A PRELIMINARY STUDY OF PHYTOCHEMICALS IN *EQUISETUM ARVENSE* & *E. RAMOSISSIMUM* (EQUISETACEAE) EXTRACTS FROM NORTHERN IRAQ

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ABSTRACT

Plant extracts of *Equisetum arvense* and *E. ramosissimum* were investigated from the Chemi Rezan and Kalar regions in Sulaymaniyah/Iraq and Iranian foothills districts during the summer of 2016.

Qualitative analysis of methanol extracts indicated the presence of alkaloids, saponins, tannins and flavonoids. By using High Performance Liquid Chromatography (HPLC), the flavonoid content of two *Equisetum* species were analyzed, and may provide useful medicinal compounds. The presence of kaempferol, kaempferol-3-O-glycoside, leutolin and querctin were found in *E. arvense*, with total flavonoid content of 179.5 µg/ ml. The flavonoids kaempferol, kaempferol-3-O-glycoside, leutolin, myrcetin, querctin and rutin were detected in *E. ramosissimum* with a total flavonoid content of 831.8 µg/ml. This is the first documented study of *Equisetum* (horsetail ferns) extracts in Iraq.

INTRODUCTION

To date, there is no detailed comprehensive study on any species of ferns in the Kurdistan region of Northern Iraq (Maulood et al., 2016). All previous investigations on ferns in Iraq referred only to fern morphology and geographical distribution (Al-Mayah et al., 2016). The genus *Equisetum* is the only remaining representative of the once abundant and diverse class Equisetopsida (Pryer et al., 2001), and recent phylogenetic studies suggest that *Equisetum* should be classified within the true ferns (Pryer et al., 2001, Smith et al., 2006). *Equisetum* consists of 30 species of rush-like and jointed perennial herbs (Sandhu & Chopra, 2010). *Equisetum arvense* L. and *E. ramosissimum* Desf. (order: Equisetales, family: Equisetaceae) are members of a very primitive family of plants found in diverse habitats, such as open grasslands, along roadsides, railway tracks, swamps, wetlands and river banks (Maulood et al., 2016, Brownsey & Perrie, 2015). Many members of Equisetaceae have an important role in nutrient recycling and soil treatment

(Marsh et al., 2000). *Equisetum arvense* (field horsetail or scouring rush) belongs to subgenus *Equisetum*, recognised by its dimorphic aerial stems, sterile erect stems with undivided lateral branches arising in whorls, and fertile unbranched stems, occasionally producing terminal strobili in mid-summer. *Equisetum ramosissimum* (branched horsetail) belongs to subgenus Hippochaetae and is recognized by its unimorphic aerial stems, sterile and fertile branched stems, fertile stems and its branches producing strobili (Maulood et al., 2016). Spores in these two fern species are monomorphic, globose, green and bear four linear-spatulate elaters (Yatskiervych & Windham, 2008).

Many secondary metabolites (phytochemicals) have been shown to provide effective treatment to a wide variety of illnesses and ailments, and are being embraced by the public (Rajesh et al., 2014). Among these phytochemicals alkaloids, carotenoids and phenolics have been studied (Do Momtae et al., 2004) and have been used in the treatment of kidney and bladder disturbances in folk medicine (Briksin, 2000). *Equisetum telmateia* Ehrh. extracts have been used in traditional medicine for their anti-inflammatory and diuretic properites (Correia et al., 2005). Specifically, the ethyl acetate portion from HPLC showed strong antioxidant activity, with flavan-3-ol, kaempferol, and phenolic acid derivatives detected.

Recent investigations showed that the studied plants have anti-inflammatory, anti-microbial, anti-cancer and anticonvulsant effects (Radolovic et al., 2006). *Equisetum* species were reported to contain several flavonoids, alkaloids, phenolics, triterpenoids, saponins and phytosterols (Yatskiervych & Windham, 2008, Stajner et al., 2009). Flavonoids and phenolic acids are the most important groups of secondary metabolites and bioactive compounds in plants and good sources of natural antioxidants in human diets (Jain et al., 2016). *Equisetum arvense* extracts have also been found to have high antioxidant activity, especially in the ethyl acetate fraction. Specifically, among the phenolic compounds, isoquercitrin and di-E-caffeoyl-meso-tartaric were found in the fractions with high antioxidant activity (Mimica-Dukic et al., 2008).

Extracts of *E. arvense*, *E. sylvaticum* L., *E. fluviatile* L., *E. palustre* L. and *E. telmateia* Ehrh. were examined and assessed for antioxidant activity, phenol content and flavonoid profiles. The phenol content ranged from 92-349 μmol/g, with kaempferol-, quercetin- glycosides and caffeic acid derivatives identified as the major components, and *E. telmateia* showing the greatest antioxidant capacity (Milovanovic et al., 2007).

The goal of the present investigation is to quantify the flavonoid content in these *Equisetum* species in Iraq, which have not been studied in this region to date.

MATERIALS AND METHODS

Equisetum arvense and *E. ramosissimum* (Figure 1 A and B) samples were collected from Chemi Rezan and Kalar regions during summer of 2016. Dr. Ihsan Al-Shehbaz of Missouri Botanical Gardens identified the plant samples. Voucher samples (No. 4, 19, 5, *E. arvense* & No. 4, 19, 5, *E. ramosissimum* were deposited at the Howler Botanical Garden in the Erbil Governorate (Ismail, 2018). Geographical aspects of the study area are represented in Table 1.

The collected plants were brought to the laboratory and the aerial stems washed with tap water, followed by distilled water. The stems were then shade-dried at room temperature for 7-10 days and ground to a fine powder using an electrical grinder, and then the powdered samples were stored in an airtight container. A 10 g aliquot of plant powder was extracted with 100 ml of methanol (BDH) using a soxhlet apparatus and was decanted through filter paper (Whatman No.1). After filtration, the supernatant was

Table 1: Geographical characters of the study area

Regions	Chemi Rezan	Kalar
Elevation (m)	722	247
Longitude	34° 95. 050 N	34° 42. 821N
Latitude	39° 64. 065 E	045° 27. 867 E

further dried in a vacuum desiccator and then the dried extracts were stored in vials at 4°C. Phytochemical studies of the dried extracts was performed in the Biotech research laboratory, College of Science for Women, University of Baghdad. The detection of active compounds in plant materials was performed following a standard procedure (Harborne, 1998). To test for alkaloids, 1 ml plant methanolic extract was added to 2 ml Mayer's reagent. The turbidity or precipitation of green colour was observed, indicating the presence of alkaloids. To test for flavonoids, 3 ml plant methanolic extract was mixed with 4 ml 1N NaOH in a test tube, and the formation of dark yellow colour was observed, indicating the presence of flavonoids. To test for saponins, plant extract weighing 0.5 g was dissolved in 3 ml boiling distilled water in a test tube, allowed to cool and shaken well to mix thoroughly, where the appearance of foam indicates the presence of saponins.

Figure 1. A. *Equisetum arvense*, B. *E. ramossisimum*, collected from Chemi Rezan and Kalar regions north of Iraq.

Table 2. The retention time and the area of standard flavonoids

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

To test for tannins, plant extract weighing 0.5 g was boiled in 20 ml distilled water and then filtered and 1 ml filtrate plant extract was mixed with 5% $FeCl_3$ (1 ml). The appearance of brownish green colour indicates the presence of tannins.

HPLC was used for quantitative analysis following standard procedure from Suarez et al., 2005. The dried methanolic extract was dissolved in 1 ml HPLC methanol after being filtered through Millipore filter paper (pore diameter 0.45µm), and 20µl of filtrate extract injected into HPLC instrument by an auto sampler according to the optimum conditions. The main compounds were separated on FLC column: C18-DB, 3µm particle size (50 x 2 mm ID) column, mobile phase: linear gradient of solvent A 0.05% trifluoracetic acid in deionized water: solvent B was 0.05% TFA in methanol pH 2.4 gradient program from 0 % to 100 % 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 concentration of standard x dilution factor.**

The separation occurred on liquid chromatography Shimadzu 10 AV – LC equipped with binary delivery pump model LC – 10A. The eluted peaks were monitored by UV-Vis spectrophotometer. The retention time and the area of standard flavonoids are presented in Table 2.

RESULTS

Phytochemical screening:

The qualitative analysis of methanolic extracts showed the presence of alkaloids, saponins, tannins and flavonoids in *E. arvense* and *E. ramosissimum*, Table 3.

HPLC analysis:

The results of quantitative analysis of methanol extracts showed the flavonoids concentrations in *E. arvense* and *E. ramosissimum*, given in Table 4. The qualitative study of methanol plants extracts of *E. arvense* and *E. ramosissimum* showed the presence of four groups bioactive compounds: saponins, tanins, alkaloids and flavonoids (Table 4). The results of phytochemical screening in these two species are in accord with the reported studies in true fernesh et al., 2014; Asgarpanah & Elnaz, 2012; Hoque et al., 2016).

The results of HPLC analysis of fern extracts was revealed in the presence of the four flavonoids kaempferol, kaempferol-3-o- glycoside, leutolin and querectin in *E. arvense* and the six flavonoids kaempferol, kaempferol-3-o- glycoside, leutolin, myrcetin,

Table 3. Qualitative analysis of phytochemicals in plants

Plants	*E. arvense*	*E. ramossisimum*
Alkaloids	+	+
Flavonoids	+	+
Tannins	+	+
Saponins	+	+

quercetin and rutin in *E. ramosissimum* (Table 4). Leutolin was recorded in the highest concentration (100.6 µg/ml) *in E. arvense* but myrcetin had the highest concentration (223.5 µg/ml) in *E. ramosissimum* (Table 4). The results showed a higher concentration of total flavonoid (831.8 µg/ml) in *E. ramosissimum* compared with the concentration of total flavonoid (179.5µg/ml) in *E. arvense* (Table 4).

DISCUSSION

The high concentration of total flavonoids in *E. ramosissimum* from Kalar region may indicate a key role in protecting the plant against environmental stresses especially abiotic conditions such as high temperature (Borges et al., 2013). Phenolic and other active compounds act as a chemical interface between plants and environment (Gobbo-Neto & Lopez, 2007). Flavonoids not only participate in protection against harmful abiotic factors, but also allow interactions with other organisms (plants, animals and microorganisms) and their reactions to environmental stresses (Mierziek et al., 2014). Concentrations of phenolic compounds like flavonoids can be influenced by environment changes and there was a positive correlation between the phenolic compound's concentration and the environmental stresses (Monterio et al., 2006). It has been reported that flavonoids also directly interact with biological membranes, reducing their fluidity and making them more resistant to many oxidative factors (Harborne & Williams, 2000). The changes in the concentrations of chemical contents may be used as criterion in estimating the degree of stresses and plant responses to environmental factors. However, such changes in flavonoid concentrations can affect directly the quality of the fern for medicinal use (Al-Khasreji et al., 2017).

Table 4. Quantitative analysis of flavonoids (µg/ ml) in plants

Plants	*E. arvense*	*E. ramosissimum*
Quercetin	9.9	121.6
Rutin	-----	93.8
Kaempferol	26.6	177.6
Kaempferol_3_o_glycoside	42.4	145
Luteolin	100.6	70.3
Myracetin	-----	223.5
TotalFlavonoids	179.5	831.8

Absent: (-----)

CONCLUSIONS

The current study revealed that there are several bioactive compounds into two investigated *Equisetum* species. The investigation gathered information about the quantitative of flavonoids to provide the basis for future clinical trials and other medicinal research.

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