An ecological study of the fern *Asplenium Trichomanes* L. in Iraq with a reference to its phytochemical and antioxidant activity

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Abstract. A number of ferns are known to have ornamental, medicinal and ethnobotanical importance all over the world. In spite of the presence of numerous fern species such as *Equisetum, Adiantum, Asplenium and Cheilanthes species* all over our country (Iraq) particularly the Kurdistan part, still, such information in this area is quite rare. *Asplenium Trichomanes* L.is a genus of one of the widest groups of Aspleniaceae (maidenhair spleen worts). It is a megaphyllous, homosporous fern, which was found at an elevation above 1600 m.a.s.l. Samples were collected from the Taweela area in Kurdistan of Iraq, the phytochemical and antioxidant activity of the plant was dealt with in the current investigation through the methanol extract method of ferns. The result revealed positive detection of few numbers of flavonoid compounds, among which kaempferol compound showed the highest concentration (227 µg/ml) whereas kaempferide 3-0 glycoside showed the lowest concentration (130.3 µg/ml), six phenolic compounds were identified through HPLC technique among which (Kaempferol, Quercetin, Myrstin, Rutin, Kea,3-o-glycoside, and Leutolin), also anti-oxidant activity recorded (1c50) it had exceeded (2.27 µg/m1). In contrast the positive control (Ascorbic acid) 0.018 mg/ml. The present study has been devoted to the distribution of *Asplenium Trichomanes* L in Iraq with reference to it is a phytochemical and anti-oxidant activity for the first time in the region. In years to come, undoubtedly this will follow with more investigations on fern wealth in Iraq so it will reduce the existing gap in the knowledge in this respect.

Keywords: *Asplenium*, Ferns, Antioxidant, phytochemical, Iraq.

1. Introduction
The most recent classification of ferns shows that Polypodiopsida includes only 7 orders, 33 families, and 300 genera with only 8861 species [1]. Some of which being medicinal, and ethnobotanical importance in habitat conservation [2]. In fact, ferns constitute the major class of Pteridophytes, meanwhile, it has been reported for their pharmacological uses to treat parasitic infections, bleeding, diarrhea, and cold [3]. Aspleniaceae is one of the wide and large groups of ferns within Polypodiales. Actually, the taxa *Asplenium* (Aspleniaceae, Aspleniidae) is a large genus consist of 650 species [4]. In Iraq, this fern was studied by Townsend & Guest [5] they investigate only the geographical distribution of *Asplenium. Trichomanes*. Recently few local investigations were carried out about the distribution, ecology and Phytochemistry of some ferns: such as *Equisetum, Adiantum, Asplenium and Cheilanthes species* [6, 7, 8, 9]. At the beginning of the 21st century, recently the important of antioxidant and bioactive compounds in ferns in general and particularly in various spieces of Asplenium have been well documented [10, 11, 12]. The present project has been confined to bioactive compounds (phytochemical and antioxidant activity) and distribution of *Asplenium Trichomanes* L. for the first time in Iraq for the best of our knowledge this is the first attempt in this respect carried out in Iraq and nearby countries aiming to document bio information's about *Asplenium*.
This may draw the attention to utilize fern wealth in near future and will encourage further studies.

2. Materials and methods

2.1. Collection of the fern samples
The Specimens (mature sporophyte) were collected during April- May 2017 from the Tweela region in Sulaymaniayh district Northwest of Iraq: coordinates Lat. 35° 13. 002 N, Long. 046° 11. 345 E at elevation (1633 m a.s.l). The collected specimens were identified, then were voucher specimens have been deposited with No. (4, 17, 1, AS. tr) in the Hawler Botanical Garden – Erbil city which is officially being under the supervision of the Erbil governorate through a scientific board. The annual mean of meteorological data for the study site were as follows: temperature 36 cº, rainfall 176 mm and humidity 70%. The scientific classification was followed according to [9].

2.2. Preparation of crude extract
The plant samples (mature sporophyte) collection of the mature sporophyte of the fern Asplenium. Trichomenes were carried out in the field in plastic bags. Then after return to the lab, samples were washed with tap and distilled water, then after samples left for a week on the benches to dry up at room temperature. Samples then crashed and grounded to powder. 10 grams of the powder was used for extraction procedure by applying 99% methanol. Using shaker water bath for a period of 12 hours at 40 cº temperature. The obtained extract from filtration through Whatman No.1 filter paper the supernatant left to evaporate in order to get a semi-solid material (final extract) which was kept in a container at 4 cº (13).

2.3. Phytochemical screening
Qualitative analysis of the plant extracts carried out in advanced Biotech. Laboratory of College of Science - Baghdad University, following the procedure described by [13].
- Flavonoids test. One ml of plant extract was dissolved in (4 ml) of 1N NaOH in a test tube, the formation of a dark color was observed indicates the presence of flavonoids.
- Alkaloids test. One ml of plant extract added to (2 ml) Myers reagent, turbidity green color was observed, indicates the presence of alkaloids.
- Saponins test. Plant extract (0.5 gm) was mixed with (5 ml) boiling distilled water in test tube allowed to cool and shaking well too, the formation of foam indicates positive detection of saponins.
- Tannins test. The dried crude extract (0.5 gm) was dissolved in (10 ml) boiled distilled water in a test tube and then filtered. One ml of the filtrate plant extracts mixed with 5% FeCl3 (1 ml), formation of brownish color indicates positive detection of tannins.

3. HPLC analysis
The dried crude extract was dissolved in 100 ml mobile phases, after filtering through a filter paper and a 0.45 µm membrane filter (Millipore 0.45 µ), the extract was injected into an HPLC instrument by an Autosampler according to the optimum condition. The main compound was 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% trifluoracetic acid (TFA acid) in deionized water: solvent B was 0.05% TFA in methanol, pH, and 2.5 gradient programs from 0% B to 100%. B for 10 minutes. Flow rate 1.1 ml/ min. Detection: UV at 280 nm. All these analyses were performed in Baghdad University- Science college.
4. Calculations
Calculation of the result was made through the following equation:
concentration = area of sample / area of standard × conc. of standard × dilution factor. The result was expressed in µg/ml.

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 [14, 15].

The antioxidant activity was expressed by an IC50 according to the Graph Pad program [16]. HPLC analysis showed six major and clear peaks in the retention time range of 1.25-6.20 min.

5. Result and discussion

5.1. Habitat
The fern is known to occur in mountainous and rocky habitats such as cliffs and crevices. It grows in temperate arctic areas from sea level up to 3000 m (1,4,26) whereas in the Kurdistan region of Iraq it was found at 1630 m a.s.l. in Taweela site northeast of Iraq.

5.2. Morphology
The fern Asplenium trichomonas L. which is the concern of this paper is megaphyllous, homosporous, it grows in tufts from short creeping brown-blackish rhizome, 1 cm rhizome thick, densely covered with scales. The fronds are long, narrow, gradually tapering toward the tip, open dichotomous venation, oppositely arranged at the rachis and sessile. Fronds are divided into small yellow-green pinnae (10-15 pairs) with serrate margin, glabrous above and covered with hairs below. The rachis of the frond dark-brown with dense scales. The fronds long may reach 20-30 cm. Sori bright brown, narrow, ellipsoidal shape bearing underside the pinnae which contain sporangia with spores (Figure 1a-c).
6. Phytochemistry analysis
The preliminary examination of the methanolic extract of aerial parts of the fern (Asplenium trichomonas L.) was done by various tests that suggested the presence of major components viz., alkaloids, flavonoids, saponins and tannins (Table 1). The results of HPLC analysis of the fern extract indicate the presence of six flavonoids, which were: kaempferol, kaempferol-3-O-glycoside, myrestin, rutine, querctine and leutolin (Table 2).

| Plant         | Flavonoid | Alkaloid | Saponnin | Tannin |
|---------------|-----------|----------|----------|--------|
| Asplenium trichomonas | ++        | ++       | ++       | ++     |

Table 1. Qualitative analysis of the phytochemicals in fern extract.

| Plant extract | Kaempferol | Quercetin | Myrestin | Rutin  | Kea.3-O-glycosid | Leutolin |
|---------------|------------|-----------|----------|--------|-----------------|----------|
| Asplenium trichomonas | 257       | 149.5     | 151.3    | 182.7  | 130.6           | 186.9    |

Table 2. Quantitative analysis of flavonoids in fern extract(µg/ml).

7. 2-2 Dihenyl -1- picroly hydrazil (DPPH) scavenging activity
The result of antioxidant activity, DPPH IC50 for the methanolic extract of the fern (Asplenium trichomonas L.) was about 2.212 µg/ml in comparison with DPPH IC50 of Ascorbic acid (positive control) = 0.018 µg / ml.

Total flavonoids content: The results of the qualitative study revealed positive detections, the finding showed the presence of four bioactive compounds: flavonoids, alkaloids, saponins and tannins table (1). The results of quantitative analysis, on the other hand, were showing that the methanolic extract of A. Trichomanes L. contains these six flavonoid compounds: querctin, rutin, kaempferol, kaempferol – 3-O- glycoside, myrestin and leutolin table (2). However, different concentrations of these compounds. We’re also observed and reported elsewhere [15]. In the present study, Kaempferol was recorded to have the highest concentration (227 µg/ml) that may indicate a key role in protecting the fern against environmental stresses as it was referred to by [16], in fact, kaempferol-3-O- glycoside did record the lowest concentration (130 µg/ml) in the present investigations.
The results indicate that the total flavonoids content in fern extract was about \( 1060 \, \mu g/ \text{ml} \). That consist of Kaempferol, Quercetin, Myrstin, Rutin, Kea.3-o-glycoside and Leutolin. The variability of flavonoid concentration in the present study may be due to the stresses created by environmental factors such as humidity, altitude and temperature. The concentration of phenolic compounds similar to flavonoids may be influenced and effected by environmental variations [17,18, 19]. So, phenolics and other Antioxidants compounds may have a role as a chemical interface between the plant (fern) and environmental factors [10], variation in their level may be act as a criterion in finding the degree of stresses and plant responses to nonliving ecological factors [19, 20, 21]. Phenolics exhibit a strong antioxidant activity as it is well-known as scavenger, metal cheaters, reducing agents and hydrogen donor [22]. Antioxidants naturally occurring in ferns in the form of phenolic such as flavonoids and tannins [23].

There was a linear relationship between the phenolic compounds (such as flavonoids) and DPPH radical scavenging [24]. Recently there has been an increase in the standardization of medicinal ferns of pharmacogenetic significance [25]. Recently, much attention has been devoted to ferns as potential sources of natural antioxidants, therefore several Asplenium taxa have been evaluated for their antiradical scavenging activity [13], [24]. Further research on the Asplenium taxa may help in the isolation of biologically active compounds which can finally be the subject of pharmacological activities, thus leading open up new avenues in the use of natural products for the therapeutic purpose [25], [26]. From the effects of secondary metabolites, ferns and fern allies showed strong functional activities on human health (e.g., antioxidant antibiotic, antitumor and anti-inflammatory). So, pteridophytes could be a very important plant group for many kinds of natural foods and medicines [27]. Hopefully, such fern wealth will be utilized in Kurdistan and the whole of Iraq to serve humanity and whole health in general.

8. Conclusion
1-Bio information about the fern (Asplenium trichomonas L.) in Iraqi Kurdistan have been documented for the first time, the fern extract showed the presence of Flavonoid, Alkaloid, Saponin and Tannin as phytochemical compounds. The total Flavonoid content in Asplenium trichomonas L. extract in the present investigation was about \( 1060 \, \mu g/ \text{ml} \) within which Kaempferol was the dominant whereas Kea.3-o-glycoside concentration was the lowest. 2- The variability of concentration may be related to environmental stress factors such as humidity, temperature and altitude. Such study may enhance and encourage utilization of fern wealth in Kurdistan or the whole of Iraq in the future.

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