	+	++	+++
	TLC	-	-	-	-	-	-	-	+	++	++
Cardiac glycosides	Keller killiani's	-	-	-	-	-	-	-	-	-	±
	Kedde's	-	-	-	-	-	-	-	-	-	-
	Baljet's	-	-	-	-	-	-	-	-	-	-
coumarins	Fluorescence	+	+	+	++	+	++	+	+	+	+
	TLC	+	+	+	++	+	++	+	+	+	++
	AICl <sub>3</sub>	-	-	+	+	++	++	+++	++	+++	++
Flavonoids	Shinoda's	-	-	-	-	++	++	++	+	++	++
	Alkaline	-	-	+	-	+	+	+	+	+++	++
	TLC	-	-	+	+	+	+	+	+	+++	++
Cyanogenic glycosides		-	-	-	-	±	±	±	+	+	+
Phenolic acids		-	-	-	-	-	-	+	+	+++	+++
Anthocyanidin		-	-	-	-	-	-	+	±	+	±
Pro anthocyanidin and catchins		-	-	-	-	+	±	++	+++	++	+
Lignans		-	-	-	-	+	+	+	+++	+	++
Saponins	Froth test	-	-	-	-	+	+	+	++	++	+++
	Emulsion test	-	-	-	-	+	+	+	++	+	+++
	Haemolysis	-	-	-	-	-	±	±	+	+	++
	Salkowski's	+	++	++	++	++	+	+	+	+	+
Steroids	Liebermann'	±	+	+	++	++	++	+	++	+	++
	Burchard	±	+	+	++	++	++	+	++	+	++
	TLC	+	+	+	++	++	++	+	++	+	++
Tannins	Fe Cl <sub>3</sub>	-	-	±	-	++	+	++	+	+++	++
	Lead acetate	-	-	-	-	+	++	+	+	++	++
	Gelatin	-	-	-	-	+	+	+	+	++	++

- Indicates the absence of phytochemicals, ± couldn't determine their presence, + Indicates trace of phytochemicals, ++ Indicates the presence of phytochemicals, +++ Indicates their presence abundantly.

Sm.. in Syria have indicated that; it is a plant rich of all tested kinds of phytochemicals except cardiac glycosides and anthranoids. In addition, it needs to be studied more in depth to explore its varied active phytochemicals quantitatively and qualitatively. Moreover, many applied studies must be done to determine its medicinal activities, beside to studying its importance as a food plant source.

#### ACKNOWLEDGEMENTS

We greatly acknowledge the research laboratories of Chemistry and Pharmacognosy Departments - Aleppo University- Syria, for the technical and financial support of the research.

#### REFERENCES

1. Linz J, Stökl J, Urru I, Krügel T, Stensmyr MC, Hansson BS. Molecular Phylogeny of the Genus *Arum* (Araceae) Inferred from Multi-Locus Sequence Data and AFLPs. *Taxon* 2010; 59(2): 405-415.

2. Mayo SJ, Bogner JJ, Boyce PC. The Genera of Araceae. Royal Botanical Gardens, Kew. 1997; 370 p.
3. Gibernau M, Macquart D, Przetak G. Pollination in the Genus *Arum* a Review. *Aroideana* 2004; 27: 148-166
4. Mouterde PSJ. Nouvelle Flore du Liban et la Syrie. Éditions De L'imprimerie Catholique, Beyrouth, Liban, 1966; 3rd Ed. Vol.1, Texte, 182-185. Froulok
5. Bruce NS, Meeuse BJD. Production of Volatile Amines and Skatole at Anthesis in Some *Arum* Lily Species. *Plant Physiology* 2004; 41 (2): 343-347.
6. Urru I, Stensmyr MC, Hansson BS. Pollination by Brood-Site Deception a Review. *Phytochemistry* 2011; 72: 1655-1666.
7. Gras A, Garnatje T, Bonet MÀ, Carrió E, Mayans M, Parada M, Rigat M, Vallès J. Beyond Food and

- Medicine, But Necessary for Life, Too: Other Folk Plant Uses in Several Territories of Catalonia and the Balearic Islands. *Journal of Ethnobiology and Ethnomedicine* 2016; 1-53.
8. Łuczaj L, Končić MZ, Miličević T, Dolina K, Pandža M. Wild Vegetable Mixes Sold In the Markets of Dalmatia (Southern Croatia). *Journal of Ethnobiology and Ethnomedicine* 2013; 9 (2): 1-12.
  9. Simkova K, Polesny Z. Ethnobotanical Review of Wild Edible Plants Used in the Czech Republic. *Journal of Applied Botany and Food Quality* 2015; 88, 49 - 67.
  10. Kochmarov V, Kozuharova E, Naychov Z, Momekov G, Mincheva I. Ethnobotany and Ethnopharmacology of *Arum maculatum* L. (Araceae) In Bulgaria with an Emphasis on Its Effect Against Haemorrhoids. *International Journal of Pharmaceutical, Chemical and Biological Sciences* 2015; 5(2): 394-402.
  11. Dogan Y, Nedelcheva A, Łuczaj L, Drăgulescu C, Stefkov G, Maglajlić A, Ferrier J, Papp N, Hajdari A, Mustafa B, Dajić-Stevanović Z, Pieroni A. Of the Importance of a Leaf: the Ethnobotany of Sarma in Turkey and the Balkans. *Journal of Ethnobiology and Ethnomedicine* 2015; doi: 10.1186/s13002-015-0002-x.
  12. Caxito MLC, Correia RR, Gomes ACC, Justo G, Coelho MGP, Sakuragui CM, Kuster RM, Sabino KCC. In Vitro Antileukemic Activity of *Xanthosoma sagittifolium* (Taioaba) Leaf Extract. *Evidence-Based Complementary and Alternative Medicine* 2015; 1-10.
  13. Gueye M, Diouf M. Traditional Leafy Vegetables in Senegal: Diversity and Medicinal Uses. *African Journal of Traditional, Complementary and Alternative Medicines* 2007; 4 (4): 469 - 475.
  14. Archita B, Sanjeet K, Kumar JP. A Review on Amorphophallus species: Important Medicinal Wild Food Crops of Odisha. *International Journal of Pharmacy & Life Sciences* 2014; 5 (5): 3512-3516.
  15. Medeiros MFT, Albuquerque UP. Food Flora in 17th Century Northeast Region of Brazil in *Historia Naturalis Brasiliae*. *J. of Ethnobiology and Ethnomedicine* 2014; 88: 49 - 67.
  16. Ferreres F, Gonçalves RF, Gil-Izquierdo A, Valentão P, Silva AMS, Silva JB, Santos D, Andrade PB. Further Knowledge on the Phenolic Profile of *Colocasia esculenta* (L.) Shott. *Journal of Agricultural and Food Chemistry* 2012; 60: 7005–7015.
  17. Zhang YH, Xu, H, Wang CF, Huai H. Diversity of Wetland Plants Used Traditionally in China: a Literature Review. *Journal of Ethnobiology and Ethnomedicine* 2014; 1-18.
  18. Pieroni AA, Nedelcheva A, Hajdari B, Mustafa B, Scaltriti K, Cianfaglione C, Quave L. Local Knowledge on Plants and Domestic Remedies in the Mountain Villages of Peshkopia (Eastern Albania). *Journal of Mountain Science* 2014; 11(1): 180-194.
  19. Afifi FU, Abu-Dahab R. Phytochemical Screening and Biological Activities of *Eminium spiculatum* (Blume) Kuntze (family Araceae). *Natural Product Research* 2011; 1-5.
  20. Patel DK, Kumar R, Laloo D, Hemalatha S. Diabetes mellitus: An overview on its Pharmacological Aspects and Reported Medicinal Plants Having Antidiabetic Activity. *Asian Pacific Journal of Tropical Biomedicine* 2012; 2(5): 411-420.
  21. Murraya AP, Faraonia MB, Castroa MJ, Alzaa NP, Cavallaroa V. Natural AChE Inhibitors from Plants and their Contribution to Alzheimer's Disease Therapy. *Current Neuropharmacology* 2013; 11: 388-413.
  22. Dring JV, Kite GC, Nash RJ, Reynolds T. Chemicals in Aroids: a Survey, Including New Results for Polyhydroxy Alkaloids and Alkylresorcinols. *Botanical Journal of the Linnean Society* 1995; 117 (1): 1-12.
  23. Meija J, Soukup VG. Phenyl-terminated Fatty Acids in Seeds of Various Aroids. *Phytochemistry* 2004; 65 (15): 2229-2237.
  24. El-Desouky SK, Ryu SY, Kim YK. Piperazirum, a Novel Bioactive Alkaloid from *Arum palaestinum* Boiss. *Tetrahedron Letters* 2007; 23 (4): 4015-4017.
  25. Hudaib M, Mohammad M, Bustanji Y, Tayyeb R, Yousef M, Aburjeie M, Aburjai T. Ethnopharmacological Survey of Medicinal Plants in Jordan, Mujib Nature Reserve and Surrounding Area. *Journal of Ethnopharmacology* 2008; 120: 63-71.
  26. Jaradat NA, Al-Ramahi R, Zaid AN, Ayesh OI, Eid AM. Ethnopharmacological Survey of Herbal Remedies Used for Treatment of Various Types of Cancer and Their Methods of Preparations in the West Bank-Palestine. *BMC Complementary and Alternative Medicine* 2016; 1-12.
  27. Afifi-Yazar FU, Kasabri V, Abu-Dahab R. Medicinal Plants from Jordan in the Treatment of Cancer: Traditional Uses vs. *In vitro* and *In Vivo* Evaluations - Part 1 Lectures GA Conference Mini Reviews. *Planta Med* 2011; 77(11): 1203-1209.
  28. Khalil A, Dababneh B. Inhibition of Phytopathogenic Fungi by Extracts from Medicinal Plants in Jordan. *Journal Biol Sci.* 2007; 7: 579-581.
  29. El-Desouky SK, Kim KH, Ryu SY, Eweas AF, Gamal-Eldeen AM, Kim YK. A New Pyrrole Alkaloid Isolated from *Arum palaestinum* Boiss. and its Biological Activities. *Arch Pharm Res.* 2007; 30: 927-931.
  30. Abu-Reidah IM, Ali-Shtayeha MS, Jamous RM, Arráez-Román D, Segura-Carretero A. Comprehensive Metabolite Profiling of *Arum palaestinum* (Araceae) Leaves by Using Liquid Chromatography-Tandem Mass Spectrometry. *Food Research International* 2015; 70: 74–8.
  31. Farid MM, Hussein SR, Ibrahim LF, El Desouky MA, Elsayed AM, El Oqlah AA, Saker MM. Cytotoxic Activity and Phytochemical Analysis of *Arum palaestinum* Boiss. *Asian Pacific Journal of Tropical Biomedicine* 2015; 5 (11): 944-947.
  32. Fatima A, Yee HF. *In Silico* Screening of Mutated K-Ras Inhibitors from Malaysian *Typhonium flagelliforme* for Non-Small Cell Lung Cancer. *Advances in Bioinformatics* 2014; 1-7.



33. Thomas E, Semo L, Morales M, Noza Z, Nunez H, Cayuba A, Noza M, Humaday N, Vaya J, Damme PV. Ethnomedicinal Practices and Medicinal Plant Knowledge of the Yuracarés and Trinitarios from Indigenous Territory and National Park Isiboro-Sécure, Bolivian Amazon. *Journal of Ethnopharmacology* 2011; (133): 153–163.
34. Caw H. The Distribution of Calcium Oxalate Crystals in Genus *Dieffenbachia* Schott. and the Relationship between Environmental Factors and Crystal Quantity and Quality. Master Thesis of Science, University of Florida 2003; 68p.
35. Marciano da Costa L, Tronto J, Constantino VRL, Fonseca MKA, Oliveira AP, Rocha da Costa MB. Extraction and Concentration of Biogenic Calcium Oxalate from Plant Leaves. *Ci. Solo* 2009; 33: 729-733.
36. Andersen OM, Markham KR. Flavonoids: chemistry, biochemistry, and applications. 1 st Ed. CRC Press, Taylor & Francis Group, NW, Boca Raton, USA. 2006, 1198.
37. Zhao YX, Liu Y, Liu F, Zheng W. Aqueous Two-phase Systems with Ultrasonic Extraction Used for Extracting Phenolic Compounds from *Inonotus obliquus*. *Chinese Herbal Medicines* 2013; 5(1): 67-72.
38. Aniszewski T. Alkaloids - Secrets of Life Alkaloid Chemistry, Biological Significance, Applications and Ecological Role. Elsevier Amsterdam, Netherland, 1st Ed. 2007, 316.
39. Hajnos MW, Sherma J, Kowalska T. Thin Layer Chromatography in Phytochemistry. CRC Press Taylor & Francis Group, LLC, Boca Raton, FL the USA. 2008, 874 pages.
40. Singh AP. A Treatise on Phytochemistry. Emedia Science Ltd. South Nutfield, UK. 2002.
41. Khan AM, Qureshi RA, Ullah F, Gilani SA, Nosheen A, Sahreen S, Laghari MK, Laghari MY, Ur-Rehman S, Hussain I, Murad W. Phytochemical Analysis of Selected Medicinal Plants of Margalla Hills and Surroundings. *Journal of Medicinal Plants Research* 2011; 5(25): 6017-6023.
42. Monajjed H, Hasan Aga I. Phytochemistry and Extraction. Practical Issue. 2 nd Ed. The Office of Books and University Publications, Damascus University, Syria, 1997, 340.
43. Harborne JB. Phytochemical Methods, A Guide To Modern Techniques of Plant Analysis. Second edition, Published by Chapman and Hall, New York, The USA. 1984, 300.
44. Ahuja J, Suresh J, Deep A, Ravi MP. Phytochemical Screening of Aerial Parts of *Artemisia parviflora* Roxb.: A Medicinal Plant. *Der Pharmacia Lettre* 2011; 3 (6):116-124.
45. Ganjewala D, Kumar S, Devi SA, Ambika K. Advances in Cyanogenic Glycosides Biosynthesis and Analyses in Plants: A Review. *Acta Biologica Szegediensis* 2010; 54(1):1-14.
46. Eze SO, Kanu CQ. Phytochemical and Nutritive Composition Analysis of *Solanum eathupicum* L. *Journal of Pharmaceutical and Scientific Innovation* 2014; 3(4): 358-362.
47. Gonzalez-Laredo RF, Flores De La Hoya ME, Quintero-Ramos MJ, Karchesy JJ. Flavonoid and Cyanogenic Contents of Chaya (Spinach Tree). *Plant Foods for Human Nutrition* 2003; 58: 1-8.
48. Prabhu A Comparative Preliminary Phytochemical Screening on the Leaves, Stems and the Roots of Three *viburnum* Linn. Species. *Der Pharmacia Sinica* 2011; 2 (3):81-93.
49. Mir MA, Sawhney SS, Jassal M S. Qualitative and Quantitative Analysis of Phytochemicals of *Taraxacum officinale*, Wudpecker. *Journal of Pharmacy and Pharmacology* 2013; 2(1): 001 - 005.
50. Harsha N, Sridevi V, Lakshmi MVC, Rani K, Vani NDS. Phytochemical Analysis of Some Selected Spices. *International Journal of Innovative Research in Science, Engineering and Technology* 2013; 2, (11): 1-4.
51. Nabatanzi A, Kabasa JD, Nakalembe I. Phytochemicals and Antioxidant Properties of Five Wild Edible Plants Consumed by Pregnant Women in Buikwe District, Uganda. *International J. of Pharmacognosy and Phytochemical Research* 2015; 7(6): 1267-1271.
52. Markham KR. Techniques of Flavonoid Identification. 1 st Ed. Published by Academic Press, New York USA. 1982, 113.