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Evaluation of proximate, free radical scavenging activity, and phytochemical analysis of *Equisetum Arvense* L. extracts

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Medicinal properties of *Equisetum arvense* L. have been recognised since ancient times and are used in the treatment of various diseases. It is reported to contain 5-8 % elemental silica. Recently, silica has been reported to promote collagen synthesis and help bone mineralization. It was therefore hypothesized that its inclusion to the basic supplement of calcium, vitamin D, and zinc may prove beneficial to osteoporotic patients. The present study was thus undertaken. Its nutritional activity and radical scavenging potential were also included in the study as these are other aspects reported to help patients of osteoporosis. The results show that ethanolic extract of aerial parts of *E. arvense* may prove beneficial in the anabolic treatment of osteoporosis by boosting bone formation activity.

Keywords: *Equisetum arvense* L.**,** Phytochemical, Proximate, Radical scavenging activity.

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Introduction

The genus *Equisetum* consists of 30 species of rush-like and conspicuously jointed perennial herbs[1]. The species in the genus *Equisetum* are reported to be rich in silica content in the whole plant kingdom[2]. The rush like appearance of the plant is due to deposition of silicates on the stem. Among the 30 species, *E. arvense* L. is mostly studied and is traditionally used for cardiovascular problems, kidney problems, HIV induced cytopathy, bone related disorders and also used as collagen promoting agent in cosmetics, antioxidant, astringent, etc[3]. It is commonly known as horsetail and is a strange-looking plant with creeping, string like rootstock. In *E. arvense*, leaves are reduced to small scales, fused into sheaths and distributed along the stems. The roots are present at the nodes and produce numerous hollow stems. The hollow stems are of two types: the fertile and the sterile or vegetative stem. The sterile or vegetative stem is green bottlebrush like with branches at stem nodes while the fertile stem is brownish, unbranched, thick, producing strobilus containing spores and is smaller than sterile stem. For medicinal purpose, aerial parts, mainly sterile stems are used as they are reported to be rich in silica. Silica in this plant is mostly found in the form of silicic acid[4].

Osteoporosis is a multifactorial disease associated with inflammation and increased oxidative stress. Nutritional and plant based medicines targeting these associated maladies are expected to bring relieving effects to the patients[5]. The current available drugs (antiresorptive and anabolic) are costly and associated with various side effects[6,7]. In order to overcome this situation, scientists have turned to formulating a therapy based on the use of nutrients and medicinal plants. The literature mentions that *E. arvense* has antioxidant, free radical scavenging, and anti-inflammatory activities but no systematic study is available. Silica containing *E. arvense* is suggested for the treatment of osteoporosis as it helps in the absorption and use of calcium and also in the formation of collagen[4,8]. Alterations of collagen properties are therefore likely to affect the mechanical properties of bone and increase fracture susceptibility[9]. Plant secondary metabolites such as flavonoids (quercetin, kaempferol, luteolin, and apigenin) and triterpenoids (oleanolic acid, betulinic acid, and ursolic acid) have shown protective effect in osteoporotic bone loss[10-12]. *E. arvense* is reported to contain all these metabolites. Very few references are available evaluating its anabolic effect for

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osteoporosis treatment. In view of the studies reporting anabolic effect of silica on bone metabolism, this plant was selected to study its anabolic potential. To ensure the authentication and to determine the standardisation parameters for preventing adulteration, thus leading to safety and efficacy of the plant, pharmacognostic study[13] was undertaken. In order to use this plant as a medicinal drug in oral dosage forms for the treatment of osteoporosis, it was necessary to characterize its proximate composition and silica content to distinguish the plant from its adulterants. Also, free radical scavenging activity and phytochemical composition of different extracts were studied to determine the best solvent system extracting the metabolites that play an important role in reducing the risk of osteoporosis. In the present work, pharmacognostic parameters (proximate composition, elemental analysis especially silica content in the whole plant, DPPH free radical scavenging activity, and phytochemical screening) of *E. arvense* have been reported.

Plate 1 — Whole plant of *E. arvense*

Materials and Methods

Chemicals and solvents used in the study were of analytical grade and purchased from Himedia Laboratories Pvt. Ltd, Mumbai and local firms.

Collection and extraction of *E. arvense*

The whole plant of *E. arvense* (Plate 1) was collected from Vainganga River in Bhandara District of Maharashtra, India in the month of October, 2011 and was authenticated by University Department of Botany, Rashtrasanth Tukadoji Maharaj Nagpur University, Nagpur by depositing a voucher specimen (No. R/9698). The aerial part (sterile stem) of *E. arvense* was washed with distilled water to remove all the adhering soil and dirt particles. The stems were then shade-dried at room temperature (25±2 °C) for 7-8 days as shade drying influences high yield of phenolic compounds as reported earlier[14]. The dried stems were powdered and stored in air-tight containers. Dried powder (20 g) was extracted with distilled water, 95 % ethanol, hydro-methanol (1:1), and hexane using a Soxhlet apparatus. The extract was vacuum concentrated, dried and weighed to determine its yield. The yield of the extract was: aqueous extract (34.87 %), ethanolic extract (17.89 %), hydro-methanolic extract (16.52 %), and hexane (6.67 %).

Proximate analysis

Proximate analysis of *E. arvense* was performed according to the standard procedure. The recommended methods of the Association of Official Analytical Chemists (AOAC)[15] were used for the determination of moisture, ash, fat, and protein content. Total carbohydrate content was calculated by difference. The gross energy content of a food (metabolizable energy values) was calculated using energy conversion factors for the protein, fat, and carbohydrate. The caloric value was calculated by the sum of the percentages of proteins and carbohydrates multiplied by a factor of 4 (kcal/g) and total lipids multiplied by a factor of 9 (kcal/g)[16]. The results were expressed as percentage composition of the representative constituents.

Elemental analysis

Silica estimation

Silica, a trace element and major mineral in ash was measured in acid insoluble ash (wet ashing) of the sample by AOAC method[17].

Antioxidant activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity of the extracts (aqueous, ethanolic, and hydro-methanolic) was determined by Pérez-Jiménez method[18]. Aqueous, ethanolic, and hydromethanolic extract stock solutions (1.0 mg/mL) were diluted to final concentrations of 300 µg/mL in methanol. About 1 mL of 0.6 mM DPPH was mixed with 2.5 mL of sample solutions in different

concentrations and allowed to react at room temperature. Methanol (1.0 mL) and plant extract solution (2.5 mL) without DPPH served as blank. For negative control, 1 mL of 0.6 mM DPPH with 2.5 mL of methanol was used. Standard solution of ascorbic acid (1 mg/mL) was considered as positive control. After 30 min of incubation, the absorbance was measured at 518 nm and converted into the percentage antioxidant activity (AA %) using the following formula:

AA %= 100- {[(Δ sample- Δ blank) *100]/ Δ control}

Phytochemical analysis

Qualitative phytochemical screening of different extracts (aqueous, ethanolic, hydromethanolic, and hexane extracts) of *E. arvense* was done according to the standard procedures[19] to determine the secondary metabolites like sterols, tri-terpenes, saponins, glycosides, alkaloids, tannins, and flavonoids.

Statistical analysis

Statistical analysis was done by AnalyseIt software (Version 2.26) by Microsoft Partner, Silver Independent Software Vendor (ISV) for Windows Excel. The values given are mean±standard error (S.E).

Results

Proximate analysis

The result of proximate analysis of *E. arvense* is presented in Table 1. The moisture content in *E. arvense* was found to be 5.62 %. The total ash content was found to be 2.17 %. Carbohydrates formed the major portion of organic matter and contributed more caloric value compared to protein and fat content. The decreasing order of the nutrients present in *E. arvense* is carbohydrate > protein > fat > moisture > ash. The caloric value of *E. arvense* was found to be 424.06 Kcal.

Elemental analysis

Silica was estimated in the ash. The total yield of acid insoluble ash was found to be 1.87 g % and contained 40.72 % of silica. This is equal to 7.62 % silica in the plant.

DPPH free radical scavenging activity

DPPH is a free radical, stable at room temperature, which produces a violet solution in methanol. It is reduced in the presence of an antioxidant molecule, giving rise to uncoloured methanol solutions. The use of DPPH provides an easy and rapid way to evaluate antioxidants. The free radical scavenging activity of the three different extracts of *E. arvense* is given in Table 2. A significant difference was observed in DPPH free radical scavenging activity of ethanolic and hydromethanolic extracts of *E. arvense* when compared to the aqueous extract.

Table 1 — Proximate analysis of *E. arvense*

S. No.	Food component	Content
1	Moisture	5.62 %
2	Ash	2.17 %
3	Fat	11.3 %
4	Protein	19.67 %
5	Carbohydrate	60.92 %
6	Caloric value (kcal/kJ)	424.06 kcal /1174.27 kJ

Table 2 — DPPH free radical scavenging activity of different extracts of *E. arvense*

S. No.	Extracts	EC_{50}
1	Aqueous extract	94.86±0.23
2	Ethanolic extract	96.89±0.20*
3	Hydromethanolic extract	96.87±0.21*

Values are represented as mean±S.E. * indicates $p < 0.05$ as compared to Aqueous extract (n=3)

Phytochemical analysis

The result of the phytochemical analysis of *E. arvense* is presented in Table 3.

Discussion

Pharmacognosy is the study of medicines derived from natural sources[13]. The medicinal and nutritional potentials of sterile stems of *E. arvense* and its different extracts (aqueous, ethanolic, and hydromethanolic) were assessed in the present study through proximate, elemental, and phytochemical analyses. Also, free radical scavenging of different extracts was studied by DPPH method. Proximate analysis helps to understand the nature and properties of the medicinal plant to be used. Water, an essential constituent in food composition databases, is the most variable component especially in plants. The moisture content, an important parameter for storage of *E. arvense* was 5.62 % as shade drying for 7-8 days brought down the initial large moisture content to enable its prolonged storage[20]. Low moisture content also indicates the low content of volatile matter and it also reduces the risk of putrification as well as proliferation of mold. Ash value is an indicator of quality and purity of crude drugs, especially in the powder form. Ashing the sample removes all traces of organic matter, which may otherwise interfere in an analytical determination. In the present study, sterile

Table 3 — Qualitative phytochemical analysis of aerial parts (sterile stems) of *E. arvense*

Phytochemical screening		Aqueous extract	Ethanolic extract	Hydro-Methanolic extract (1:1)	Hexane extract
Sterols/ Triterpenes	Salkowski test	+	++	++	+++
	Liebermann test	+	++	+	+++
Saponin	Foam test	+	+	+	–
Glycosides	Keller Killani test	+	+	++	+++
	Conc. H_2SO_4	+	++	++	+++
	Salkowski test	+	++	++	+++
Alkaloids	Mayer's test	+	+++	+	+
	Wagner's test	+	+++	++	+
Tannins	$FeCl_3$ test	+	–	+	–
	Gelatin test	+	–	+	–
Flavonoids	$FeCl_3$ test	+	+++	++	–
	NaOH test	+	+++	++	–

Note: Low (+); Average (++); High (+++); Absence (–)

stems of *E. arvense* were ashed. The ash content was low (2.17 %) because leaves in *E. arvense* are reduced to small scales fused into sheaths and distributed along the stems. As leaves contain more amounts of ash than stems[21], ash content was found to be very less in *E. arvense*. Carbohydrate is an important energy constituent. It can be utilised to yield energy, polymers of carbohydrates act as energy storage molecules and their derivatives are found in a number of biological molecules including coenzymes and the nucleic acids[22]. Carbohydrate content was more in the sterile stems of *E. arvense*, thus, contributing more caloric value. The energy values of medicinal plants are mainly used to translate medicinal samples intakes as intakes of food components[23]. The result obtained from proximate analysis establishes the nutrient and energy value of *E. arvense* and value as a medicinal drug in oral dosage form. For silica estimation, ashing of the powder was done by wet ashing using acid, as wet ashing prevents formation of insoluble silicates[24]. Silica is one of the important plant nutrient found most abundantly in the plant ash[24]. Silica content in *E. arvense* was found to be 7.62 %. This is in agreement with the reported range of 5-8 %[1]. From the phytochemical analysis, it was observed that ethanolic and hydromethanolic extracts of *E. arvense* showed more presence of alkaloids and flavonoids compared to aqueous and hexane extracts. When compared to hydromethanolic extract, ethanolic extract showed very high presence of these metabolites. Alkaloids are one of the most efficient therapeutically significant bioactive substances in plants. Flavonoids are known to have anti-oxidant activity and they provide protection against inflammation as well[25]. Oxidative stress and inflammation are some causes implicated in osteoporosis. The result of phytochemical analysis was found to be consistent with the result of free radical scavenging activity by DPPH method, where ethanolic and hydromethanolic extract showed highest free radical scavenging activity compared to aqueous extract. The results of phytochemical and free radical scavenging analyses showed that ethanol followed by hydromethanol extracted maximum phytochemicals from *E. arvense*. The results indicate that ethanolic extract has strong potential for medicinal use and would also serve as agents for the treatment of a wide range of diseases and infections.

Conclusion

The study shows that sterile stem of *E. arvense* has high medicinal and nutritive values and that it can be used as a medicinal drug in oral dosage form. Also, the ethanolic extract of the aerial parts (sterile stems) of *E. arvense*, which is rich in antioxidants, may prove beneficial in anabolic treatment of osteoporosis.

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