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Phytochemical and Antioxidant Activity of Asplenium Species (Spleenworts) Extracts from Northern Districts of Iraq

Abstract- The main objective of the present investigation was to ascertain of different phytochemicals in two ferns: *Asplenium scolopendrium* L. from Malakan-Erbil district and *A. trichomanes* L. from Tweela-Sulaymaniyah district during Spring 2016. Antioxidant activity and total flavonoids contents were determined in methanol extracts. In qualitative analysis, the active compounds such as alkaloids, tanins, saponins and flavonoids were screened. In quantitative analysis, total flavonoids were quantified 234.7 µg/ml with IC50 of 113 mg/ml in *A. scolopendrium* but in *A. trichomanes* total flavonoids 1061 µg/ml. with IC50 of 2.271 mg/ml comparison with Ascorbic acid IC50 of 0.0186 mg/ml as positive control.

Keywords- *Aspleniaceae*, phytochemistry, Flavonoids, Antioxidant

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1. Introduction

The spleenworts family (*Aspleniaceae*) includes just one very large genus: *Asplenium*, a cosmopolitan and important genus of the *Aspleniaceae* family, order Polypodiales, comprises 650 species distributed among the world [1]. Phytochemical investigations on the ferns have led to characterization many secondary metabolites [2]. Plants produce secondary metabolites not simply to adapt to their environment but also to resist themselves against several environmental stresses [3]. *Asplenium trichomanes* L. (commonly known as maidenhair spleenworts), the fern grows in aggregate form, the laevis are long, gradually tapering towards the tip, they bear long narrow sori to both sides of midrib, widespread in tropics and mountainous regions prefers acidic rocks such as sandstone and cliffs [4]. *Asplenium scolopendrium* L. (Commonly known as harts tongue fern), the frond is simple, narrowly oblong, undivided, bright green color, heart shape base, sori elongated, a row on either side of midrib [5]. Active compounds of ferns mainly belong to the phenolics group, flavonoids, alkaloids and terpenoids families [6], and *Asplenium* species, mainly sporophytes have been only partially phytochemically characterized [7]. In the ten last years, much attention has been devoted to ferns as potential sources of natural antioxidants and

several *Asplenium* species have been evaluated for their antioxidant potential [8]. To the knowledge, this is the first investigation focus on flavonoids quantitatively and qualitatively of *Asplenium* species from North of Iraq.

2. Materials and Methods*I. Collection of plant materials*

A. trichomanes and *A. scolopendrium* (mature sporophyte) were collected from two sites (Mallakan in Erbil district and Tweela in Sulaymaniyah district) from Iraqi Kurdistan and were confined by the Missouri Botanical Garden in USA. Voucher samples (4, 17, 1, Ph. SC & 4, 17, 1, As. tr.) of the ferns were deposited in the Herbarium of Howler Botanical Garden- Erbil City. Geographical characters and metrological data of the study sites are presented in Table 1 and 2.

Table 1: Geographical characters of the studied sites

Sites	Temperature (c°)	Rainfall (mm)	Humidity (%)
Malakan	39	120	59
Tweela	36	175.5	65

Table 2: Metrological characters of the studied sites

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Sites	Elevation (m)	Longitude	Latitude
Malakan	559	44° 26. 533 E	36° 37. 496 N
Tweela	1680	046° 11.435 E	35° 13. 002 N

II. 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 dried at room temperature for a week. The dried plants were ground to fine powder and then the powdered samples were stored at 4° c for future analysis. The plant powder (10 gm) was extracted with 100 ml of methanol (BDH) 99%, using shaker water bath for (12 h) at 40 c° temperature. 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 containers covered with aluminum foils at 4 c°.

III. Phytochemical screening

Qualitative analysis of the plants extracts cured out in advanced Biotech. Laboratory of Baghdad University – College of science, following procedure described by [9].

a. Flavonoids test

One ml of plant extract was mixed with (4 ml) of 1N NaOH in a test tube, formation of a dark color was observed indicates the presence of flavonoids.

b. Alkaloides test

One ml of plant extract added to (2 ml) Myers reagent, turbidity green color was observed, indicates the presence of alkaloids.

c. Saponins test

Plant extract (0.5 gm) dissolved in (5 ml) boiling distilled water in test tube allowed to cool and shaking well to mix thoroughly, the appearance of foam indicates the presence of saponins.

d. Tanins test

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 extract mixed with 5% FeCl₃ (1 ml), appearance of brownish color indicates the presence of tanins.

HPLC analysis

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 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% trifluoroacetic 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.

3. Conclusion

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 [10].

HPLC analysis revealed six major peaks in the retention time range of 1.25 - 6.20 min. presented in Table 3.

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

No.	Subject	Retention time (min)	Area
1	Querctine	1.25	67880
2	Rutine	2.54	98186
3	Leutoline	3.47	114892
4	Kaempferol	4.37	109560
5	Kaempferol-3-O-glycosid	5.29	107439
6	Myrcetin	6.20	75818

Free Radical scavenging

The oxidative activity of the crude extracts was measured on the basis of the scavenging activity of the stable 1, 1, diphenyl 2- picrylhydrazyl (DPPH) free radical. One milliliter of (0.1 mM) DPPH solution in methanol was mixed with 1 ml of plants extracts solution of various concentrations. DPPH solution was added to the solutions were prepared with plant extracts and standard antioxidant substances and stirred. Each mixture was kept in the dark chamber at room temperature for 30 min and absorbance changes were measured at 517 nm with a Shimadzu- 1063 spectrophotometer. Blank sample was prepared (DPPH + methanol) and standard solution of ascorbic acid was considered as positive control (without extract). Absorbance values converted

into the percentage antioxidant activity (AA %) using the following equation: $AA \% = (Ac - As) / Ac \times 100$. Where Ac is the absorbance of the control and as is the absorbance of the sample. Lower observed absorbance values are related of higher antioxidant potential. The antioxidant activity of the extract was expressed as IC₅₀. The IC₅₀ was defined as the concentration ($\mu\text{g/ml}$) of extract that scavenged 50% the scavenging activity in the reaction mixture and is obtained from calibration curve according to the statistical graphpad program [11].

4. Results and Discussion

The results of qualitative study revealed the presence of four active compounds presented in Table 4. The results of HPLC analysis of plant extracts presented in Table 5.

Table 4: Qualitative analysis of phytochemicals in the plants

Plants	Alkaloids	Flavonoids	Tanins	saponins
<i>A. scolopendrium</i>	+	+	+	+
<i>A. trichomanes</i>	+	+	+	+

Table 5: Quantitative analysis of flavonoids ($\mu\text{g/ml}$) in the plants

Plants	Quercetin	Rutin	Kaempferol	kaempferol-3-O-glycosid	luteolin	myracetin	total flavonoids
<i>A. scolopendrium</i>	25.1	41.5	24.7	100.4	43	-----	234.5
<i>A. trichomanes</i>	149.5	182.7	227	130.7	216.9	151.3	1061

Scavenging activity

The results IC₅₀ values for the plant extracts presented in Table 6.

Table 6: IC₅₀ values (mg/ml) for the studied plants

Sampls	IC ₅₀ (mg/ml)
<i>A. scolopendrium</i>	113
<i>A. trichomanes</i>	2.212
Ascorbic acid (positive controle)	0.018

Total flavonoids were determined for quantitative analysis using methanol extracts for two ferns mature sporophyte. The results showed that *A. scolopendrium* contains five flavonoid compounds: quercetin, rutin, kaempferol, kaempferol-3-O-glycoside and leutolin but *Asplenium trichomanes* contains six flavonoids Table 5. The results show differences in concentration of these compounds between two fern species. In comparison to other compounds, kaempferol recorded the highest concentration 227 $\mu\text{g/ml}$ in *Asplenium trichomanes* but kaempferol -3-O- glycoside recorded the highest concentration 100.4 $\mu\text{g/ml}$ in *A. scolopendrium* while kaempferol-3-O-glycoside recorded lowest concentration 130.7 $\mu\text{g/ml}$ in *A. trichomanes* and

kaempferol 24.7 $\mu\text{g/ml}$ in *P. scolopendrium* Table 5. Results also showed that the total flavonoids content in *A. trichomanes* in Tweela region 1061 $\mu\text{g/ml}$ was higher than in *A. scolopendrium* in Malakan region 234.7 $\mu\text{g/ml}$ (Table 5). The differences in total flavonoid concentrations between two ferns may be linked to stresses created by environmental factors (like elevation, humidity and temperature) associated with two regions. Concentrations of phenolics like flavonoids can be influenced by environmental and geographical variations [12, 13, and 14]. So phenolic compounds and other active compounds act as bioactive secondary metabolites interface between the ferns and environmental factors [15], and the changes in their concentrations may be used as criterion in estimating the degree of stresses and plant responses to ecological factors (abiotic) [14,16].

The antioxidant activity of methanol extracts in *A. trichomanes* and *A. scolopendrium* were estimated by DPPH free radical scavenging according to IC₅₀ value comparison with ascorbic acid as positive control. The results showed that IC₅₀ of 2.27 mg/ml in *A. trichomanes* but IC₅₀ of 113 mg/ml in *A.*

scolopendrium, in comparison with ascorbic acid (vitamin C) IC₅₀ of 0.018 mg/ml (Table 6). Phenolics exhibit strong antioxidant activity as well-known scavenger, metal chelators, reducing agents and hydrogen donor [17]. Naturally anti oxidative occurring in ferns in form phenolic compounds such as flavonoids and tannins [18]. The lower IC₅₀ values leads to stronger scavenging radical capacity, there were a linear relationship between the flavonoid content and antioxidant activity, this relationship was indicated by [19], which agree with the results of this investigation. *Asplenium trichomanes* recorded high IC₅₀ value because its flavonoid content higher than *A. scolopendrium* table 6, this results agreed with [11,20]. Recently, the scientific studies in certain that many of drugs and antibiotic components from plant secondary metabolites. Many recent reports indicates that the synergize reacts (synergic) affected into antioxidant activity of study ferns [21,22]. The last but not the least the results of antioxidant activity of studied ferns agreed with total flavonoid content.

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