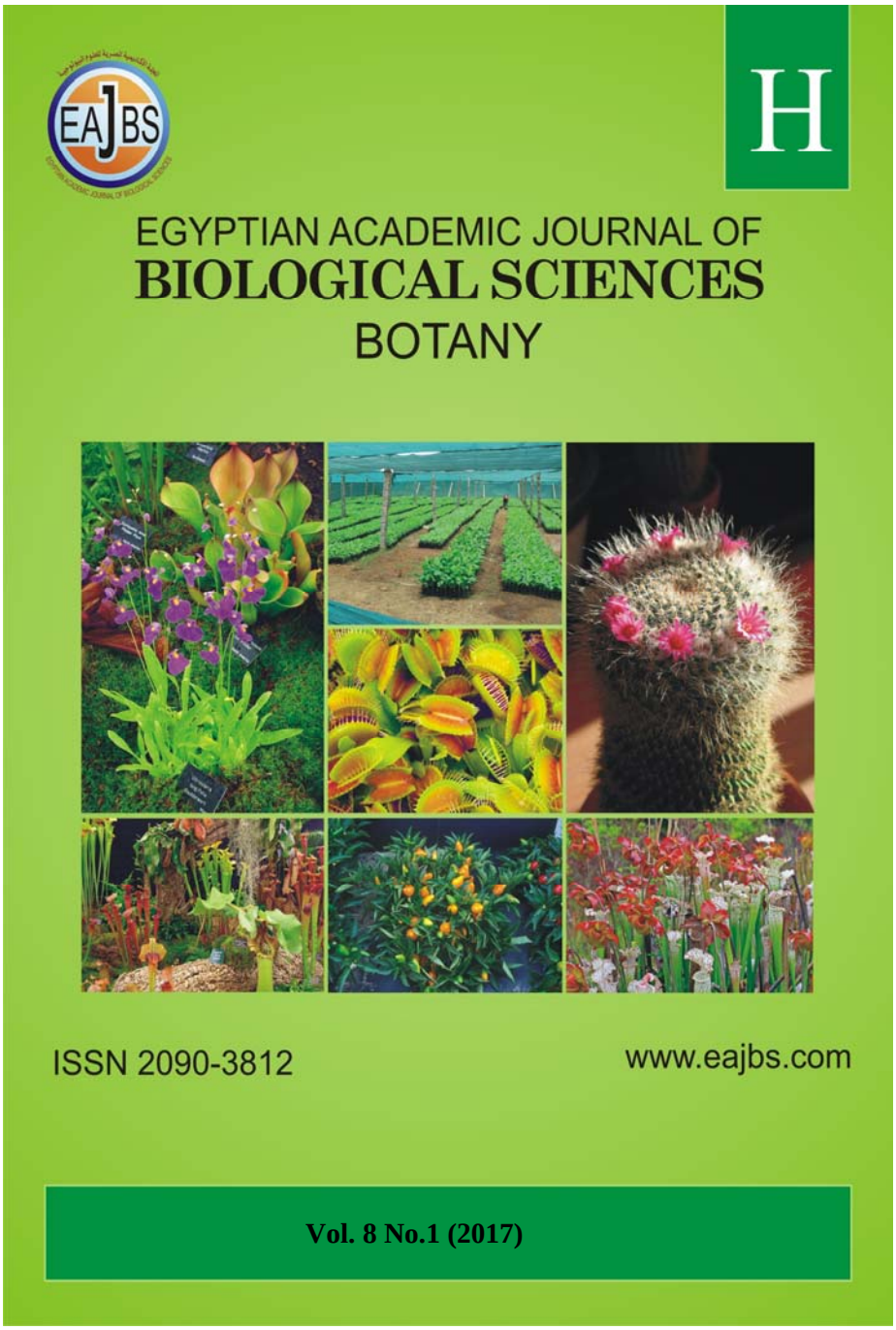

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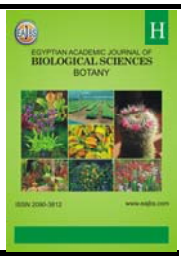

Flavonoid Contents of *Adiantum capillus-veneris* L. Growing in Two Different Districts from Iraqi Kurdistan Northern Iraq

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ABSTRACT

The present study was conducted to determine flavonoid contents in aerial parts of *Adiantum capillus- veneris* L. (Pteridaceae) collected from two different sites: Gali Ali Bek in Erbil district and Kalar in Sulaimaniyah district, in Iraqi Kurdistan-Northern Iraq by using HPLC technique. Six flavonoid compounds: Kaempferol, Kaempferol-3-O-glycoside, Luteolin, myricetin, quercetin and rutin were identified in *A.capillus-veneris* growing at both sites. These compounds showed differences in their concentrations at each site and between sites. It was concluded that flavonoid content of this fern was interrelated with site attributes.

INTRODUCTION

Adiantum capillus veneris L.(Family:Pteridaceae) is a fern species worldwide distribution, including Iraq and bordering countries (Al-Rawi and Chakravarty,2014 , Khodaie et al.2015 ; Al-Snafi, 2015). It is known as kuzburat – elber, Krafs al-bir , Shaar – ul- jibal in Arabic ,maidenhair fern in English, hansaaraja in Ayurvedic , Kazbaratul Ber in Unani, avenca in Brazil (Ahmed et al. 2012, Singh et al. ,2013 , Al-Rawi and Chakravarty ,2014, Al-Snafi , 2015). This fern is often found growing on moist, protected and shaded sandstone or limestone cliffs (Ansari and Ekhlasi-Kazaj, 2012, Ahmed et al. 2012, Al-Snafi, 2015, Khan et al., 2017). *A. capillus veneris* is small, rhizomatous, erect and evergreen herb up to 30 cm in height with black and wiry stipe (Ansari and Ekhlasi-Kazaj, 2012, Al-Snafi, 2015, Khan et al., 2017). This species has a long history of use in indigenous medicine systems and was used as anti-parasitic, anti-inflammatory, antitussive, antidandruff , astringent , demulcent , depurative , emetic , expectorant , febrifuge , laxative , stimulant and tonic (Ansari and Ekhlasi-Kazaj , 2012 , Ahmed et al. , 2012 , Al-Snafi , 2015 , Khan et al. , 2017) .Extracts from *A.capillus-veneris* had shown good microbiological activities (Pan et al., 2011 , Ansari and Ekhlasi-Kazaj , 2012 , Ishaq et al. , 2014 , Al-Snafi , 2015 , Ahmed and Nawel , 2016 , Khan et al. , 2017). Regarding the phytochemical content, many active compounds such as flavonoids, terpenoids, phenyl propanoids, steroids have been isolated from different species of the genus *Adiantum* (Brahmachari et al. , 2003 , Pan et al. , 2011 , Yuan et al., 2012 , Ansari

and Ekhlasi- Kazaj , 2012 , Ishaq et al. , 2014 , Al- Snafi , 2015 , Khodaie et al. , 2015 ,Ahmed et al. ,2015, Khan et al. , 2017) . Biological activities (antibacterial and antifungal effects) attributed to this fern might be due to its phenolic compounds , among them flavonoids as a group of polyphenol compounds with known roles in scavenging free radicals , inhibition of oxidative enzymes and anti- inflammatory effect (Singh et al, 2008 , Yuan et al. , 2012, Mierziak et al. , 2014) . However, the role of phenolic compounds, such as flavonoids, in protecting plants from environmental stresses was well documented in litratures (Alonso-Amelot et al, 2001, 2004; Chanishvili et al, 2007; Borges et al, 2013; Manan et al, 2015). Despite wide use of medicinal herbs (including ferns) in Iraq and other Arab countries , very few reports are available on active phytochemicals and biological activities of these plants (Al- Rawi and Chakravarty, 2014, Molan & Mahdy 2014). Therefore, the present study was conducted to determine flavonoids content of *Adiantum capillus- veneris* growing in two different sites from Iraqi Kurdistan / Northern Iraq in order to draw relationship between active content of this fern and site attributes.

MATERIALS AND METHODS

Adiantum capillus -veneris samples (mature sporophyte) were collected from two sites (Gali Ali Beck in Erbil district and Kalar in Suliamaniyah district) from Iraqi Kurdistan / Northern Iraq and were confirmed by Prof. Ihsan Shahbaz of the Mizzory Botanical Garden in USA. *A. capillus- veneris* L. samples were deposited in Herbarium of Erbil Botanical Garden. Geographical aspects and meteorological data of the sites are presented in Table 1& 2.

Table (1): Geographical characters of the studied sites.

Sites	Elevation (m)	Longitude	Latitude
Gali Ali Beck	559	36° 37 490 E	44° 26 540 N
Kalar	883	34° 56 501 E	45° 44 084 N

Meteorological Data:

These data were recorded at Meteorological station, Erbil and Sulaimaniyah Governorates, and are represented in Table (2).

Table (2): Metrological characters of the studied sites.

Sites	Temperature (c°)	Rainfall (mm)	Humidity (%)
Gali Ali Beck	28	65	42
Kalar	31	55.5	32

Soil Samples Collection:

Soil of studied sites were collected during April, 2016, the collected samples were brought to the laboratory in plastic bags. The soil samples were dried using hot air oven at temperature 70°c for 3 h and kept at room temperature for further analysis.

Preparation of plant extracts: The collected plant samples were brought to the laboratory in plastic bags and the aerial parts of plant were separated and washed with tap water followed by distilled water. The plant was blotted on the blotting paper and spread out at room temperature in shade for a week. The shade dried samples were ground to fine powder using electrical grinder and then the powdered samples were

stored in refrigerator at 4° c for further analysis. The plant powder (5 gm) were extracted with 50 ml of methanol (BDH) 99%, using shaker water bath (12 h) at a temperature 40 °c. The methanol extraction were filtered through filter paper (Whatman No.1), after filtration the supernatant was evaporated at room temperature to obtain extract as semi-solid materials and then the extract was stored in sealed vials at (-4°c) for further analysis .

HPLC analysis: Suarez et.al. (2005)

The dried crud extract was dissolved in 100 ml mobile phase, after filtering through a filter paper and a 0.45 mm membrane filter (Millipore), the extract was injected into HPLC instrument by an auto sampler according to the optimum condition. The main compound were separated on FLC (fast liquid Chromatographic column) under the optimum condition column: C18-DB, 3µm particle size (50X 2.0 mm I.D) column, mobile phase: linear gradient of, solvent A 0.05% trifloroacetic acid (TFA acid) in deionized water: solvent B was 0.05% TFA in methanol, pH, 2.5 gradient program from 0% B to 100% B for 10 minutes.

Flow rate 1.1 ml/ min.

Detection: UV at 280 nm.

Calculation:

Concentration of sample µg/ ml = area of sample/ area of standard X conc. of standard X dilution factor. The separation occurred on liquid chromatography Shimadzu 10 AV- LC equipped with binary delivery pump model LC- 10A Shimadzu, Japan) the eluted peaks were monitored by UV-Vis 10 A- SPD spectrophotometer.The data were printed on LC-6A integrate, (Shimadzu).

The retention time and the area of standard flavonoids were recorded in Fig (1). HPLC analysis revealed six major peaks in the retention time range of 1.25-6.20 min. (Table3).

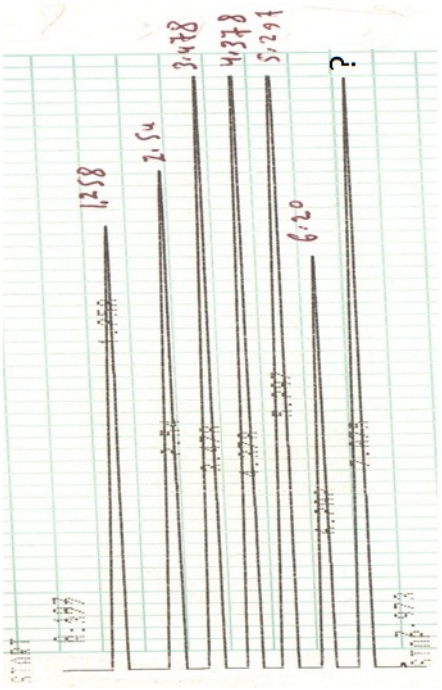

Fig. 1: Major peaks of retention time and the area of standard flavonoid compounds.

Table (3): The retention time and the area of standard flavonoid compounds.

No.	Subject	Retention time (min)	Area
1	Quercetin	1.25	67880
2	Rutin	2.54	98186
3	Luteolin	3.47	114892
4	Kaempferol	4.37	109560
5	Kaempferol-3-O-glycoside	5.29	107439
6	Myricetin	6.20	75818

RESULTS AND DISCUSSION

In this study, soil analysis revealed clear differences in soil characteristics including soil color, texture, TOC, ionic contents, and EC between the two sites studied (i.e., Kalar and Gali Ali Beck sites) (Tables 4 and 5). Soil pH in both sites was in weak alkaline side (pH 7.2-7.8) (Table 5) . Both sites, as parts of Erbil and Sulaimaniyah districts, were also differed in geographical (altitude and latitude) and meteorological aspects (humidity, temperature, and Rainfall) (Tables 1 and 2).

Table (4): Soil properties of the studied sites.

Site	Color	Texture	Total organic content% (TOC)
Gali Ali Beck	Light red	Sandy stone	5.6
Kalar	Light brown	Sandy clay	12.4

Table (5): Soil chemical characters of the studied sites.

Site	pH	EC µsem./cm	CO_3 (ppm)	NO_3 (ppm)	PO_4 (ppm)	Ca (ppm)	Mg (ppm)	K (ppm)	Na (ppm)
Gali Ali Beck	7.2	260	240	3.0	0.5	40	32	40	32
Kalar	7.8	440	155	0.8	0.13	44	40	28	68

Six flavonoid compounds: kaempferol , kaempferol-3-O-glycoside,luteolin, myricetin, Quercetin, and rutin were identified in *Adiantum capillus-veneris* L. growing at both sites (Table 6). Table 6 shows differences in concentration of these compounds in each studied site and between sites as well. In comparison to other compounds, kamphaerol-3-O-glycoside and myricetin recorded the highest concentrations (135.5 and 90 µg/ml, respectively) in Gali Ali Beck while rutin and luteolin recorded the highest concentrations in Kalar site (315.2 and 209.3 µg/ml, respectively) .However, kaempferol (44.4µg/ml) and quercetin (14.1µg/ml) showed the lowest concentration in Gali Ali Beck and Kalar sites, respectively (Table 6) .

Table (6): Total flavonoids (µg / ml) for the studied sites.

Site	Quercetin	Rutin	Kaempferol	Kaempferol-3-o-glycoside	Luteolin	Myricetin	Total flavonoids
Gali Ali Beck	63.3	68.6	44.4	135.5	84	90	485.8
Kalar	14.1	315.2	35.4	59	209.3	30.8	664.5

The high content of kaempferol 3- O- glycoside and rutin in *A. capillus -veneris* in studied sites may indicate a key role to play by these two compounds in protecting the fern against environmental stresses (Alonso-Amelot et al,2004) knowing that kaempferol glycosides and rutin are the main flavonoids isolated from *A. capillus – veneris* (Pan et al. , 2011 , Nilforoushzadeh et al. , 2014). Beside this, luteolin (209.3µg/ml) and rutin (315.2 µg/ml) showed higher concentrations at Kalar site than in Gali Ali Beck site (Table 6) and this variation may assume that the two compounds react to environment in a manner separating them from other flavonoids recorded at both investigated sites (Table 6) . Results also showed that total flavonoid content in Kalar (664.5µm/ml) was higher than in Gali Ali Beck (485,8µm/ml) (Table 6) and this may be linked to stresses created by environmental factors (like altitude , humidity , temperature and soil physicochemical properties) associated with the two sites . Concentrations of phenolics like flavonoids can be influenced by environmental changes (Hatano et al., 1986, Salminen et al. 2001, Monteiro et al., 2006, Borges et al., 2015). So phenolic compounds and other active compounds act as chemical interface between plants and environment (Gobbo-Neto &Lopes, 2007) and changes in their concentrations may be used as criterion in estimating the degree of stresses and plant responses to environmental factors. Such changes in flavonoid concentrations can affect directly the quality of the fern for medicinal use. The present study revealed that differences in sites attributes (soil characteristics, altitude and climatic factors) were clearly reflected on flavonoid content of the fern .Thus, the site and its environmental factors were interrelated with flavonoid content of *A. capillus -veneris*.

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