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Determination of Alkaloids Constituents in Some Ferns by Using High Performance Liquid Chromatography Technique – Iraq

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Abstract:

Ferns are considered as important medical herbs. They produce a wide range of secondary metabolites that could be useful in treating different diseases. However, they still remain underexplored in medical aspects in Iraq. Hence, the purpose of this study was to identify alkaloids components in the methanol extracts of four fern species (*Asplenium ceterach* L., *A. scolopendrium* L., *Cheilanthes pteridioides* (Richard) C. Chr. from Malakan region in Erbil Governorate and *Equisetum ramosissimum* Desf. from Chemi Rezan in Sulaimaniyah Governorate, of northen Iraq during March - June 2018. The research was conducted by using High performance liquid chromatography (HPLC) technique to identify the alkaloid compounds. Seven types of alkaloids (martensine, almazoline, caulerpin, hordenine, corilagin, colocynthis and isoquinoline) were detected in four fern methanolic extracts. The results showed that the alkaloids varied in concentrations between the studied localities as well as in variance of the alkaloid content between these species. The results revealed that caulerpin was recorded as the highest concentration (563.3 µg/ ml), while isoquinoline was recorded as the lowest (23.7 µg/ ml) in both *A. scolopendrium* and *C. pteridioides*. In this study the highest total alkaloid content was recorded 1460.1 µg/ ml in *E. ramosissimum* extract and the lowest content (603.8 µg/ ml) was recorded in *C. pteridioides* extract during the study period.

Keywords : Ferns, Alkaloids, HPLC, Iraq.

تحديد المركبات القلويدية في بعض أنواع الخنشاريات بإستخدام تقنية الكروماتوغرافيا السائل عالية الدقة –العراق

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الخلاصة:

تعد السرخسيات اعشاب طبية مهمة، إذ تنتج مدى واسع من مركبات الايض الثانوي التي تعد من المركبات ذات الاهمية الطبية في معالجة أنواع مختلفة من الامراض وبالرغم من هذه الأهمية الطبية الا انها

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لازالت غير مكتشفة في العراق .لذا فان الهدف من الدراسةالحالية هو تسليط الضوء على تشخيص المركبات القلوية في المستخلص الكحولي في اربعة أنواع من السرخسيات وهي *Asplenium ceterach* L., *A.scolopendrium* L., *Cheilanthes pteridioides* (Richard),C. Chr. من منطقة ملكان في محافظة أربيل والنوع *Equisetum ramosissimum* Desf. من منطقة جمي ريزان في محافظة السليمانية شمال العراق خلال الفترة من آذار لغاية حزيران من عام ٢٠١٨ .

تم في هذا البحث استخدام تقنية الكروماتوغرافيا السائل عالية الدقة لتحديد المحتوى القلويدي للخنشارياتأربعة، اذ تم تشخيص سبعة أنواع من القلويدات في المستخلص الكحولي لاربعة أنواع من الخنشاريات، والمركبات هي :, martensine, almazoline, caulerpin و , hordenine, corilagin, colocynthis colocynthis isoquinoline. أوضحت النتائج وجود تغاير في تراكيز القلويدات باختلاف انواع النباتات وأماكن تواجدها،كما اوضحت الدراسة ان مركب caulerpin قد سجل أعلى تركيز والذي بلغ (563.3مايكروغرام\ مل) بينما سجل مركب Isoqurnolin اقل تركيز وبلغ (23.7 مايكروغرام\مل) قي كل من النباتين *A. scolopendrium* و *C. pteridiodes* . سجلت اعلى تركيز من المحتوى الكلي للقلويدات (1460.1 مايكروغرام\مل) في مستخلص النوع *E. ramosissimum* واقل محتوى (603.8 مايكروغرام\مل) في مستخلص النوع *C. pteridiodes* خلال فترة الدراسة.

1. Introduction:

In the kingdom plantae, ferns represent an important phylogenetic bridge between lower and higher plants because they have an ability to produce very unique types of secondary metabolites, many of which are not produce in other vascular plants [1]. Based on the scientific literatures, very few studies have already been done on the phytochemical composition of ferns, even though their ethnobotanical importance has been studied by numerous authors [2]. Herbal use of ferns has become more common throughout the world. Ethnobotanical investigations can provide insight into other medical systems that differ from the biomedical model [3]. One of the most interesting types of plant secondary metabolites are the alkaloids, mostly on account of their involvement in ecological interactions [4] , and of their pharmacological activities which has led to their wide use as drugs [5]. The beginning of 21st century brought up this trend in medicine discovery. Plants contain secondary metabolites such as alkaloids, phenols, saponins, tannins and flavonoids that are used for the remedy and cure of certain diseases [6]. The medicinal value of the pteridophytes has been known for several years Ho *et al.*,2011[7]and Mir *et al.*,2013[8] reported that antimicrobial and antioxidant properties of ferns are remarkable compared to the higher plants, probably due to the presence of a large number of defensive biochemical compounds. Traditionally people have used pteridophytes as medicine and antibacterial agents. Studies on the pharmacology and phytochemistry of ferns have shown that they contain a wide array of alkaloids, flavonoids, polyphenols, terpenoids and steroids [9, 10, 11, 12, 13].

2. Materials and methods

Study area

The samples were collected from Malakan region in Erbil Governorate which has a height of 559 meters above sea level (m.a.s.l), with 112.5 mm as an average annual rainfall, with temperature being39°C and 65% humidity , GPS : Lat. 44° 26 540 N, Long. 36° 37 490 E. and Chemi Rezan region in Sulaimanyah Governorate has a height 736 m a.s.l, with average annual rainfall 171.5 mm per year, temperature bring 33ºC and having 82% humidity, GPS: Lat. 34° 96.050 N, Long. 39° 64.065 E**.** This research was held from March to June 2018. The scientific classification of the species as followed by [14] .

Division: Pteridophyta

Class: Polypodiopsida

Order: Polypodiales
Family: Pteridaceae
Subfamily: Cheilanthioideae
Genus: *Cheilanthes*
Species: *C. pteridioides* (Richard) C.Chr. (lip fern)
Order: Polypodiales
Family: Aspleniaceae (Spleenworts)
Subfamily: Asplenioideae
Genus*: Asplenium*
Species*: A. ceterach* L. (Rustyback fern).
Species*: A. scolopendrium* L**.** (Heart tango fern).
Order: Equisetales
Family: Equisetacea
Genus: *Equisetum*
Subgenus: Hippochaet
Species*: E. ramosissimum* Desf. (Branched horsetail).

Plant material and extract preparation:

Specimen of fern species were collected from Chemi Rezan region in Sulaimaniyah Governorate and Malakan region in Erbil Governorate. Samples used in this investigation were aerial parts (mature sporophyte) of ferns that have been identified by Prof. Dr. Maulood. B.K Chairman of Howler Botanical Garden-Erbil (Figure (1a-d.)
The samples were dried in the shade for 10-14 days before grinding them to a fine powder which was then stored for future analysis.

Methanol extraction:

Ten grams of each plant powder was added to 100 ml of methanol in a conical flask, plugged with cotton wool. The supernatant was collected after 24 hours. The solvent was later evaporated to make semisolid crude extract and stored at - 4℃ in a tightly sealed container for chemical analysis afterwards.

Qualitative phytochemical screening:

The qualitative phytochemical analysis of methanol extracts of *Cheilanthes pteridioides, Asplenium ceterach, A. scolopendrium and Equisetum ramosissimum* were conducted in materials laboratories in the Ministry of Technology and Science, following the procedures laid down by Harborne[15].

Quantitative phytochemical Screening :

The quantitative amounts of the alkaloids were determined using standard procedures as described by Suarez *et al.* [16] and Sulasmi *et al.* [17] .
High Performance Liquid Chromatography (HPLC):

Ten mgs of each crude extract was dissolved in 50 ml of methanol (HPLC grade) to get 200 ppm which was further diluted by dissolving 1 ml of this solution to 50 ml methanol.
Procedure: 20 µl of standard and sample were injected to HPLC and then recorded the chromatogram. The content sof alkaloids were calculated for each sample in comparison with standards, Rt. ranged (1.41-8.39) (Table 1).

Analytical method:

HPLC estimation of alkaloids was performed on Shimadzu 10A HPLC system, equipped with 10Avp UV detector. For estimation of alkaloids, the extracts of alkaloids according to

enclosed procedure were separated on FLC (Fast Liquid Chromatographic) column, 3um particle size, phenomenex C-8 (50 x 4.6 mm I.D), mobile phase: mixture of acetonitrile: Methanol: Ortho phosphoric acid (55:45:1 v/v)
UV detection set at 280 nm, flow rate 1.0 ml/min.

Calculation:
Concentration of sample µg/ ml = area of sample /area of standard X concentration of standard X dilution factor[16] .

Table 1: Retention time and area of the standard alkaloids

N.	Subjects	Ret. Time minute	Area
1	Isoquinoline	1.41	**77909**
2	Corilagin	2.24	**115807**
3	Colocynthis	4.05	**102518**
4	Hordenine	5.40	**106323**
5	Caulerpin	6.22	**148669**
6	Almazoline	7.37	**143413**
7	Martensine	8.39	**114305**

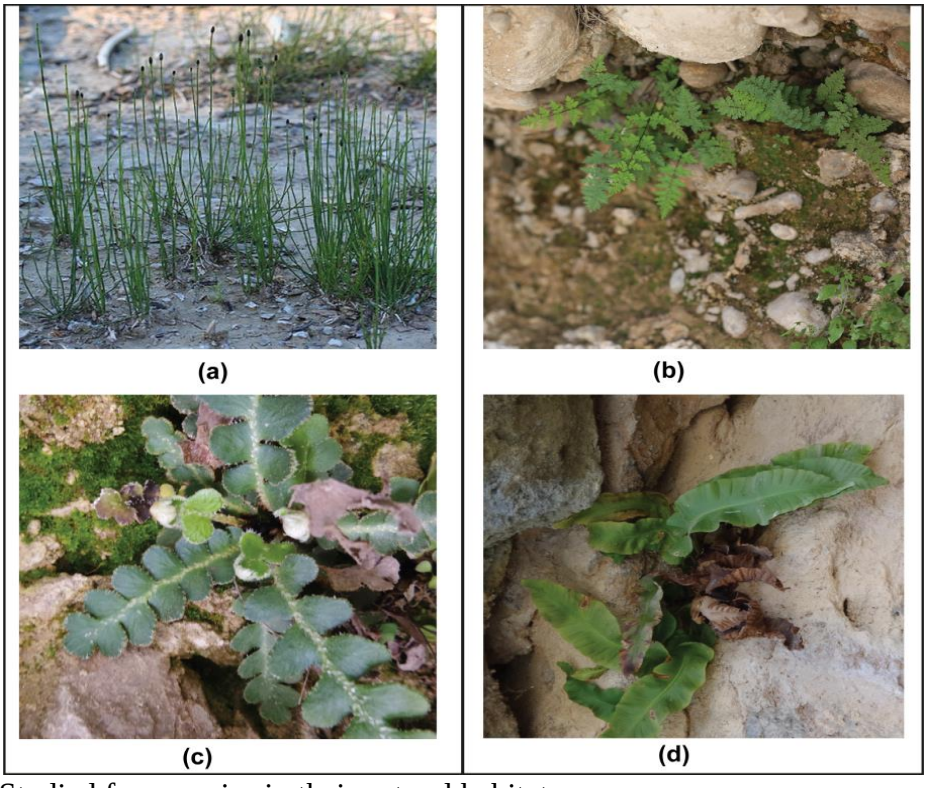

Figure 1: Studied fern species in their natural habitats
(a) *Equisetum ramossisimum* (b) *Cheilanthes pteridioides* (c) *Asplenium ceterach* d) *A. scolopendrium*

3. Results and Discussion:

The study was conducted to examine the presence of bioactive compounds generaly and the kinds of alkaloids, specially in four fern species through High Perphormancy Liquid Chromatography (HPLC) method. The results of qualitative study showed that these ferns contain a variety of secondary metabolits (Table 1). Leaves and other parts of fern sporophytes were found to be rich in phytochemical constituents such as flavonoids, saponins, tanins and alkaloids as reported by other recent studies [1, 11, 12, 18, 19].

Therefore, we performed HPLC to examine the presence of alkaloids in four fern species. Our results showed that the ferns exhibit variability in total and the number of alkaloids between them. Interestingly, the species *Cheilanthes pteridioides* recorded 6 compounds. We also found that among the four ferns, both *Asplenium ceterach and Equisetum ramosissimum* had seven compounds. Finally, *Asplenium scolopendrium* recorded just four compounds. The result have been summarized in Table 2.

Table 2 : The concentrations (µg/ml) of alkaloids in ferns methanol extracts

Alkaloid compounds	*Asplenium scolopendrium*	*Asplenium ceterach*	*Cheilanthes pteridioides*	*Equisetum ramosissimum*
Isoquinoline	137.8	59.8	23.7	**139.1**
Corilagin	54.1	40.6	37.3	**49.3**
Colocynthis	--------	313.6	--------	**304.3**
Hordenine	375.7	91.8	263.1	**426.4**
Caulerpin	563.3	107.2	147.8	**324.1**
Almazoline	-------	69.7	158.2	**77.6**
Martensine	----------	163.7	33.7	**139.3**
Total	**1180.9**	**846.4**	**663.8**	1460.1

- **(------) Absent**

Several studies have used HPLC technique as a flexible method in the analysis of alkaloids [20]. In this study, our results showed that all of the samples (aerial parts) contained alkaloids based on the R_t of the standard compounds. We further compared the R_t of the standard compounds in concentrations of the alkaloids in our samples (Table 2).

Interestingly, we found extreme differences in concentrations of the alkaloids in each studied site and between two sites as well. Our results revealed that *E. ramosissimum* from Chemi Rezan region in Sulaymaniyah Governorate contains 7 alkaloids with the highest total alkaloids (1460.1 µg/ ml) overall investigated ferns. While *Cheilanthes pteridioide*s from Malakan region in Erbil Governorate recorded the lowest concentration of total alkaloids (663.8 µg/ ml) (Table 2). Some local studies have stated that these ferns herbal positively contains alkaloids, flavonoids, tannins, flavonoids and saponins [21, 22, 23]. It could be hypothesized that the differences between the studied ferns in total alkaloids may be due to the variability in the environmental factors and the geographical habitats between two different districts. A previous research found that secondary metabolites accumulation is strongly dependent on environmental factors such as temperature, humidity, climate and latitude [22, 23, 24]. The total production of secondary metabolites in plants may form maximally related to the environment, biotic, abiotic stresses and nutrient [25], light [26], climate [27], altitude [6] and latitude [22]. Therefore, we focused our analysis on the concentrations of alkaloids to investigate the effects of these factors on the alkaloids contents of the studied ferns in Iraq. In Chemi Rezan region, the fern species survival at elevation and environment conditions varied than of Malakan region which affected the alkaloid contents in the studied fern [25, 26, 27]. Thee environmental factors influence the biosynthesis of alkaloids [28]. The synthesis and accumulation of various secondary metabolites were modified by more than one factor. Many environmental factors simultaneously influence the secondary metabolite compounds [29]. Phytochemical studies on

ferns have revealed that they contain a wide range of alkaloids [11]. So, it is important for phytologists to consider not only the environmental conditions in the future investigations but also the nature of the ferns as they affect the type of secondary metabolite production**.** So far, there have been no studies investigating on the alkaloid constituents in the fern extracts: *A. ceterach, A. scolopendrium, Cheilanthes petrioides and E. ramosissimum.* Recently, researchers have started to focus on the natural products which are used in the folk medicine. The results obtained in the present study are encouraging for further research on phytochemical of Iraqi pteridophytes in other parts of the country.

4.Conclusions:

The study revealed the presence of many alkaloid constituents in the fern species. Some of the dominant alkaloids found in fern extracts were hordenine in *Equisetum ramosissimum* from Sulaimaniyah district and caulerpin in *A. scolopendrium* from Erbil Governorate.
So, it is important for pharmacologists and phytologists to consider not only the nature of the plant but also environmental conditions because they affect the type of secondary metabolite production.

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