ABSTRACT

Objectives: ethanolic extract of *E. arvense* was fractionated to three portions according to their polarity and each fraction was examined for cytotoxic effect. Methods: The aerial part of the plant was extracted with ethanol, the ethanol was evaporated and water was added and the solution was partitioned with ethyl acetate. Ethanol, aqueous and ethyl acetate layers were examined for cytotoxicity. HPLC analysis was performed for the ethyl acetate fraction. Results: The ethyl acetate layer showed significant percent of cytotoxicity. HPLC analysis of this extract showed the occurrence of both flavonoids and flavonoid glycoside. Conclusion: Flavonoids in the ethyl acetate layer exhibit a high percent of cytotoxicity.

KEYWORDS: *Equisetum arvense*, flavonoids and cytotoxicity.

INTRODUCTION

*Equisetum arvense* L., Family: Eqisetaceae, common name horse tails is a perennial herb with 0.1 m high. The stem is branching and evergreen. The root is fibrous and dark in color (brown to black). The rhizome of the plant is long and its width is up to 0.2 cm. The leaves are renewed each year and arrange in whorls shape Figure 1.[1]

![Figure 1.](image1)

![Figure 2.](image2)
The color of the leaves is darkening in color as going to the margin of the leaves being brown in color at the end of the leave Figure 2. The distance between each tooth of the leave is up to 0.3 cm. During March and April the plant is red brown in color with a large number of leaves with emerging of three or more leaves from one node.[2] Green spore powder is usually sprinkled from the brown leaves. In summer and specifically in June the stem is elongated up to 140 mm in high with a large number of rough and square branches.[3] Phytochemical studies of this plant showed the presence of various compounds like flavonoids (isoquercetine and apeginin), caffeic acid and traces of the pyridine alkaloids (nicotine and palustrine). In addition to that the plant was reported to contain silicic acid and minerals.[4] The plant is used traditionally to stop bleeding, heal ulcers and wounds and treat tuberculosis and kidney problems.[5] The new approach in using of this plant is the cytotoxic effects. It has been reported that crude proteins extracted from Equisetum arvense L. inhibit the proliferation of cultured cancer cells.[6] The collapse of mitochondrial transmembrane potential, was all observed in cells cultured for 48 h with the herb extract of the plant.[7] Screening of some plant extracts for inhibitory effects on HIV-1 and its essential enzymes shows that water extract of aerial parts of Equisetum arvense possesses inhibitory effect on HIV-1 induced cytopathy.[8]

MATERIAL AND METHODS

Plant material

Plant material was collected from Mkeshefa town in Salahaddin province and was authenticated by the Iraqi National Herbs Center in Abughreb. The plant was collected during June and dried in shade at room temperature.

Extraction

Powdered plant (50 g) was macerated with ethanol (70%, 150 mL) for 24 hour with periods of shaking on a magnetic stirrer. The extract was filtered to get rid of plant ashes, then the filtrate was concentrated to 60 ml. and divided into two fractions; the first (20 mL.) is evaporated by rotary evaporator to dryness and taken as the Ethanolic extract. The other fraction (40 mL) was taken and 25 ml. of distilled water is added, then extracted by ethyl acetate (30 mL ×3), both layers were separated and collected, the ethyl acetate layer was dried by anhydrous sodium sulphate. Both layers were evaporated to dryness and collected as ethyl acetate and aqueous samples.
Cytotoxicity
All the three dried extracts were dissolved in RPMI as medium in a concentration of (25 µg/ml) and incubated with viable human HeLa cells (human cervical carcinoma cells) for 24 hours, then viability or growth inhibition was measured by MTT method after comparing with negative control.

HPLC analysis of the ethyl acetate layer
HPLC was performed using Schimadzu apparatus, performed in Science and Technology Ministry. Column: phenomenex C-18, 3micrometer particle size (50×2.0 mm I.D).

Mobile phase: linear gradient of solvent A 0.1% formic acid: solvent B was (6:3:1, v/v) of acetonitrile: methanol: 0.1% formic acid, gradient program from 0% B to 100% B for 10 minutes Flow rate 1.2ml/min.

RESULTS AND DISCUSSION
Ethyl acetate, Ethanolic and aqueous extracts show the ability of plant extract to inhibit cell growth as follows.

Ethyl acetate layer
Upon measuring the OD value for the wells incubated with ethyl acetate extract, the average of all readings was 0.2622 (Figure 3).

1- Ethanolic extracts
Upon measuring the OD value for the wells incubated with Ethanolic extract, the average of all readings was 0.352 (Figure 4).
2- Aqueous extract
Upon measuring the OD value for the wells incubated with aqueous extract, the average of all readings was 0.3902 (Figure 5).

3- Control wells
Upon measuring the OD value for the control wells that were incubated alone without any extracted compound, the average of all readings was 0.407 (Figure 6).
Calculations
To estimate the activity of each extract separately, the viable survived cells after incubation is calculated by the following equation, the OD value of each extract is divided by that of the control wells and multiplied by 100.

**Ethyl acetate layer**
0.2622/0.407 × 100 = 64.4% of cells survived. Therefore, 35.57% of cells died, which represent the percentage of cellular growth inhibition.

**Ethanolic extract**
0.352/0.407 × 100 = 84.48% of cells survived.

Therefore, 13.51% of cells died, which represent the percentage of cellular growth inhibition.

**Aqueous extract**
0.3902/0.407 × 100 = 95.87% of cells survived.

Therefore, 4.12% of cells died, which represent the percentage of cellular growth inhibition. The percent of growth inhibition is represented by the excel report Figure 7.

![Growth inhibition](image)

**Figure 7: Percent of growth inhibition.**

**HPLC analysis of the ethyl acetate layer**
HPLC analysis of the ethyl acetate layer reveals the presence of iso-quercetrine flavonoid retention (time 3.7) as a major component of the ethyl acetate extract Figure 8.
The flavonoid isoquercetrine reported to posses cytotoxic effect both in *vitro* and in *vivo*.[9]

Ethyl acetate is known to be the best enriching solvent for isoquercetrine flavonoids.[10] The highest amount of flavonoids is present in ethyl acetate extract, thus the highest percentage of cellular growth inhibition was observed in the ethyl acetate extract.[11]

**CONCLUSION**

Equisetum arvense of Iraq contains flavonoids mainly isoquercetine which exhibit a significant cytotoxicity on Hela cells. Lower percent of inhibition by the aqueous layer after removal of the flavonoids by partition with ethyl acetate indicate the role of flavoniods in this growth inhibition.

**REFERENCES**


