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A Comparative Study on Flavonoid Content of Cheilanthes Pteridioides (Richard.) C. Chr.1905 in Two Different Districts - Iraq

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Abstract

The studied regions were comprised of Erbil and Garmian districts, there were differences in geographic locations and habitat. The collected ferns were from 1st in Mallakan region, and 2nd in Kalar region from Erbil and Garmian districts respectively. Four active compounds qualitatively (flavonoids, tannins, alkaloids and saponins) were screened in two studied regions. Flavonoid compounds screened in the Mallakan region represent four flavonoids which were: Myrectin, Kaempferole and Rutine , but in Kalar region represent: Kaempherol, Myrectin, Qurectin and Rutin. A phytochemical quantitative study revealed that total flavonoids in Kalar region (540 µg/ml) were higher than total flavonoids in the Mallakan region (280 µg/ml). The study aimed to determine the flavonoids of Cheilanthes pteridioids (Richard) C. Chr. (Pteridaceae) in two different districts North of Iraq.

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1. Introduction

Ferns are regarded as rich in bioactive compounds in plants [1]. Pteridophytes have measurable indications and they may reflect the environmental factors changes [2]. They grow in moist and shaded regions and they were found from sea level to the highest mountains and are of much interest [3]. Several authors have studied the medicinal properties of Pteridophytes [4-6]. Some species of fern grow in water tropics and sub-tropics and some occur in arctic regions and few in deserts, tolerating extreme heat and drought [7]. Cheilanthes genus belongs subfamily Cheilanthoideae, family Pteridaceae, and these taxa are worldwide distributed, particularly in southwestern North America [8]. Cheilanthes Sw. is a diverse taxon composed of about 150 species [9]. The cheilanthioid ferns are found on rock edges, bluffs, in cavities and overhanging rocks, The leaves, often densely covered with trichomes, spring directly from the rootstocks Figure 1, in addition, some species prefer limestone or calcareous sandstone [10]. Members of cheilanthioid ferns thrive in xeric and too-dry habitats for most other ferns [11]. Recently scientific research focused on the phytochemistry of pteridophytes because this group of plants contains bioactive compounds such as flavonoids, terpenes, alkaloids, saponins and tannins as a natural product [12-17]. This paper regards one of the series of investigations which is dealing with ferns that have not received any attention to deal with scientifically so far in Iraq.

2. Materials & Methods

A. Study area: The metrological data were received from metrology stations in Erbil and Sulaimaniyah Governorates Table 1. GPS from Mallakan site: elevation (559 m a.s.l), Lat. 36º 37. 496 N, Long. 44º 26. 533 E. and Kalar site: (233 m a.s. l), Lat. 34º 42. 821 N, Long. 045º 27. 867 E.

B. Plant samples collection: Cheilanthes pteridioides (mature sporophyte) were collected from two studied sites. The plant was classified according to (Smith et al. 2006). The aerial parts of samples were cleaned and dried at room temperature for (7-10 days) and ground to very fine pieces, then the samples were stored at -4 °C.

C. Crude extract preparation: 10 g ground samples dissolved in 100 ml of alcohol and using a shaker water bath to extract the sample for 12 hours. The supernatant was collected and the

solvent was evaporated to prepare crude extract [19].

D. Phytochemical assay: Phytochemical screening qualitatively was done according to procedure using by [20].
E. Quantitative analysis: The bioactive compounds were determined quantitatively following standard method using by [21].
F. HPLC analysis: Following the standard method in [22], the dried crud extract was dissolved in the 100 ml mobile phase, after filtering through a filter paper and a 0.45 mm membrane filter (Millipore 0.45 µ), the extract was injected into HPLC instrument by an autosampler, according to the optimum condition. The main compounds were separated on the FLC (Fast Liquid Chromatographic) column under the optimum condition Column.
G. Mobile phase: Linear gradient of, solvent A 0,1%formic acid, gradient program from 0%B to 100%B for 10 minutes.
H. Flow rate: 1.2 ml/min.
I. Detection: UV 280 nm.

Figure 1. Cheilanthes pteridioides (Richard.) C. Chr.(Kalar region). Plant scientific classification by [18] as follows: Division: Pteridophyta, Class: Polypodiopsida, Order: Polypodiales, Family: Pteridaceae, Genus: Cheilanthes, Species: C. pteridiods (Richard) C. Chr. 1905.

J. Calculation:

$$Concentration_{Sample}\left(\frac{\mu g}{ml}\right) = \frac{Area_{sample}}{Area_{standard}} \times Concentration_{standard} \times Dilution\ factor \quad \ldots (1)$$

The separation occurred on liquid chromatography Shimadzu 10 AV-LC equipped with binary delivery pump model LC-10A Shimadzu, the eluted peaks were monitored by UV-Vis 10 A-SPD spectrophotometer. The retention time and the area of standard flavonoid compounds: quercetin, rutin, luteolin, kaempferol, kaempferol-3-O-glycosids, and myricetin.

Table 1. The meteorological data (annual maen) from studied sites.

Regions	Temp. (c)	Rainfule. (mm)	Humimidity. (%)
Malakan/ Erbil	36	120	69
Kalar/ Garmian	40	58.5	55

3. Results and Discussion

Qualitative phytochemical screening of plant methanol extracts in two studied sites revealed positive tests for bioactive compounds table 2.

Table 2. Results of qualitative screening in studied sites. (++) presence

Plant materials	Active compounds	Kalar	Mallakan
sporophyte aerial parts	Flavonoids	++	++
	Saponins	++	++
	Taninns	++	++
	Alkaloids	++	++

Many researchers have authenticated that the aerial parts of the ferns contain many bioactive compounds such as flavonoids, saponins, tannins and alkaloids [23]. Flavonoid compounds were assayed in the plant extract by using High-Performance Liquid Technique (HPLC). Three and four bioactive compounds were obtained in Malakan and Kalar respectively which are summarized in table 3.

Table 3. Flavonoids compounds concentrations (µg/ml) in studied sites. (--------): Absent

Flavonoids	*Malakan*	*Kalar*
Querctine	--------	101
Kaempferole	110	200
Myrectine	33	69
Rutine	137	170
Total	280	540

The quantitative phytochemical screening of the fern extract showed differences in these flavonoid

compounds in the regions and between them. In comparison to other compounds, kaempferol and rutine (200 and 137 µg/ml) were recorded in Malakan and Kalar respectively Table 3, while myrectine was recorded as the lowest concentrations (69 and 33 µg/ml) in the two studied regions Table 3. Kaempferole was recorded the high concentration in the Kalar region may indicate that this compound is important in fern resistance to ecological factors [24]. The fern total flavonoids (540 µg/ml) in Kalar region were higher than that recorded (280 µg/ml) in the Malakan region Table 3 and this may be linked with environmental factors (temperature and humidity) associated with the two studied regions table 1. The phenolic compounds were influenced by ecological variations (geographical position, temperature and climatic factors) [24, 25]. So, the bioactive compounds like flavonoids which interacted between the ferns and environmental factors and the variations of concentrations may be as a result of Eco physiological responses to the environment [26]. The results showed differences in geographical position between the two studied regions were clearly reflected in the chemical constituents of the two different regions [27]. Furthermore, the bioactive compounds can be influenced by environmental factors in the studied regions [28, 29]. Thus, the ecological factors were interrelated with the chemical constituents of the investigated fen different habitats. This report gives a piece of good information for future research, especially for medicinal ferns in Iraq.

4. Conclusions

In the studied sites the environmental factors affected clearly the flavonoid content in the methanol extract of the investigated fern. There was a relationship between the abiotic stresses such as (temperature, and humidity) and the concentration of flavonoid contents.

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