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Biochemical, Phytochemical and Antioxidant Composition of *Equisetum debile* Roxb

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Abstract

Biochemical, phytochemical and antioxidant composition of *Equistem debile* R. stem was determined. The *E. debile* extract showed the presence of reducing and non reducing sugars, total sugars, free amino acids, water soluble proteins and salt soluble protein. Phytochemical screening confirmed the presence of tannins, saponins, flavonoids, terpenoids, cardiac glycosides and total phenolic acid contents. Trolox equivalent total antioxidant activity of methanolic extract determined by DPPH radical scavenging assay was found to be lower than that determined by Phosphomolybdenum assay while the ascorbic acid equivalent total antioxidant activity determined by DPPH radical scavenging assay was found to be higher than that determined by Phosphomolybdenum assay. High value of $ABTS^{+}$ radical scavenging activity and low values of DPPH and hydroxyl radical scavenging activity and reducing power have been observed in *E. debile* extract as compared to Trolox and ascorbic acid. The data provides useful information regarding the nutritional and medicinal importance of *E. debile* and suggest that this plant possesses good antioxidant activity.

Keywords: Biochemical composition; *Equisetum debile* roxb; Phytochemical composition; Antioxidant properties; Radical scavenging activity

Introduction

Equistem debile Roxb, a member of family Equisetacea, is widely distributed in Africa and Asia. *E. debile* has been traditionally used in New Guinea to polish ornaments and is still being used in cleaning aluminium cooking pots. *E. debile* stem has a great medicinal importance and used as folk medicine for the treatment of joint pain and jaundice [1]. It is used as an efficient drug for relief from exterior syndrome, nephrotic syndrome, epidemic conjunctivitis, urethritis, ulcerative blepharitis hepatitis, and diarrhea [2-4]. *E. debile* is highly effective in treatment of urinary tract infections, intestinal bleeding, hemorrhoid blood, woman menstrual problems, pediatric apistaxis, smooth muscles apoptosis, removal of kidney stones, gonorrhoea and bone fracture [2,5-9].

Chemically, *E. debile* stem constitute nicotine, kaempferol-3-sophoroside, coumaric acid, p-hydroxybenzoic acid, ferulic acid, 5-hydroxymethyl-2-furfuraldehyde, equisetumoside, guaiacylglycerol-b-coniferyl ether, glycoside, 3a-O-b-D-glucopyranoside, isolariciresinol, sterols, flavonoids, debilignanoside, debilitriol, equisetumine, polyphenols and phenolic acids including caffeic and protocatechuic acids [10-12]. It shows the free radical scavenging, antioxidants, cytotoxic, antibacterial, antifungal activities [13,14]. The alcoholic extracts of *E. debile* stem significantly decreased the triglyceride and cholesterol levels of experimental rats and rabbits without influencing the β-apoprotein level and body weight [15].

Previously, studies have been performed on phytochemical and antioxidant properties of other members of family Equisetaceae such as *Equisetum maximum* and *Equisetum diffusum* [16]. However, limited data on the biochemical, phytochemical and antioxidant properties of *E. debile* has been reported in literature. Therefore, the present study is designed to characterize the stem of *E. debile* for its biochemical, phytochemical and antioxidant properties. The study would provide valuable information regarding the medicinal and pharmaceutical importance of *E. debile* for the researchers.

Material and Methods

Sampling

The stem sample of *E. debile* R. was collected from damp areas of Bait Hazara, located along the bank of River Indus, Dera Ghazi Khan, Punjab, Pakistan. The stem was cut into pieces with a sharp knife and dried under shade. The dried sample was grinded to fine powder using electric grinder (National MJ-176-R) and stored in air tight jars for further analysis.

Biochemical analysis

Sugars and free amino acids were extracted in 75% aqueous methanol for 24 hr at solid to solvent ratio 1:25 w/v [17]. Total sugars and reducing sugar contents were estimated by the method developed by Travelyan and Harrison [18] and Hulme and Naraian [19] respectively. Non reducing sugars content was calculated by taking the difference of total sugars and reducing sugars. The free amino acid content was determined by the method described by Hamilton and Slyke [20].

Water soluble and salt soluble protein contents of *E. debile* stem were obtained by successive extraction in distilled water and 0.5 M ammonium sulfate solution respectively for 4 hours. The protein content of each fraction was determined by Biuret's method [21].

Phytochemical analysis

Screening: Aqueous and ethanolic extracts of *E. debile* were

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subjected to Phytochemical screening by following the procedures as described by Trease and Evans [22] and Harborne [23].

Test for tannins: Few drops of ferric chloride solution (0.1%) were added to the test tube containing 2 mL water extract (2%) of *E. debile.* The blue black brownish or green coloration was observed indicating the presence of tannins.

Test for saponins: The water extract of *E. debile* (5 mL) was mixed with 2.5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with olive oil (2 drops) and shaken vigorously. The emulsification observed indicated the presence of saponins.

Test for flavonoids: Ethanolic extract (75%) of *E. debile* was treated with three drops of aluminium hydroxide solution (1%). A yellow coloration observed showed the presence of flavonoids.

Test for terpenoids (Salkowski test): Ethanolic extract of *E. debile* (5 mL) was mixed with 2 mL of chloroform. 3 mL of concentrated sulphuric acid was added along the side wall of test tube. A reddish brown coloration observed at the interface of two layer showed the presence of terpenoids.

Test for cardiac glycosides (Keller-Killani test): Ethanolic extract of *E. debile* (5 mL) was treated with glacial acetic acid (2 mL) and ferric chloride (0.1 mL) solution. To this, the addition of concentrated sulphuric acid (1 mL) resulted in to the formation of brown ring at the interface of two layers, which indicated the deoxysugar characteristic of cardenolides.

Estimation

a. Tannins: Tannins were extracted in 20% methanol using method as described by Harborne [23] and estimated by following the method of Makkar [24] as modified by Fagbemi et al. [25]. The tannins content were calculated as g/100 g dry weight from standard curve of tannic acid (R^2=*0.9944*).

b. Saponins: Saponins were determined by method of Obadoni and Ochuko [26]. The sample (20 g) was extracted twice with equal volumes of 20% ethanol (100 mL) at 55°C with continuous stirring. The extracts were combined and volume was reduced to 40 mL by evaporation in boiling water bath. The concentrate was extracted twice with diethyl ether (20 mL) and the aqueous layer was further extracted twice with n-butanol (60 mL). The butanolic extract was washed twice with 5% aqueous NaCl solution (10 mL) and evaporated to dryness in boiling water bath. The residue was weighed and saponins contents were calculated as.

$$Saponins\ Content\ \ (g/100g\ dry\ weight) = W_r \div W_s \times 100$$

where W_r is the weight of the residue and W_s is the weight of the sample.

c. Total flavonoids: Flavonoids were extracted in 70% ethanol for 30 min at 25 ± 5°C and estimated by method of Michalaska et al. [27]. Briefly, an aliquot of ethanolic extract (1 mL) was mixed with 5% $NaNO_3$ solution (1 mL) and allowed to react for 6 min. The mixture was diluted up to 25 mL with 50% ethanol after the addition of 10% aluminium chloride solution (1 mL). The reaction mixture was incubated at room temperature (25°C) for 15 min and absorbance was recorded at 510 nm. The total flavonoids content were calculated as mg catechin equivalent/100 g dry weight from the calibration curve of catechin standard solution (R^2=*0.9929*).

d. Total phenolic contents (TPC): The sample (5 g) was extracted twice in 70% methanol (50 mL) at room temperature for 24 h. TPC in crude methanolic extract was determined using the method of Taga et al. [28]. The methanolic extract (1 mL) was treated with Folin-Ciocalteu's reagent (0.1 mL) followed by the addition 2% Na_2CO_3 solution (2 mL). The mixture was allowed to stand for 30 min and absorbance was measured at 750 nm. TPC was calculated as g/100 g dry weight from the standard curve of gallic acid (R^2=*0.996*).

Antioxidant analysis

Preparation of methanolic extract: Sample (5 g) was socked in 75% methanol (50 mL) for 4 hour. The solvent was evaporated to dryness and residue (10 mg) was dissolve in 75% methanol (100 mL) and used for antioxidant analysis.

Total antioxidant content: The Trolox and ascorbic acid equivalent total antioxidant activity of methanolic extract was determined by 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical and phosphomolybdenum assays using the methods described by Shad et al. [29] and Prieto et al. [30] respectively.

DPPH radical assay: The methanolic extract (1 mL) was mixed with 40 µM methanolic DPPH solution (3 mL) and allowed to stand in dark (30 min). The absorbance of reaction mixture was recorded at 517 nm and the total antioxidant content was calculated as g/100g of extract from standard curve of Trolox (R^2=*0.993*) as well as ascorbic acid (R^2=*0.990*).

Phosphomolybdenum assay: The methanolic extract (1 mL) was mixed with the reagent solution (1 mL) prepared by mixing 0.6 M Sulphuric acid, 28 mM sodium phosphate solution and 4mM ammonium molybdate solution in ratio of 1:1:1 v/v/v. The reaction mixture was incubated at 95°C for 90 min and cooled to room temperature. The absorbance was measured at 695 nm and total antioxidant content was calculated as mg/100 g extract from standard curves of Trolox (R^2=*0.932*) and ascorbic acid (R^2=*0.997*).

Radical scavenging activity

a. DPPH radical scavenging activity: DPPH radical scavenging activity was determined by the method described by Shad et al. [17]. The methanolic extract (1 mL) was mixed with 40 µM DPPH solution (3 mL) and allowed to stand in dark for 30 min. The absorbance of reaction mixture was recorded at 517 nm and DPPH radical scavenging activity was calculated by following equation:

$$DPPH\ radical\ \ scavenging\ activity\ (\%) = 100 \times Abs_0 - Abs_{30} \div Abs_0$$

where;

Abs_0 =Absorbance of control (DPPH solution without sample or standard)

Abs_{30}=Absorbance of the reaction mixture after 30 min.

To find out the IC_{50} values, the methanolic extract (1 mL) of different concentrations (0.02, 0.04,… 0.1 mg/mL) were mixed with DPPH solution (3 mL) and absorbance was noted after 30 min at 517 nm. The IC_{50} value was calculated by linear regression equation (R^2=*0.9880*) obtained from different concentrations of *E. debile* extract.

b. Hydroxyl radical scavenging activity: Hydroxyl radical scavenging ability was determined by improved method of Smirnoff et al. [31]. Methanolic extract (0.1 mL) was mixed with 9 mM $FeSO_4$ solution (1 mL), 9 mM salicylic acid solution (1 mL) and 8.8 mM H_2O_2 (1 mL). The reaction mixture was incubated at 37°C for 30 min and absorbance was recorded at 510 nm. Trolox and ascorbic acid

were used as standard antioxidants for the comparison. The hydroxyl radical scavenging ability of the extract was calculated by the following equation:

$$Hydroxyl\ \ radical\ \ scavenging\ \ activity\ (\%) = \left[1 - (A_s - A_b) \div A_c\right] \times 100$$

where;

A_s=Absorbance of the sample

A_b=Absorbance of blank (without salicylic acid)

A_c=Absorbance of control (without sample).

c. ABTS⁺ radical scavenging activity: The antioxidant activity of methanolic extract in terms of 2, 2-azino-bis (3-ethylebenzothiazoline-6-sulfonic acid) cation radical ($ABTS^{+\cdot}$) scavenging activity was determined by the method described by Re et al. [32]. In order to generate $ABTS^{+}$ radicals, 7 mM ABTS solution was treated with 2.45 mM potassium persulphate solution in a proportion of 1:1 v/v and allowed to stand for 16 h. The solution was diluted with 80% ethanol until the absorbance was set at 0.700 ± 0.05. The sample (1 mL) was treated with $ABTS^{+\cdot}$ solution (1 mL) and allowed to stand for 6 min. Absorbance was recorded at 734 nm and $ABTS^{+}$ radical scavenging activity was calculated using following equation:

$$ABTS^{+}\ radical\ \ scavenging\ \ activity\ (\%) = \left[1 - (A_s - A_b) \div A_c\right] \times 100$$

Where;

A_b=Absorbance of blank (without sample)

A_s=Absorbance of the sample and

A_c=Absorbance of control (without extract).

d. Reducing power: The reducing power of the extract of *E. debile* was estimated by the method of Oyaizu [33]. Methanolic extract (2.5 mL) was taken into a test tube. Phosphate buffer, pH 6.6 (2.5 mL) and 1% potassium ferricyanide solution (2.5 mL) was added into the test tube and incubated at 50°C for 20 min. After that, 10% Trichloroacetic acid solution (2.5 mL) was added in to the same test tube and centrifuged at 1000 × g for 8 min. The supernatant (5 mL) was diluted with distilled water (5 mL) and treated with 0.1% ferric chloride solution (1 mL), the absorbance (700 nm) after 5 min and reducing power was expressed in terms of an increase in absorbance. Trolox and ascorbic acid were used as standard antioxidants for comparison.

Statistical analysis

The results were expressed as mean ± standard deviation of three replicates. The data on radical scavenging activity, IC50 for DPPH radical inhibition and reducing power was analyzed for comparison by applying Tuky's multiple range test at 95% confidence level ($p \leq 0.05$) using statistical software SPSS, version 12.0.

Results and Discussion

Table 1 presents the results for biochemical composition of *E. debile* stem. The total sugars, reducing sugars, non-reducing sugars and free amino acids content in ethanolic extract were found to be 6.36 ± 0.26, 0.04 ± 0.01, 6.29 ± 0.26 and 0.15 ± 0.01 g/100 g dry weight respectively. The water soluble and salt soluble proteins content were estimated to be 1.65 ± 0.11, and 1.58 ± 0.25 g/100 g dry weight respectively. Previously, no data about biochemical composition of *E. debile* stem has been reported in literature.

Phytochemicals such as tannins, saponins and flavonoids contribute to the medicinal characteristics of plants. Tannins are polyphenols and act as anti-irritant. They also posses antisecretolytic, antiphlogistic, antiparasitic and antimicrobial properties [34]. Saponins are a special class of glycosides which have soapy characteristics and antifungal activity [35]. Flavonoids are the phytochemical compounds which show antioxidant activity due to hydroxy phenol groups in their chemical structure [36,37]. The results obtained from phytochemical analysis have been summarized in Table 2. The phytochemical screening indicated the presence of Tannins, Saponins, Flavonoids, Terpenoids, Cardiac glycosides in *E. debile* stem. The quantitative analysis of some of the phytochemical compounds showed that total tannins, saponins, total flavonoids and total phenolic content were found to be 1.033 ± 0.022, 0.34 ± 0.08, 0.114 ± 0.02 and 3.58 ± 0.25 g/100 g dry weight respectively. This phytochemical composition was in comparison to the stem of *C. intybus* that have been reported to be 1.32 ± 0.04, 0.26 ± 0.01, 0.08 ± 0.03, 2.09 ± 0.21 g/100g dry weight for total tannins, saponins, total flavonoids and total phenolic content respectively [38]. The *E. debile* stem and *C. intybus* have been traditionally used for the treatment of jaundice [1].

Parameters	Value (g/100 g dry weight)
Sugars	
Total	6.36 ± 0.26
Reducing	0.04 ± 0.01
Non Reducing	6.29 ± 0.26
Proteins	
Water soluble	1.65 ± 0.11
Salt soluble	1.58 ± 0.25
Total salt soluble	3.38 ± 0.66
Free amino acids	0.15 ± 0.01

Table 1: Biochemical composition of *E. debile* stem.

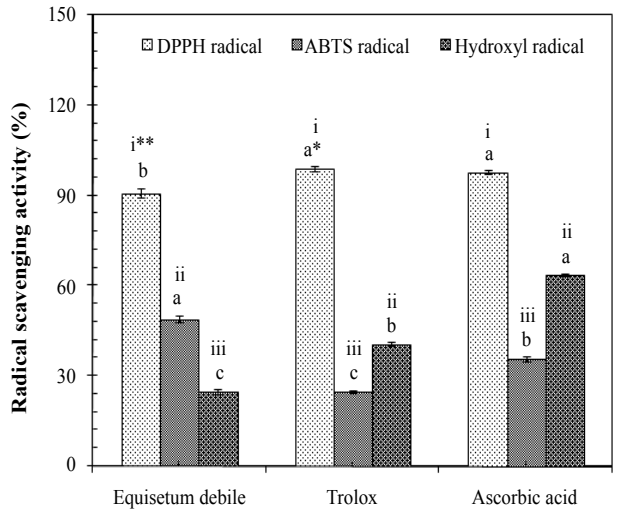
	Screening	Content (g/100 g dry weight)
Tanins	+	1.03 ± 0.02
Saponins	+	0.34 ± 0.08
Total Flavonoids	+	0.15 ± 0.02
Total phenolic content	+	1.37 ± 0.88
Terpenoids	+	ND*
Cardiac glycosides	+	ND
*ND: not determined		

Table 2: Phytochemical composition of different parts of *E. debile* stem.

The results for total extract yield and total antioxidant content of methanolic extract of *E. debile* stem are presented in Table 3. Total extract yield was found to be 11.07 g/100 g dry weight. Trolox equivalent as well as ascorbic acid equivalent total antioxidant content of methanolic extract of *E. debile* stem estimated by DPPH radical scavenging assay was found to be 52.33 ± 10.50 and 76.60 ± 11.5 mg/100 g extract respectively. On the other hand, the same activity determined by phosphomolybdenum assay was found to be 79.12 ± 9.0, 77.92 ± 8.2 mg/100 g extract respectively. Previous studies concerning the scavenger activities of *Equisetum arvense, Equisetum ramosissimum* L., and *Equisetum telmateia* L. showed high scavenger abilities against DPPH, NO, and OH radicals and also high total antioxidant capacity according to the fluorescence recovery after photobleaching method [39]. The methanolic extract of *E. hyemale*, using DPPH method, exhibited a percentage of antiradical activity of 24.29% at the concentration of 100 ug/mL [40,41]. The antioxidants are the compounds which prevent the oxidative damage to macromolecules by scavenging the free radicals produced in various biochemical

	Total extract yield (g/100 g dry weight)	Total antioxidant content (g/100 g dry weight)	
		Trolox equivalent	Ascorbic acid equivalent
	11.7 ± 0.58		
DPPH assay	-	5.23 ± 2.50	7.86 ± 4.11
Phosphomolybdenum assay	-	7.91 ± 4.07	7.00 ± 3.48

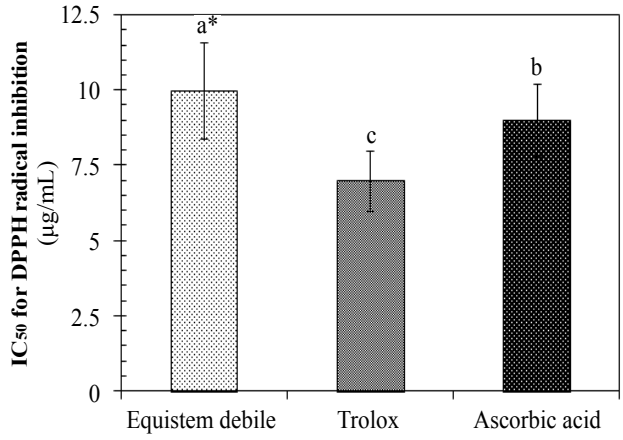
Table 3: Total extract yield and total antioxidant content of methanolic extract of *E. debile* stem.



*The bars labeled with different alphabets show significant difference among the scavenging activities of different antioxidants against a particular radical at 95% confidence level ($p<0.05$).

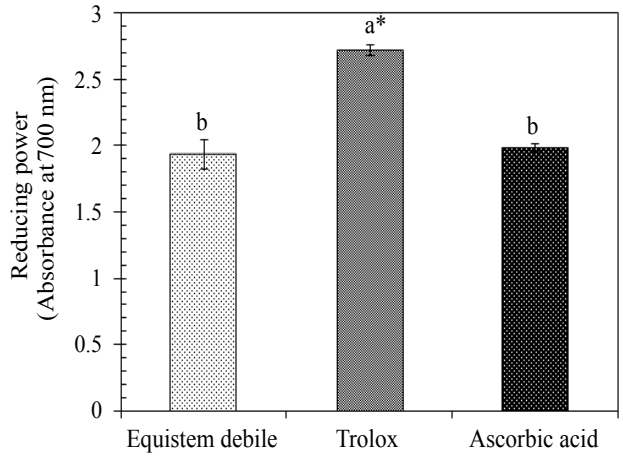
**The bars labeled with different numbers show significant difference among the scavenging activity of a particular antioxidant against different radicals at 95% confidence level ($p<0.05$).

Figure 1: Radical scavenging activities of methanolic extract of *E. debile* stem compared to that of Trolox and ascorbic acid.



*The bars labeled with different letters are significantly different at 95% confidence level ($p=0.01$).

Figure 2: Comparative values of IC50 of methanolic extract of *E. debile* stem, Trolox and ascorbic acid against DPPH radical.



*The bars labeled with different letters are significantly different at 95% confidence level ($p=0.001$).

Figure 3: Reducing power of methanolic extract of *E. debile* stem compared to that of Trolox and ascorbic acid.

processes occurring in human body [42]. The flavanoids and phenolic acids are the most important phytochemical compounds which posses medicinal potential owing to their antioxidant properties. The foods rich in antioxidant compounds have been proved to be effective in decreasing the risk of cardiovascular mortality, destruction of cancer cells and preventing the oxidative lung damage [43,44].

The free radical scavenging ability of methanolic extract of *E. debile* comparative to Trolox and ascorbic acid was determined using DPPH radical, hydroxyl radical and $ABTS^+$ radical. The results have been presented in Figure 1. The DPPH radical scavenging, hydroxyl radical scavenging and $ABTS^+$ radical scavenging activities of *E. debile* extract, Trolox and ascorbic acid was found to be 96.83 ± 0.60, 24.78 ± 0.82, and 49.03 ± 0.30%, 98.98 ± 0.10, 40.60 ± 0.22 and 24.82 ± 0.20% and 97.89 ± 0.20, 63.60 ± 0.44 and 35.65 ± 0.80% respectively. A statistically significant difference ($p<0.05$) was observed among the free radical scavenging activities of *E. debile* extract, Trolox and ascorbic acid. The DPPH radical and hydroxyl radical scavenging activity of *E. debile* was found to be comparatively low than those of Trolox and ascorbic acid. On the other hand, each of the *E. debile* extract and standard antioxidants were found to show greater ability to scavenge DPPH radical as compared to hydroxyl and $ABTS^+$ radical. The determination of IC_{50} values showed that the concentration of *E. debile* extract required for 50% inhibition of DPPH radical was found to be 10.02 ± 0.01 µg/ml. The IC_{50} value of *E. debile* extract was found to be significantly high than those of Trolox and ascorbic acid (Figure 2). However, IC_{50} value at µg level indicates high antiradical activity. The present results for DPPH radical scavenging activity stem were found to be higher than those reported by Khan et al. [14] for *E. debile*.

The reducing properties of the antioxidant compounds are based on their hydrogen donating ability to terminate the free radical chain reactions [45,46]. The reducing power of *E. debile* extract was found to be significantly lower than those of Trolox and ascorbic acid (Figure 3).

Conclusion

E. debile, a traditionally important medicinal plant, possesses great nutritional and medicinal value. It contains a significant amount of biochemical, phytochemical and antioxidant compounds with good radical scavenging ability. It may be a valuable candidate for pharmaceutical formulations particularly used for the management of oxidative stress caused by the reactive oxygen and nitrogen species.

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