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Phytochemical Study, Hplc Analysis of Vitamins, and Elemental Mapping of Iraqi *Cardaria Daraba* L

Duaa Abbas Zughair[1*], Luma Amer Musa[2], Hala Hamid Hammadi[3]
[1]Department of Pharmacognosy and Medicinal Plants, College of Pharmacy, Mustansiriyah University, Alqadisia, Baghdad, Iraq
[2,3]Department of Pharmaceutical Chemistry, College of Pharmacy, Mustansiriyah University, Alqadisia, Baghdad, Iraq
*Duaa19882013@uomustansiriyah.edu.iq

Abstract. Cardaria belongs to Brassicaceae family which was originally known as Cruciferae and is commonly referred to as mustard or Cabbage family. *C.draba* has numerous signifigant therapeutic activities including, carminative, antiscorbutic, antioxidant, and laxative. The presence of numerous significant secondary metabolites is responsible for this function. Alkaloids, flavonoids, saponins, terpenoids, tannins, and leucoanthocyanins are all found in the aerial parts of *C.draba*. Elemental microanalysis by electron-excited X-ray spectrometry is a powerful characterization technique that has more than 60 year history. It gives comprehensive details on the constituents of substances by interrupting the characteristic X-rays that are generated by the collision of the sample with the electron beam. Despite the medicinal importance of *C.daraba*, very little work has been done in Iraq to investigate their phytochemical constituents. the present study aims to screen the phytochemical compositions of the Iraqi grown plant and to identify the presence and amounts of essential elements Atomic absorption spectrophotometer (AAS) and Field Emission Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy (FESEM-EDS) were used to identify the elements present in the plant. The antioxidant activity was determined using the DPPH method while HPLC was used to determine the vitamins in the plant. Plant extract exhibited radical scavenging activity in a dose-related manner in relation to the standard (ascorbic acid) and the IC50 value was 2.96 mg/ml. AAS analysis of Iraqi *C.daraba* revealed that the level of Fe, Zn, Cu, Ca, and Mg were 89, 46, 0.1, 13.5, and 21.5 ppm, respectively. FESEM-EDS mapping of the ethanoilc plant extract revealed the presence of oxygen (O), sodium (Na), potassium (K), chlorine (Cl), silicon (Si), selenium (Se), strontium (Sr), and thalium (Th), with percent mass 58.42%, 3.26%, 4.73%, 3.09%, 19.48%, 1.73%, 5.62%, and 7.74% respectively.

Highlights:

1. Iraqi Cardaria daraba L. contains alkaloids, flavonoids, tannins, terpenoids, and saponins with notable antioxidant activity (IC_{50} = 2.96 mg/ml).
2. HPLC analysis revealed high levels of vitamin C (42.8 mg/ml) and vitamin A (49.8 mg/ml) in the plant extract.
3. AAS and FESEM-EDS analyses confirmed the presence of essential elements such as Fe, Zn, Ca, Mg, Se, and Sr, supporting its medicinal potential.

Keywords: C.daraba HPLC, C.daraba FESEM/EDS, Vitamins in C.daraba, C. daraba antioxidant, Essential elements in C.daraba.

Introduction

Cardaria belongs to Brassicaceae family which was originally known as Cruciferae and is commonly referred to as mustard or Cabbage family. There are over 3,700 species and 385 genera in

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this family, Figure 1 [1]. The plant name comes from the Greek word kardia which means heart because the fruits have a heart like shape [2]. Is a perennial weed [3], has stiff, erect, branching stems, which are about 10 to 80 cm tall. Ash-colored soft hairs cover the stems irregularly [4]. Most of the mature leaves were alternative, simple, toothed range in size from 4 to 10 cm, and have petioles [5]. The flower clusters are somewhat conical and resemble corymbs; each bloom is about 2 mm across [6]. The fruit has inverted heart shape and is about 3 to 4 mm long [7]. *C.draba* is indigenous to Eastern Europe, North America, and Africa, including Algeria, as well as Western Asia, which include Iraq, Iran, Turkey, Armenia, and Syria [8]. Since ancient time, herbal remedies have been used in treatment for a wide range of illness. Because they are safer and better to the human body, phytomedicines have gained popularity recently. Medicinal plants are used alone or in combination to manufacture a range of therapies and they are also serving as the primary supply of raw ingredients for the different therapies [9]. *C.draba* has numerous signifigant therapeutic activities including, carminative, antiscorbutic [10], antioxidant, and laxative [11]. In addition, two gram (+) bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two gram (-) bacteria (*Pseudomonas aeruginosa* and *Escherechia coli*) are susceptible to the plant [12]. The presence of numerous significant secondary metabolites is responsible for this function. Alkaloids, flavonoids, saponins, terpenoids, tannins, and leucoanthocyanins are all found in the aerial parts of *C.draba* [13]. Glucosinalbin and glucoraphanin are two active forms of glucosinolates found in *C.draba* [14]. Glucoraphanin can be hydrolyzed by myrosinase to give an essential type of isothiocyanate (4-[methylsulfinyl] butyl isothiocyanate). This isothiocyanate can exhibit antibacterial effect against *Helicobacter pylori* and also it has antioxidant properties [15]. It has been established that over forty elements are crucial to life system for continued existence of both plants and mammals. An element is deemed crucial if it is a vital component of biological systems that carries out critical roles or if lowering its exposure below a particular threshold consistently leads in a decrease in a significant physiological activity. Screening for bioactive elements of plant origin and determining the elemental contents of commonly used medicinal plants is very critical. Elemental microanalysis by electron-excited X-ray spectrometry is a powerful characterization technique that has more than 60 year history. It gives comprehensive details on the chemical makeup of substances by intercepting the distinctive X-rays that are generated by the interaction of the sample with the electron beam. Despite the medicinal importance of *C.daraba*, very little work has been done in Iraq to investigate their phytochemical constituents so the present study aims to screen the phytochemical compositions of the Iraqi grown plant and to identify the presence and amounts of essential elements using Atomic absorption spectrophotometer (AAS) and Field Emission Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy (FESEM-EDS).

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Figure 1. *C.daraba* L

Materials and Methods

Preliminary phytochemical screening of *Cardaria daraba* L. [16]

After the plant was shade dried, the leaves were cleaned with water, allowed to air dry, and then processed into a powder using an electric blender. About 10 gram of powdered plant material was boiled with 100 milliliters ethanol and water separately. After that, both extracts were filtered with Whatman filter paper and employed in the preliminary tests.

Test for tannins (Ferric chloride test)

Ten percent of FeCl3 solution was used for tannins test, 2 milliliters of the aqueous plant matter was blended with few drops of the reaction compounds. Green-blackish or blackish-blue hues will verify the existence of tannins.

Test for saponins (Froth test)

Five milliliters of aqueous plant extract was combined with an equal volume of distilled water and shaken thoroughly for fifteen minutes. The formation of I centimeter foam which is stable for 1 minute ensure the presence of saponins.

Test for flavonoids (Alkaline reagent test)

Ethanolic extract (3ml) was mixed with 1 ml of ten percent sodium hydroxide, the formation of intense yellow color indicates the presence of flavonoids.

Test for terpenoids (Salkowski's test)

Five milliliters of ethanolic plant extract was combined with two milliliters of chloroform. This step was followed by the cautious addition of three milliliters concentrated sulfuric acid. The reddish brown color of the interphase implies a positive result.

Test for alkaloids (Wagner's reagent test)

Wagner's reagent was used to estimate the presence of alkaloid. Two milliliters of ethanolic plant matter was combined with the reaction compound (few drops) , a reddish-brown precipitate indicates a positive result.

Estimation of total flavonoids contents

Preparation of the standards and sample solution

To prepare a concentration of 1 mg/ml, ethanol was used as a solvent for the standard material (quercetin). From the stock solution five serially diluted solutions was prepared (100, 50, 25, 12.5, and 6.25µg/ml) which are needed to plot the calibration curve. Plant leaves were washed with tab water, allowed to dry at room temperature, and then pulverized into a fine powder. About 2.5 gram of leaves powder was soaked with 25 milliliters of 85% ethanol for 24 hours. The extract was filtered and evaporated by the rotary evaporator. The dry extract was solubilized in ethanol to give a concentration of 1mg/ml, which was used for the estimation of the total phytochemical contents [17].

Estimation of the total flavonoids content

For the estimation of total flavonoids content, one milliliters of the sample at a concentration of 1 mg/ml was combined with 4 milliliters of distilled water, and then 0.3 ml of five percent sodium nitrate was added. After 5 minutes, 0.3 ml of ten percent AlCl3 was added to the previous mixture and let stand for 5 minutes, and then 2 ml of one molar NaOH was included, to make a volume of 10 ml distilled water was added and the mixture was incubated for one half hour at room temperature. At a wave length of 510 nm, the absorbance was measured utilizing a UV-visible spectrophotometer.

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Methanol instead of the plant extract was used in the previous mixture as a blank. The total flavonoids content was expressed as µg quercetin equivalent/mg of dry plant extract using the calibration curve of quercetin [18].

Estimation of antioxidant activity

Stable 2,2 diphenyl-1-picrylhydrazyl (DPPH) radical was used to estimate the ability of the ethanolic leaves extract to donate hydrogen and scavenge free radicals. From the stock solution of the ethanolic extract (2 mg/1ml) five serially diluted solutions (2, 1, 0.5, 0.25, and 0.125 mg/ml) were prepared using methanol as a solvent. About 2 milliliters of 0.1 mM DPPH (4 mg of DPPH in 100 ml of absolute methanol) was mixed with 1 ml of each concentration. The absorbance of this mixture was measured at 517 nm in a UV-visible spectrophotometer after 30 minutes of incubation at room temperature in the dark. The plant extract was replaced with methanol to make the blank .As a reference standard, ascorbic acid (Vitamin C) was employed. The percentage inhibition of DPPH was estimated by application the following equation:

$$\%inhibition= A0 - A1 / A0 \times 100$$

The absorbance of the DPPH is denoted by A0 prior to the test material being added, and A1 subsequent to the test material being added. The half-maximal inhibitory concentration (IC50) was calculated from the relationship curve of % DPPH inhibition versus sample concentration [19].

High-performance liquid chromatography (HPLC)

High-performance liquid chromatography (HPLC) analysis was used to estimate the presence of vitamin C and A in the ethanolic extract of *C.darba* (1mg/ml). The analysis was performed at the College of Pharmacy/ Mustansiriyah University using a Shimadzu LC-20AD liquid chromatography system equipped with a DGU-20A5 degasser, SPD-20A UV-visible detector, and reversed-phase RP-C18 column with a 250 mm x 4.6 mm internal diameter (id) and a particle size of 5 µm. The separation was performed with isocratic elution of acetonitrile-water (50:50) and elution rate of 0.8 ml/min [20].

Atomic absorption spectroscopy (AAS) [21]

Sample preparation

The leaves were thoroughly washed with water to reduce contamination from remained soil particles. The samples were then allowed to air dry in the laboratory. The dried sample was crushed into fine particles with mortar and pestle to increase the surface area and this powder was used for metal extraction.

Procedure for metal extraction

In a beaker concentrated HNO3 (40 ml) was added to 2 gram of fine particles of *C.daraba*, the beaker was covered and left over night. Then, it was heated at 105 C° and the sample was cooled just after the vapor appeared and three milliliters of concentrated HCLO4 was added. Next, the cover of the beaker was removed and the sample was heated until it dry. Following cooling, two milliliters of concentrated HCL together with three milliliters of distilled water were added to the cooled sample. The sample was filtered in a flask and the volume completed to 25 ml with distilled water.

AAS conditions

The sample was analyzed for the presence of important elements utilizing Atomic absorption spectrometer (Shimadzu, BUCK SIENTIFIC 210/211VGP). The percentages of various elements in the solution have been determined by the corresponding standard calibration curves obtained by using

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standard AR grade solutions of the elements Fe^{+2}, Zn^{+2} , Cu^{+2}, Ca^{+2}, and Mg^{+2}. AAS parameters were optimized for wavelength, fuel gas and auxiliary gas using different hollow cathode lamps. Fuel gas was acetylene and the support gas was air. The wavelength was 213.9, 248.3, 324.8, 422.7, and 285.2 nm for Fe^{+2}, Zn^{+2}, Cu^{+2}, Ca^{+2}, and Mg^{+2}, respectively.

FESEM image and EDS mapping

OM (Leica Microscopy DM6000M, Germany) was used for preliminary elemental exploration of the ethanolic plant extract (1mg/ml). Raman microprobe spectroscopy (Thermo Fisher DXR, Waltham, MA, USA) with an Ar+laser (excitation wavelength 532 nm, 1 to 5 mW, and beam spot approximately 1 μm), FESEM (FEI NOVA Nano SEM with an operating voltage of 5 kV , Hillsboro,OR, USA), and energy dispersive spectroscopy (EDS) analysis (Oxford X-max 80, Oxfordshire, UK) were employed to characterize the samples. X-ray photoelectron spectroscopy (XPS, Thermo Scientific ESCALAB 250) with amonochromatized Al KαX-ray source (1,486.6 eV pho-tons) was used [22].

Results and Discussion

The phytochemical tests revealed the presence of flavonoids, alkaloids, tannins, terpenoids, and saponins. Table 1 illustrates the phytochemical tests of the plant.

Table 1. Phytochemical screening of Iraqi *Cardaria daraba* L.

Test	Positive Indicator	Result
Saponin Test	Foam observed 1cm in high	+ ve
Tannins Test	Dark green	+ ve
Flavonoid Test	Yellow color	+ ve
Alkaloid Test	Reddish-brown precipitate	+ ve
Terpenes Test	Reddish-brown precipitate	+ ve

Total flavonoids content was estimated using the aluminium chloride method and it was calculated from the regression equation of the calibration curve ($y=0.002x$, $R^2=0.6329$), expressed as μg quercetin equivalent/mg of dry plant extract, Figure 2. The total flavonoids content of the plant ethanolic leaves extract was 89 μg quercetin equivalent/mg of dry plant extract.

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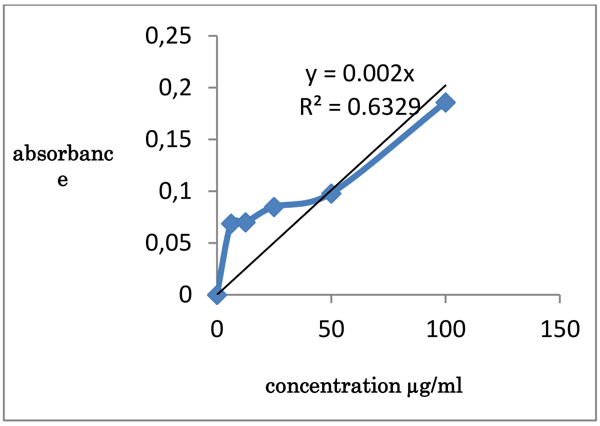

Figure 2. Calibration curve of quercetin for total flavonoids estimation.

The generation of very reactive oxygen species (ROS) with a single unpaired electron oxidative stress and is a key contributor in the pathophysiology of a number physiological illness, including cellular damage, aging, cancer, as well as liver, brain, cardiovascular and renal disorders [23,24]. Reactive oxygen radicals are produced by radiation, chemicals, toxin, deep fried and spicy meals, physical stress, and environmental contaminants. These radicals cause aberrant proteins to develop which lower the immune's system reserves [25]. Natural diets high in flavonoids and phenols and exhibiting antioxidant activity have encouraged interest in food science and nutrition, recently [26]. These substances are plant secondary metabolites with an aromatic ring containing a minimum one hydroxyl group [27]. Because of their hydroxyl group which can serve as an electron donor, flavonoids and phenols can contribute to the antioxidant activity [28]. Natural antioxidants derived from leafy vegetables are crucial for defending against the effects of free radicals [29].

Several researches verifying that consuming leafy plant vegetables full of phenols and flavonoids with powerful antioxidant activity are linked with reducing the occurrence of cardiovascular diseases, cancer, diabetes and neurodegenerative diseases [30]. Five serially diluted solutions (2-1-0.5-0.25-0.125 mg/ml) of the ethanolic plant extract were tested by DPPH method. DPPH solution can become discolored in proportion to the number of antioxidants since they can donate an electron to DPPH and forming DPPHH [31,32]. They caused the disappearance of the DPPH purple color solution in a proportional manner with the increasing of the concentration. Results were expressed as percent inhibition and summarized in Table 2. The plant extract exhibited radical scavenging activity in a dose-related manner in relation to the standard (ascorbic acid), Figure 3 and 4. The IC50 value was determined via a concentration-versus-inhibition percentage plot which was 2.96 mg/ml. Numerous polyphenolic compounds are abundant in *Cardaria daraba* L, the presence of these polyphenolic compounds contributed to the antioxidant activity of plant extract.

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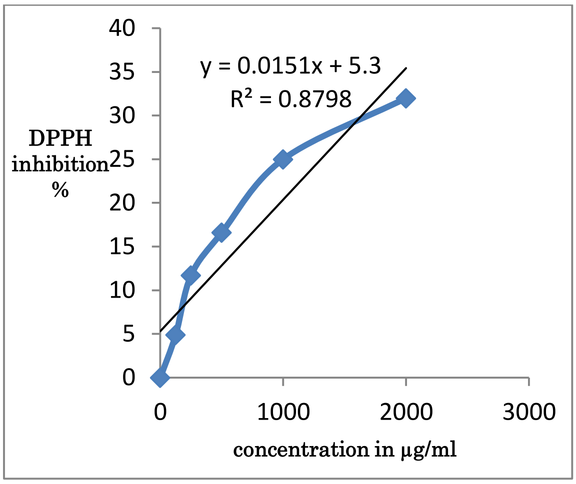

Figure 3. Curve of DPPH inhibition activity of *C.daraba* L extract

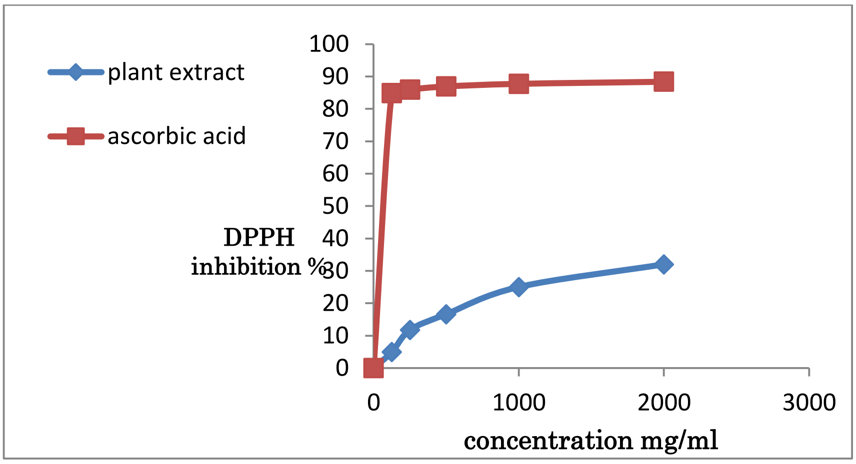

Figure 4. Dose response curve of DPPH scavenging activity of different concentrations (µg/ml) of the *C.daraba* L extract and ascorbic acid (standard antioxidant compound)

Table 2. DPPH scavenging activity of five concentrations of Iraqi *Cardaria darba* L. extract and ascorbic acid.

Concentration mg/ml	DPPH inhibition % in the presence of *C.darba* extract	DPPH inhibition% in the presence of ascorbic acid
0.125	4.9	84.83
0.25	11.7	85.96

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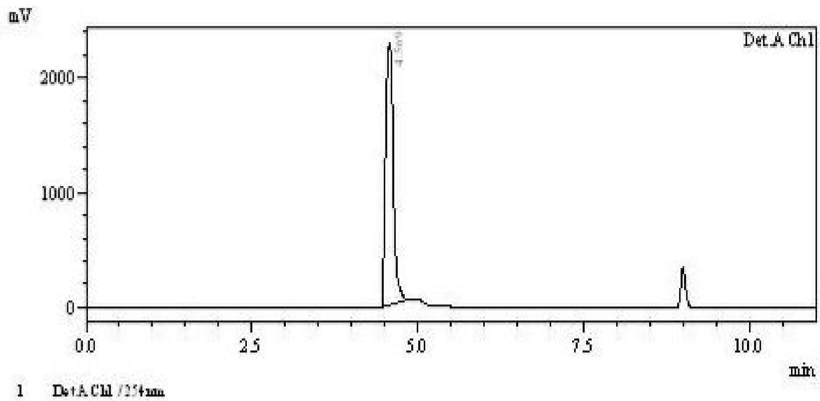
0.5	16.6	86.93
1	25	87.74
2	32	88.38

HPLC analysis of the ethanolic extract of *C.daraba* L was performed to detect the presence of vitamin C and A. The presence of these compounds was determined by comparison of the retention time (RT) of the extract with the standards. The HPLC chromatogram of Vitamin C standard revealed the appearance of peak at a RT of 4.569 min, Figure 5, while the HPLC chromatogram of the extract revealed the appearance of peak at RT of 4.480 min, Figure 6. The concentration of vitamin C in the plant extract was 42.8 mg/ml of plant extract. The HPLC chromatogram of vitamin A standard revealed the appearance of peak at a RT of 4.580, Figure 7, while for extract the peak appeared in the 4.733 min, Figure 8. The concentration of vitamin A in the plant extract was 49.8 mg/ml of plant extract. A water soluble organic compound, Vitamin C (also known as ascorbic acid, ascorbate) is essential to several biological functions. It is extensively dispersed throughout plant cells, where it performs several vital functions. Vitamin C is a strong antioxidant that can remove a variety of reactive oxygen species. It is also essential in electron transport, hydroxylation processes, and oxidative catabolism of aromatic compound [33]. Vitamin C is mostly founded in fruits and vegetables, including citrus fruits, hips, strawberries, peppers, tomatoes, cabbage, spinach and more [34].



PeakTable
Detector A Ch1 254nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.569	18290341	2285738	100.000	100.000
Total		18290341	2285738	100.000	100.000

Figure 5. HPLC chromatogram of vitamin C standard

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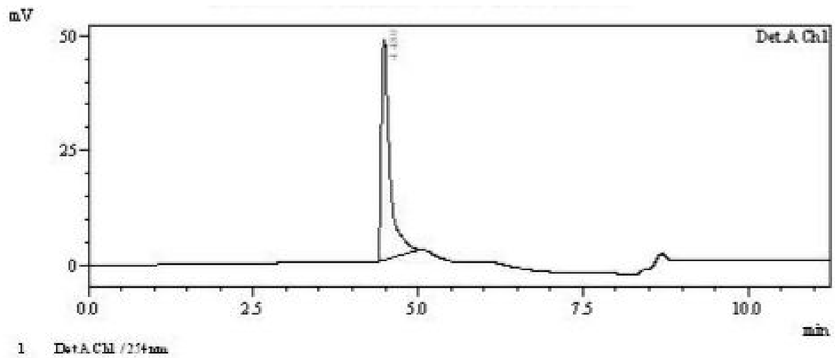
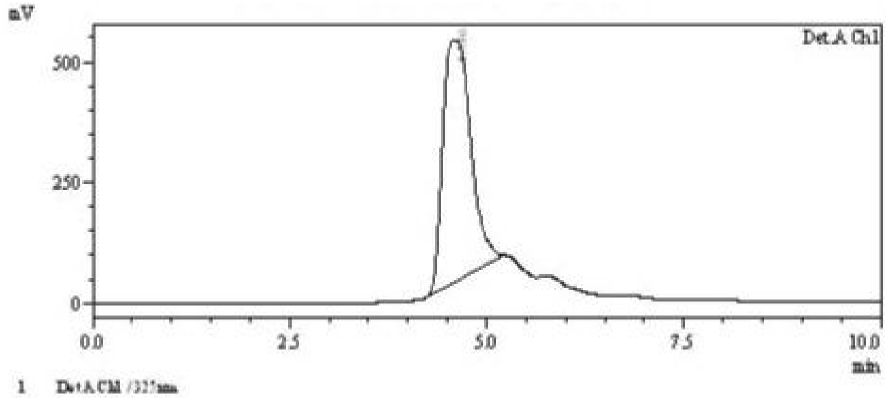


PeakTable

Detector A Ch1 254nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.480	427442	47835	100.000	100.000
Total		427442	47835	100.000	100.000

Figure 6. HPLC chromatogram of vitamin C in *C.daraba* L ethanolic extract



PeakTable

Detector A Ch1 325nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.580	12296406	500659	100.000	100.000
Total		12296406	500659	100.000	100.000

Figure 7. HPLC chromatogram of vitamin A standard

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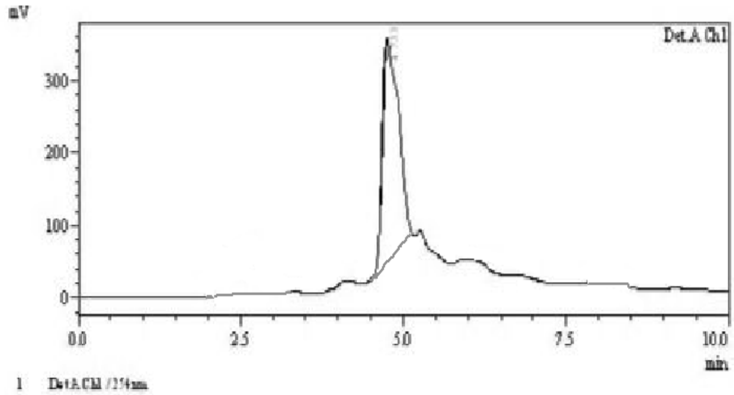

PeakTable

Detector A Ch1 254nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.733	246880	17713	100.000	100.000
Total		246880	17713	100.000	100.000

Figure 8. HPLC chromatogram of vitamin A in *C.daraba* L ethanolic extract

AAS analysis of Iraqi *C.daraba* revealed that the level of iron, zinc, Cu, Ca, and Mg were 89, 46, 0.1, 13.5, and 21.5 ppm, respectively. FESEM-EDS mapping of the ethanoilc plant extract revealed the presence of oxygen (O), sodium (Na), potassium (K), chlorine (Cl), silicon (Si), selenium (Se), strontium (Sr), and thalium (Th), with their elemental mapping and mass percentage expressed in Figure 9. Atomic absorption spectroscopy (AAS) is a spectroanalytical method which used to quantitatively detect the chemical elements and it based on the using of the absorption of free atoms to the optical light in the gaseous condition. Atomic absorption spectroscopy is based on absorption of radiation by free metallic ions. The technique is used in analytical chemistry to ascertain the quantity of a specific element in a sample. Through electrothermal vaporization, AAS can identify more than seventy elements in both solid and liquid sample and it has many of applications in toxicology, archaeology and pharmacology research. The sample in a gas state absorbs light of a particular wave length providing an excellent detection. The solid sample can be also dissolved successfully and the liquid ionizes in the gas state after being brown into a flame. The flame is illuminated with light of a certain wave length; the amount of light absorbed is proportional to the concentration of element. By setting up the standard, the quantification is achieved [35]. Elemental microanalysis by electron -excited X-ray spectrometry is a powerful characterization technique that has more than 60 years history. Physically, the technique’s principle is based on leverage in-elastic scattering of intense electron from an incident beam focused to a diameter of micrometers to nanometers to create distinctive X-rays, with the beam energy usually chosen in the range 5 KeV to 30 KeV. EDS/EDX technique has extended and exceptional applications across the physical and biological sciences. It gives detailed data on the components of substances by intercepting the characteristic X-rays that are generated by the interaction of the sample with the electron beam. This analytical process is automated by using the modern SEM-EDS in which the software is designed to analyze multiple samples with no need to adjust settings when changing them [36].

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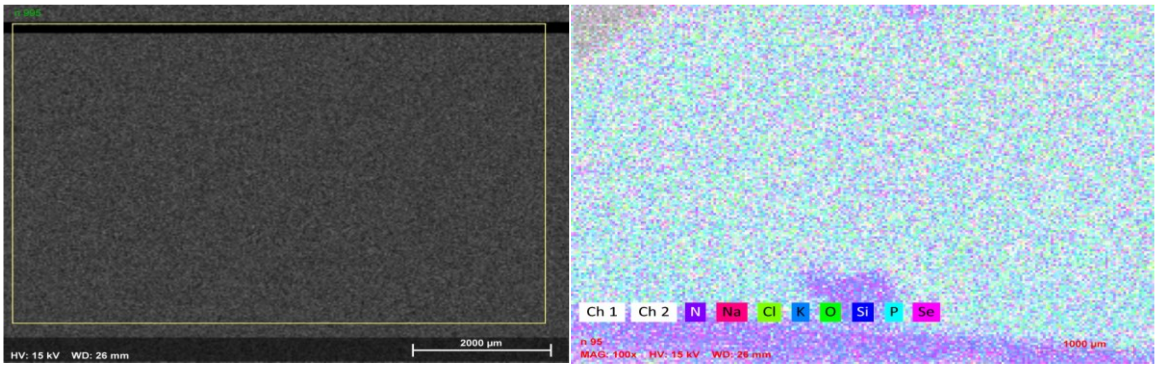
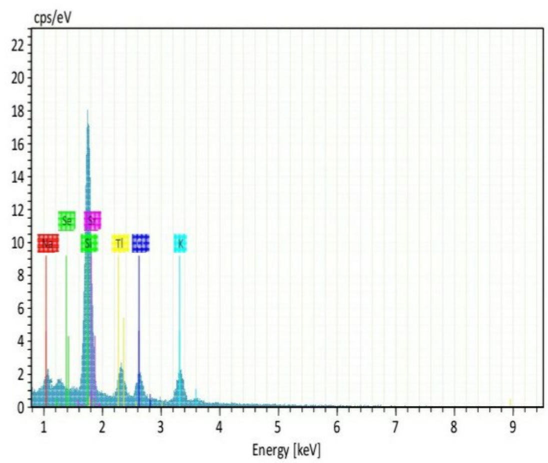
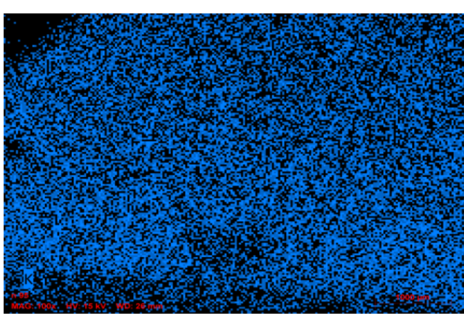


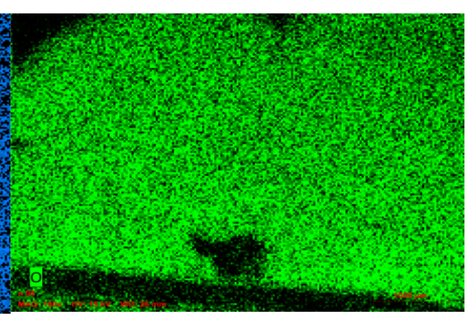
(a)



Element	At. No.	Mass [%]	Atom [%]
Oxygen	8	58.42	75.77
Sodium	11	3.26	2.95
Silicon	14	19.48	14.39
Chlorine	17	3.09	1.81
Potassium	19	4.73	2.51
Selenium	34	1.73	0.46
Strontium	38	5.62	1.33
Thallium	81	7.74	0.79
		104.07	**100.00**

(b)





(c) **(d)**

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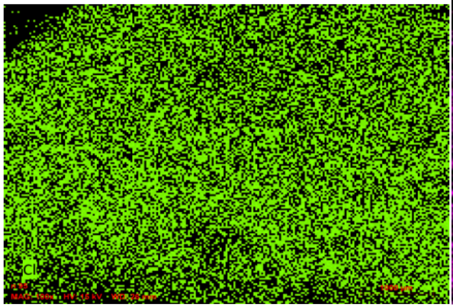

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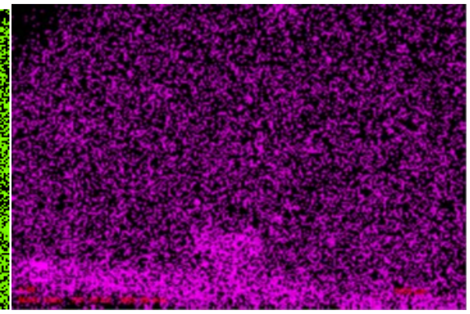

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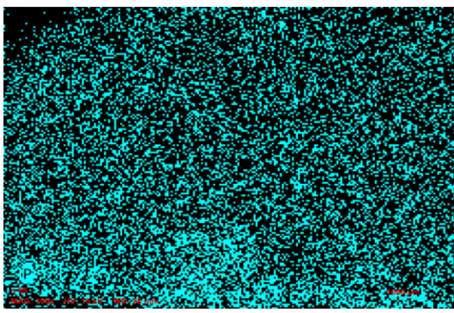

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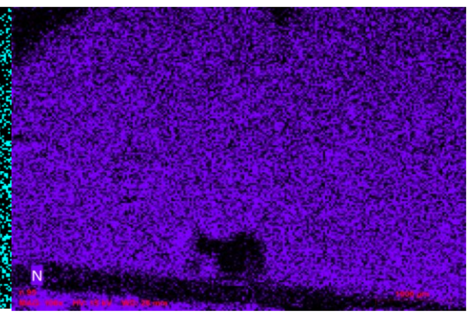

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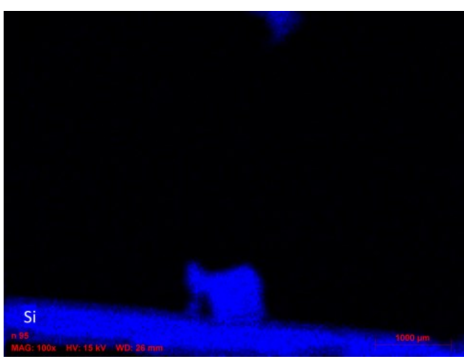
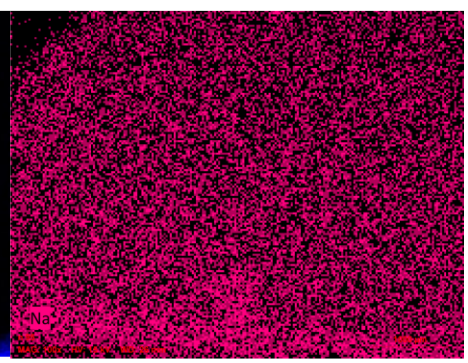


(i)



(k)

Figure 9. (a) SEM-EDS elemental mapping of Iraqi *Cardaria daraba* L , (b) Area EDS spectrum and right table for the atomic and weight percentage of varoius elements, (c) elemental mapping of **O**, (d) elemental mapping of **K**, (e) elemental mapping of **Cl**, (f) elemental mapping of **N** , g) elemental mapping of **N**a, (h) elemental mapping of P, (i) elemental mapping of **Se**, (k) elemental mapping of **Si.**

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Conclusions

Despite the medicinal importance of *C.daraba*, very little work has been done in Iraq to investigate their phytochemical constituents so the present study aims to screen the phytochemical compositions of the Iraqi grown plant and to identify the presence and amounts of essential elements using Atomic absorption spectrophotometer (AAS) and Field Emission Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy (FESEM-EDS). Elemental microanalysis by electron -excited X-ray spectrometry is a powerful characterization technique and the result revealed the presence of many elements such as Na, Cl, Sr, Si, and Se. Further elemental and phytochemical investigations of the plant are needed to detect the active phytoconstituents and essential elements quantitatively and make a correlation with the pharmacological activities of the plant using different chromatographic and analytical methods.

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