

## Evaluation of the Chemical Composition of *Equisetum ravenis*

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**Abstract:** Different crude fractions of *Equisetum ravenis* were analyzed for their chemical evaluation, vitamins and bioactive secondary metabolite. The results revealed the presence of bioactive constituents comprising alkaloids, saponins, flavonoids, phenols and tannins. The medicinal also revealed the presence of ascorbic acid, riboflavin, thiamine and niacin in different crude fractions.

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### 1. Introduction

The potential of the phytochemicals have large scale pharmacological and biological activities such as antioxidant constituents (hydrolysable tannins, phenolic acid and flavonoids) of the plant materials for the care of health and protection from coronary heart diseases, cancer, anti-carcinogenic and anti-mutagenic effects. Varieties of herbaceous vegetables are protective against various diseases, particularly cardiovascular diseases. These herbaceous plants and species are harmless sources for obtaining natural antioxidants. Antioxidant constituents can delay or inhibit the oxidation of lipids and other compounds by inhibiting the propagation of oxidation chain reaction. Primarily, antioxidant effect is due to phenolic compounds such as phenolic acid, flavonoids and phenolic diterpenes and their mode of action for antioxidant compounds is due to its redox reaction properties which can absorb and neutralize free radicals by quenching singlet and triplet oxygen. Vitamins are organic substances necessary for metabolism. Human diet does not always contain the required amount of vitamins for the normal growth and maintenance of the body function and as such cannot produce enough quantity for their body metabolism, so it can be obtained from fruits, vegetables and foods. Deficiency of vitamins can cause serious human health diseases and sometimes, very small concentrations are required for maintenance of good human health. Keeping in view the importance of phyto-chemicals, vitamins and other important constituents of these medicinal plants and their wide use by the local community and practitioners for a variety of ailments, it is therefore very vital to discern their active constituents and to provide a scientific data base line which may play a significant role in knowing the quantities of these phytochemicals, vitamins and other constituents (Iqbal H et al 2011)

### 2. Materials and Method

#### 2.1 Plant materials

The plant *Equisetum ravenis* was collected at Peshawar, KPK, Pakistan, in July 2012 and was identified by Plant taxonomist. The whole plants were air-dried for 10 days and milled into powder with electrical grinder and dipped in methanol for one month and finally obtained methanol crude fraction was partitioned in to *n*-hexene, chloroform, ethyl acetate and water fraction. These fractions were used for further study.

#### 2.2 Alkaloid determination

3 g of the sample were weighed into a 250 ml beaker and 250 ml of 25% acetic acid in ethanol was added and covered to stand for 4 h. This was filtered and the extract was concentrated using a water-bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed (Harborne JB 1973).

#### 2.3 Tannin determination

1g of the sample was weighed into 250 ml plastic bottle. 100 ml of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 100 ml volumetric flask and made up to the mark. Then 10 ml of the filtrate was pipette out into a tube and mixed with 5 ml of 0.1 M FeCl<sub>3</sub> in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured in a spectrophotometer at 120 nm wavelength, within 10 min. A blank sample was prepared and the colour also developed and read at the same wavelength. A standard was prepared using tannin acid to get 100 ppm and measured (Van-Burden TP and Robinson WC 1981).

#### 2.4 Determination of total phenols

For the analysis of the phenolic component, the fat free sample was boiled with 100 ml of ether for 30min. 10ml of the extract was pipette into a 100 ml flask, then 20ml of distilled water was added. 4ml of ammonium hydroxide solution and 10 ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 40 min for colour development. The absorbance of the solution was read using a spectrophotometer at 550 nm wavelengths (Harborne JB 1973).

### 2.5 Saponin determination

The samples were ground. 25 g of each plant samples were dispersed in 250 ml of 25% ethanol. The suspension was heated over a hot water bath for 4 h with continuous stirring at about 60°C. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separator funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated thrice. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight. The saponin content was calculated in percentage (Iqbal H et al 2011)

### 2.6 Flavonoid determination

20 g of the plant species were extracted repeatedly with 150 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper no. 42 (125 mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed (Iqbal H et al 2011)

### 2.7 Determination of riboflavin

10 g of the sample was extracted with 150 ml of 50% ethanol solution and shaken for 1 h. This was filtered into a 250 ml flask; 15 ml of the extract was pipette into 50 ml volumetric flask. 10 ml of 5% potassium permanganate and 10 ml of 30% H<sub>2</sub>O<sub>2</sub> were added and allowed to stand over a hot water bath for about 30 min. 2 ml of 40% sodium sulphate was added. This was made up to 50 ml mark and the absorbance

measured at 550 nm in a spectrophotometer (D. E. Okwu and C. Joshi 2006).

### 2.8 Determination of thiamin

10g of the sample were homogenized with ethanolic sodium hydroxide (100 ml). It was filtered into a 250 ml flask. 10 ml of the filtrate was pipette and the colour developed by addition of 10 ml of potassium dichromate and read at 360 nm. A blank sample was prepared and the colour also developed and read at the same wavelength (D. E. Okwu and C. Joshi 2006).

### 2.9 Determination of niacin

10 g of the sample was treated with 100 ml of 1 N sulphuric acid and shaken for 40 min. 5 drops of ammonia solution were added to the sample and filtered. 10 ml of the filtrate was pipette into a 50 ml volumetric flask and 5 ml potassium cyanide was added. This was acidified with 5ml of 0.02N H<sub>2</sub>SO<sub>4</sub> and absorbance measured in the spectrophotometer at 450 nm wavelengths (D. E. Okwu and C. Joshi 2006).

### 2.10 Determination of ascorbic acid (vitamin C)

10 g of the sample was weighed into an extraction tube and 200 ml of EDTA/TCA (2:1) extracting solution were mixed and the mixture shaken for 30 min. This was transferred into a centrifuge tube and centrifuged at 2900 rpm for about 25 min. It was transferred into a 250 ml volumetric flask and made up to 100 ml mark with the extracting solution. 20 ml of the extract was pipette into a volumetric flask and 1% starch indicator was added. These were added and titrated with 20% CuSO<sub>4</sub> solution to get a dark end point (Barakat M Z et al 1973)

## 3. Results and Discussion

Table 1 showing the quantitative determination of phytochemicals constituents of different crude fractions of *Equisetum ravenis*. High quantity of saponins was found in water fraction 2.36 ± 0.01. The values of Alkaloids, phenolic compounds, saponins, flavonoids and tannins were very trace in all study fractions of the plant.

Table 2, showing the results analysis of Vitamin composition of crude fractions of *Equisetum ravenis* expressed as mg/100g dry weight. Ascorbic acid (vitamin C) was found highest 21.13 ± 0.11 in Ethyl acetate fraction followed by chloroform fractions. Riboflavin, thiamine and niacin were also detected in little amount in all tested fractions of *Equisetum ravenis*.

Table 1. Phytochemical composition of the crude fractions of *Equisetum ravenis* expressed as mg/100g dry weight.

Phytochemicals	<i>n-hexane</i>	<i>Chloroform</i>	<i>Ethyl acetate</i>	<i>Water</i>
Alkaloids	0.13 ± 0.1	0.44 ± 0.01	2.44 ± 0.01	0.44 ± 0.01
Flavonoids	0.51 ± 0.11	1.56 ± 0.01	1.86 ± 0.01	1.61 ± 0.01
Phenols	0.02 ± 0.02	1.32 ± 0.01	1.22 ± 0.01	0.12 ± 0.01
Tannins	0.01 ± 0.001	0.71 ± 0.14	0.81 ± 0.14	1.81 ± 0.14
Saponins	0.01 ± 0.03	0.17 ± 0.01	0.14 ± 0.01	2.36 ± 0.01

Table 2. Vitamin composition of crude fractions of *Equisetum ravens* expressed as mg/100g dry weight.

Vitamin	<i>n</i> -hexane	Chloroform	Ethyl acetate	Water
Ascorbic acid	5.12 ± 0.11	15.13 ± 0.24	21.13 ± 0.11	11.13 ± 0.24
Riboflavin	0.15 ± 0.11	0.63 ± 0.13	0.15 ± 0.11	0.51 ± 0.13
Thiamine	0.22 ± 0.20	0.24 ± 0.12	0.23 ± 0.20	0.21 ± 0.12
Niacin	0.01 ± 0.01	0.03 ± 0.07	0.04 ± 0.11	0.02 ± 0.07

In particular, despite widespread use of wild plants as medicines in Pakistan, there have been also found the relationship of total flavonoid and phenol contents with antioxidant activity. In the longer term, plant species (or their active constituents) identified as having high levels of antioxidant activity in vitro may be of value in the design of further studies to unravel novel treatment strategies for disorders associated with free radicals induced tissue damage (F. Pourmorad et al 2006).

Saponins are a class of chemical compounds, one of many secondary metabolites found in natural sources, with saponins found in particular abundance in various plant species (Hostettmann, K. and Marston, A 1995). There is tremendous, commercially driven promotion of saponins as dietary supplements and nutraceuticals. There is evidence of the presence of saponins in traditional medicine preparations (Asl, M.N. and Hosseinzadeh, H 2008).

These plants are good source of vitamins including ascorbic acid, riboflavin, thiamine and niacin. Both plants have higher amount of ascorbic acid. Vitamin C is also a highly effective antioxidant. Even in small amounts vitamin C can protect indispensable molecules in the body, such as proteins, lipids (fats), carbohydrates, and nucleic acids (DNA and RNA) from damage by free radicals and reactive oxygen species that can be generated during normal metabolism as well as through exposure to toxins and pollutants (e.g. smoking). Vitamin C may also be able to regenerate other antioxidants such as vitamin E (Carr AC, Frei B 1999). Severe vitamin C deficiency has been known for many centuries as the potentially fatal disease, scurvy. Scurvy is rare in developed countries because it can be prevented by as little as 10 mg of vitamin C daily (Simon JA, Hudes ES 2000).

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