Quality control standardization and antioxidant activity of roots from *Eriosema chinense*

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**A B S T R A C T**

**Background:** Roots of the plant *Eriosema chinense* Vogel are used as a vegetable by the people of Northern Australia and North East India. Traditionally, the roots of the plant are used for the treatment of diarrhea by the tribal people of North East India.

**Aim:** The present study was undertaken to perform quality control standardization and to evaluate antioxidant activity of roots from *Eriosema chinense*.

**Methods:** The roots were examined macroscopically, microscopically and various physicochemical parameters were evaluated using standard guidelines. Further, quantitative estimations of different phytoconstituents along with standardization of ethanol extract with HPTLC using lupinifolin as a marker compound was also done. The extract was also evaluated for in vitro antioxidant activity using different experimental models.

**Results:** The brown coloured bulb shaped, hairy roots showed the presence of cortical cells densely filled with tannins, lignified sclerenchymatous pericyclic fibers and central lignified xylem vessels with spiral thickening. Physicochemical parameters evaluated included ash values, extractive values, loss on drying, foaming index, swelling index, foreign matter, crude fiber content, pesticide residue and heavy metal analysis which were found to be in prescribed limits. Fluorescence powder drug analysis and total number of starch grains was also evaluated while total hemolytic activity was found to be moderate. Phytochemical screening of different extracts and quantitative estimations revealed the extract to be rich in carbohydrates, flavonoid, phenols and tannins, while lupinifolin quantified by HPTLC in ethanol extract was found to be 6.48% w/w. In vitro antioxidant studies depicted a potent antioxidant activity of extract that may be attributed to the presence of higher amount of flavonoids, phenols and tannins.

**Conclusion:** The quality control standards obtained in the present study will provide referential information to researchers for proper identification and authentication of plant and will help in maintaining its pharmaceutical, botanical and economical importance.

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1. Introduction

*Eriosema chinense* Vogel (Leguminosae-Papilionoideae) is mainly distributed over the Eastern Himalayan region of India and China and is also found in countries like Thailand, Myanmar and Australia. The stem of plants is 30–50 cm in height, slender, erect, woody, little branched, and densely hairy. Leaflets are simple, linear-ligulate, and 2.5–5 cm in length. The flowers are yellow, 1–2, and borne in leaf-axils. The pods are oblong, about 2 cm in length, and densely hairy.1 The roots of the plant are eaten raw and in the form of vegetable by the people of Northern Australia and North East India. It is used as a traditional medicine for the treatment of diarrhea by the tribal people of Meghalaya (India). The decoction of grains is used as astringent, diuretic, tonic, in cold sweats and is used during parturition to promote discharge of the lochia. A decoction of the grain with powdered pepper is given for diarrhea.2 Recently, eight new prenylated flavonoids, khonklonginols A–H, together with six known compounds including five flavonoids, lupinifolinol, dehydrolupinifolin, flemichin D, eriosemaone A, lupinifolin, and one lignan, yangambin, have been reported from this plant. Pharmacologically the plant has been evaluated for its cytotoxic and anti-tubercular activity.3

In developed as well as developing countries, medicinal plants have been used as a potential source of home remedies. It has also been observed that in recent years, there is great increase in...
demand of herbal medicines in pharmaceutical industries which covers a substantial proportion of the global drug market over the counter drug products. This has resulted into a huge turnaround in fields like botany, pharmacognosy and pharmacology and has become a major pillar of streams like pharmacy, medicine, natural product chemistry and many others. The above revolution has resulted in initiation of various active research programmes to produce effective standardized extracts and to isolate new lead compounds. Herbal drugs are prone to contamination and deterioration which may lead to variation in composition of constituents resulting in little or no therapeutic efficacy. Therefore, care should be taken right from proper identification of plants, seasons and area of collection and their extraction and purification process in order to obtain quality oriented herbal formulations. To minimize the amount of adulteration and misinterpretation of herbal drugs or food materials, it is very essential to standardize it by evaluating various qualitative and quantitative parameters which may play a major role in developing referential standards of that particular drug or food materials. Even though the roots of the plant *E. chinense* is used as a vegetable food and is traditionally used for treating various ailments still, there is no any scientific data available describing its quality control profile. Therefore, the present study was carried out to evaluate different qualitative and quantitative parameters to facilitate the quality control standardization of *E. chinense*.

2. Material and methods

2.1. Authentication

The plant samples of *E. chinense* were obtained from different places in Shillong region (Khasi Hills district) and Jowai region (Jaintia Hills District) of Meghalaya (India) in May 2011 and after careful examination they were identified as *E. chinense* Vogel by Dr. B.K. Sinha (Scientist C, In-charge), Botanical Survey of India, Shillong, India. For future reference, a voucher specimen (COG/EC/14) of the plant has been deposited in Department of Pharmaceutics, Indian Institute of Technology (Banaras Hindu University), Varanasi, India. The results represented in the present study provide a mean or common observations observed in different samples of *E. chinense*.

2.2. Macroscopic and microscopic evaluation

The macroscopic evaluation of the roots was done by observing them with reference to their color, shape, size, odor and taste etc. For microscopic examination, the roots of the plant were cut and were fixed in FAA (Formalin 5 mL + Acetic acid 5 mL + 70% Ethyl alcohol 90 mL) for about 24 h. Further, as per the schedule given by Sass, the specimens were dehydrated with graded series of tertiary- butyl alcohol (TBA) followed by infiltration of the specimens by gradual addition of paraffin wax (melting point 58–60 °C) until TBA solution attained super saturation. The specimens were then casted into paraffin blocks which were further sectioned with the help of Rotary Micromtome at a thickness of 10–12 μm followed by dewaxing as described by Johansen. Staining of the sections was done using Toludine blue and whereever required, fast-green, safranin and IKI (iodine in potassium iodide (for starch)) were also used as a staining agents. Photographs of different magnifications were taken with Nikon Trinocular Microscopic unit, Model E-200, Japan.

2.3. Physicochemical standardization

The roots of the plant were dried and grounded in a mixer grinder and were used for evaluation of various physicochemical parameters. Foreign matter present in the roots was determined visually using lenses, whereas loss on drying was determined by drying specified quantity of plant material in oven to a constant weight at 110 °C. Different ash values (total ash, acid insoluble ash and water soluble ash) were determined according to the standard procedure by incinerating the plant material at a temperature between 500 and 600 °C until it is white, indicating the absence of carbon. Extractive values of the roots in different solvents were evaluated by soaking it in respective solvents for about 18 h. Hemolytic activity of the roots was evaluated by determining its capacity to produce hemolysis in ox blood sample at a particular concentration compared to standard diosgenin. In addition, foaming index (by measuring the length of the foam) and swelling index (by measuring the volume occupied by the plant material in a measuring cylinder after 3 h) were also determined using the usual procedure.5,9 Lycopodium spore method, as described by Wallis10 was used to determine the total number of starch grains in the roots. The amount of crude fiber present in the plant material was determined by boiling the plant material with 10% nitric acid followed by treating it with 2.5% NaOH.11 The powdered roots were subjected to fluorescence powder drug analysis under day light, short UV and long UV according to the methods described by Chase and Pratt.12

2.4. Heavy metal analysis

The quantitative estimations of various heavy metals present in the roots of *E. chinense* was carried out using atomic absorption spectrophotometer by adopting the procedure mentioned in WHO guidelines.9

2.5. Pesticide content

The pesticide content of the roots was determined using the WHO guideline. A mixture of 350 mL of acetonitrile:water (65:35) was added to 50 g of grinded powdered roots which was blended at high speed for 5 min followed by filtration. 250 mL of filtrate was then transferred to a separating funnel to which further 100 mL light petroleum, 10 mL of sodium chloride (40%) and 600 mL of water were added with constant shaking up to 35–45 s. Aqueous layer was discarded from the solvent layer and the later was washed twice with 100 mL portions of water to which 15 g of anhydrous sodium sulfate was added with vigorous shaking. The extract was separated followed by reducing its volume to 5–10 mL which was allowed to pass through column packed with Florisil R grade 60/100 PR, activated at 650 °C at a rate of not more than 5 mL per minute. Three different elutes were obtained using three different ratios of ether: light petroleum mixture as mobile phase i.e. Elute 1 contained 6% of ether while elute 2 and 3 contained 15% and 50% of ether. The obtained elutes were transferred to a sample holder, and burned in a suitable combustion flask flushed with oxygen. The gases produced gets absorbed in a suitable solution in the combustion flask (water for chloride and H2SO4 in case of phosphate pesticides). In case of chloride pesticides 15 mL of the solution obtained after combustion was mixed with 1 mL of ferric ammonium sulfate (0.25 mol/L) and 3 mL of mercuric thiocyanate followed by swirling it where absorbance was measured at 460 nm. For determining phosphate pesticides, 7 mL of the solution obtained after combustion was mixed with 2.2 mL of sulfuric acid (300 g/L), 0.4 mL of ammonium molybdate (40 g/L) and 0.4 mL of aminophenylsulfonic acid followed by swirling it and heating it at 100 °C for 12 min which was then measured at 820 nm.

2.6. Phytochemical standardization

Preliminary phytochemical screening of the different extracts of powdered roots obtained after cold maceration using different
solvents for the presence of various phytoconstituents was carried out. The grinded roots of the plant were extracted using ethanol (1:5 L) with Soxhlet apparatus until the whole drug was exhausted. Further, the extract was concentrated and evaporated (12.29% w/w) in a Rota evaporator and was kept in a desicator until use. Total alkaloid content in the plant material was estimated using the usual gravimetric analysis in which the plant material was first extracted with H2SO4 and was further given successive washes with chloroform and diethyl ether. The method described by Yemm and Willis was used to determine total carbohydrates in ethanol extract of E. chinense (EEC) using anthrone reagent. Total phenols and tannin contents in EEC were estimated according to the method of Hagerman et al. (1974) using Folin ciocalteau reagent. Total flavonoid and flavonol contents were determined following the methods of Kumara and Karunakaran using aluminum trichloride. Total saponin content was estimated taking dioxigenin as standard by implementing method of Baccou et al. using anisaldehyde-ethyl acetate reagent and H2SO4.

EEC was further standardized for the first time with lupinifolin after confirmation of its presence by thin layer chromatography using high performance thin layer chromatography (HPTLC). A stock solution of both EEC and standard lupinifolin in methanol was prepared in concentration of 5 mg/mL and 0.2 mg/mL respectively. The mobile phase for developing the chromatogram consisted of hexane and ethyl acetate mixture in the ratio 85:15 (v/v). The study was carried out using Camag- HPTLC instrumentation equipped with Linomat V sample applicator, Camag TLC scanner 3, Camag TLC visualizer and WINCATS 4 software for data interpretation. The Rf values were recorded and the developed plate was screened and photo-documented at ultra violet range with wavelength (λmax) of 254 nm.

2.7 In vitro antioxidant studies

Since EEC showed the presence of flavonoids in very high quantity along with phenols and tannins therefore, it was further evaluated for its in vitro antioxidant activity by using various in vitro methods. Phosphomolybdenum method was used to determine the total antioxidant capacity of EEC which is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at 695 nm in acidic pH. The assay of reducing power was performed by adopting potassium ferricyanide method using ascorbic acid as standard. The free radical scavenging activity of EEC was determined by 1,1-diphenyl-2-picryl-hydrazil (DPPH) method where ascorbic acid was used as standard and absorbance was measured at 517 nm. Nitric oxide scavenging assay was performed following the principle that, sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions. EEC was also evaluated for H2O2 scavenging activity by using standard method and percent inhibition was calculated after taking the absorbance at 230 nm. Deoxyribose method was implemented for determining the hydroxyl radical scavenging activity of EEC using butylated hydroxyl anisole (BHA) as a standard where absorbance was measured at 532 nm.

3. Results

3.1 Macroscopical and microscopical evaluation

Macroscopically, the roots of the plant are bulb shaped, dark brown in color with smooth surface bearing root hairs. Their size ranges from 1 to 3 cm in length and 0.5–1.5 cm in width with a narrow pointed base. The roots bear, characteristic odor and bitter taste (Fig. 1).

The primary structure of the root in transverse section shows tetrarch condition, where secondary growth starts quite early. The formation of cork cambium is normal. The transverse section of roots before secondary growth shows the presence of cork cells, continuous lignified sclerenchymatous pericyclic fibers followed by continuous ring of phloem encircling the slightly ridged and furrowed solid zone of xylem (Fig. 2B).

In the mature root (Fig. 2A), the cork cells are compressed, thick walled, rectangular in shape with 7–10 layers and have their sizes ranging from 40 to 50 μm in length and 8–12 μm in width. This is followed by cork cambium showing 5–8 layers of thin walled rectangular to tabular shaped parenchymatous cells (35–55 μm in length and 10–15 μm in width). Next to the cork cambium appears, thick walled discontinuous lignified sclerenchymatous pericyclic fibers having size in the range of 150–300 μm in length and 10–25 μm in width. This is followed by secondary cortex constituting 8–10 layers of irregular shaped thin walled parenchymatous cells. In this zone, few parenchymatous cells are densely filled with tannin having size in the range of 25–45 μm in length and 15–35 μm in width which appear blue with ferric chloride solution (Fig. 2D). The entire secondary cortex region shows the presence of starch grains both simple and compound and few discrete xylem vessels with spiral thickening (150–300 μm in length and 15–45 μm in width). The central portion of the transverse section consists of wide zone of lignified xylem which consists of vessels, fibers and tracheids vessels showing spiral thickening and appears pink with phloroglucinol (Fig. 2A, B and F). As growth proceeds, the number of furrows in the xylem become more deeper and are represented by radially elongated medullary rays 3–4 cells wide dissecting the xylem from 3 to 4 sides. Starch grains are present in large amount all throughout the section. Under the polarized light, the starch grains appears bright with dark background and are elliptical or circular in shape having size in the range of 8–14 μm in length and 7–12 μm in width (Fig. 2E).

Powder microscopy of roots shows the presence of sclerenchymatous pericyclic fibers (Fig. 2C) and parenchymatous cells filled with tannin appearing brown in color (Fig. 2D). The ground tissues are filled largely with starch grains which are more predominant (Fig. 2E). The grains are large, elliptical or ovate or circular with centric or eccentric hilum. The powder study also showed the presence of lignified xylem with spiral thickening (Fig. 2F).

3.2 Physicochemical standardization

The roots had 0.914% w/w of foreign matter, 3.853% w/w of moisture content and a swelling index of 7.25 mL/g, whereas length...
of the foam of powdered root was found to be less than 1 cm therefore, foaming index was reported to be less than 100. The roots showed the presence of total ash (5.612% w/w), acid insoluble ash (0.642% w/w) and water soluble ash (2.773% w/w), while extractive values in different solvents reported in % w/w were water: 5.150, methanol: 9.604, ethanol: 8.332, ethyl acetate: 4.975, chloroform: 5.778, hexane: 1.392, acetone: 5.441 and pet ether: 2.294 respectively. The total number of starch grains present in 1 mg of powder drug was found to be 162 325, while crude fiber content of roots was found to be 13.95% w/w of plant material. The hemolytic activity of roots of *E. chinense* was found to be 188.89 units per gram of powder. Fluorescence powder drug analysis of roots of *E. chinense* is represented in Table 1, whereas the results of heavy metal analysis of roots of *E. chinense* for the presence of various heavy metals are represented in Table 2.

### 3.3. Pesticide content

From the results, chlorinated pesticide present in the roots of *E. chinense* in first and the second elute was reported to be 0.479 and 0.315 mg/kg of plant material. The phosphated pesticide from the first and second elute of column was found to be 0.036 and

### Table 1
Fluorescence analysis of *E. chinense*.

<table>
<thead>
<tr>
<th>Test</th>
<th>Day light</th>
<th>Short UV</th>
<th>Long UV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Powder + 1 N NaOH in methanol</td>
<td>Corn silk</td>
<td>No fluorescence</td>
<td>Light green</td>
</tr>
<tr>
<td>Powder + 1 N NaOH in water</td>
<td>Orange red</td>
<td>Firebrick</td>
<td>Green yellow</td>
</tr>
<tr>
<td>Powder + 1 N HCl in methanol</td>
<td>Dark red</td>
<td>No fluorescence</td>
<td>Dark olive</td>
</tr>
<tr>
<td>Powder + 1 N HCl in water</td>
<td>Khaki</td>
<td>No fluorescence</td>
<td>Dark golden red</td>
</tr>
<tr>
<td>Powder + 1 N HNO₃ in methanol</td>
<td>Corn silk</td>
<td>No fluorescence</td>
<td>Green yellow</td>
</tr>
<tr>
<td>Powder + 1 N HNO₃ in water</td>
<td>Golden red</td>
<td>No fluorescence</td>
<td>Medium spring green</td>
</tr>
<tr>
<td>Powder + 5% iodine</td>
<td>Corn silk</td>
<td>No fluorescence</td>
<td>Pale green</td>
</tr>
<tr>
<td>Powder + 5% FeCl₃</td>
<td>Maroon</td>
<td>No fluorescence</td>
<td>No fluorescence</td>
</tr>
<tr>
<td>Powder + 50% KOH</td>
<td>Dark olive green</td>
<td>No fluorescence</td>
<td>Sea green</td>
</tr>
<tr>
<td>Powder + 25% ammonia saturated</td>
<td>Dark red</td>
<td>No fluorescence</td>
<td>Green yellow</td>
</tr>
<tr>
<td>Powder + picric acid saturated</td>
<td>Dark golden red</td>
<td>No fluorescence</td>
<td>Medium sea green</td>
</tr>
<tr>
<td>Powder + acetic acid</td>
<td>Dark red</td>
<td>Light green</td>
<td>Spring green</td>
</tr>
</tbody>
</table>
0.0641 mg/kg of plant material respectively while in third elute it was reported to be absent.

3.4. Phytochemical standardization

Preliminary phytochemical analysis of the EEC revealed the presence of phenols, flavonoids, tannins, alkaloids, steroids and carbohydrates as a major component (Table 3). Total phenolic content in EEC was reported to be 43.242 mg/g gallic acid equivalent while total tannin content was estimated to be 26.161 mg/g tannic acid equivalent. Total flavonoid and flavonol content were found to be 109.868 and 16.447 mg/g rutin equivalent. Total alkaloid and saponin estimated in the plant material were reported to be 0.552% w/w and 12.790 mg/g diosgenin equivalent, whereas total carbohydrate in EEC was found to be 146.807 mg/g D-fructose equivalent. The HPTLC analysis depicted well resolved peaks of EEC showing the presence of lupinifolin. The spots of the entire chromatogram were visualized under UV 254 nm and the percentage of lupinifolin (Rf 0.38) in EEC was reported to be 6.48% (w/w) (Fig. 3).

3.5. In-vitro antioxidant studies

Linear regression equation was used to determine the total antioxidant capacity of EEC and was expressed as the number of equivalent of ascorbic acid which was found to be 90.166 ± 1.641 μg/mL. Assay of reducing power is a concentration dependent reaction i.e. higher concentration indicates higher reducing power. The results demonstrated a potent reducing potential of E. chinense (0.263 ± 0.004 μg/mL) which was quite comparable with reducing power of standard ascorbic acid (0.419 ± 0.006 μg/mL). The capability of EEC to reduce DPPH by donating an electron or hydrogen to DPPH is indicative of free radical scavenging activity of the extract. The results depicted a IC50 Value of 146.357 ± 4.321 μg/mL of EEC as compared to ascorbic acid (IC50: 79.120 ± 4.016 μg/mL). A considerably moderate scavenging potential of hydrogen peroxide by EEC was observed with an IC50 value of 221.048 ± 5.055 μg/mL compared to standard rutin IC50 82.866 ± 6.396 μg/mL. Griess reagent was used to determine the nitric oxide scavenging activity which illustrated a moderate scavenging activity of EEC (IC50: 232.945 ± 4.690 μg/mL) in comparison to rutin (IC50: 76.436 ± 3.773 μg/mL). Fenton reaction was used to assess the potential of EEC in inhibiting the hydroxyl radical production through iron (II)-dependent deoxyribose damage assay. The results demonstrated a potent scavenging activity with an IC50 value of 170.234 ± 6.505 μg/mL compared to positive control BHA (IC50 71.923 ± 4.934 μg/mL).

4. Discussion

Quality control standardization of herbal drugs helps us in correct identification and authentication of the plant material. In recent years, there has been wide increase in therapeutic importance of herbal medicine thus, it is very essential to obtain a proper quality control profile for various medicinal plant used in traditional system of medicine. This may be helpful in minimizing the adulteration of these plant which occurs mainly due to improper knowledge regarding the varied geographical conditions, associated problems of different vernacular names, its morphology and microscopical characteristics. It is also said that correct identification and proper quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicine which contributes to its safety and efficacy.

Pharmacognostical evaluation of a plant/plant parts is considered to be the preliminary step in standardization of a plant that provides valuable information in terms of its morphological, microscopical and physical characteristics. Macroscopical examination of a plant/plant parts represents detailed information regarding the qualitative assessment of plant based on its morphological and sensory characters such as size, shape color, taste, odor etc while microscopical evaluation provides us extensive knowledge about the cellular arrangement of tissues. The specific microscopic characters of roots of E. chinense showed the presence of cortical cells densely filled with tannins, normal cork cambium, lignified sclerenchymatous pericyclic fibers, central lignified xylem vessels with spiral thickening and simple and compound starch gains spread all throughout the transverse section.

The results showed the presence of very low moisture content in the roots. It is very essential to control the moister content, since higher moisture content in plant material may lead to its deterioration and may therefore result in percentage variation of active constituents. The ash values represent inorganic salts naturally occurring, adhering or deliberately added to crude drug as a form of adulterant. Total ash in a plant material includes both physiological as well as nonphysiological ash while acid insoluble ash is a part of total ash and is an indicative of silica present, especially as sand and siliceous earth whereas, water soluble ash is the water soluble portion of the total ash. From the results, it was found that the roots showed the presence of higher quantity of water soluble ash compared to acid insoluble ash. There was a consistent reduction in extractive values observed with a decreasing order of solvent polarity. The amount of active chemical constituents present in plant material depends on the extractive values extracted through different solvents for which as yet no suitable chemical or biological assay exist. Swelling index of a plant material is conclusive of the therapeutic or pharmaceutical value which may be attributed to the presence of gums, mucilage, pectin and hemicelluloses. The results demonstrated a lower swelling index of roots which may be due to low quantity of the above mentioned parameters. The foaming index of a plant material is the ability of an aqueous decoction of that plant materials and their extracts to
form persistent foam.\textsuperscript{9} Fluorescence powder drug analysis is considered to be an important parameter in qualitative determination of crude drug since many compounds in plant material can be judged by their property of exhibiting florescence in daylight or in ultra violet range (e.g. alkaloids like berberine).\textsuperscript{4} The phenomenon of hemolysis is common in plants containing mainly saponins which cause hemoglobin to diffuse into the surrounding medium.\textsuperscript{9} This principle is used for the determination of hemolytic activity of plant material which demonstrated a medium hemolytic activity of roots of \textit{E. chinense}.\textsuperscript{154}

The heavy metals analysis performed in the present study showed the presence of As, Cr, Cu, Fe, Ni, Pb which were found to be in accordance with the prescribed limits of WHO. Heavy metals may accumulate in plants either through foliage or root systems and therefore, it is very essential to determine the level of toxic metals in the medicinal plants. The main contributing factor includes environmental pollutants, industrial and traffic emissions, agricultural expedients such as cadmium-containing dung, organic mercury fungicides and insecticide containing lead and arsenate. Heavy metal analysis also provides valuable information regarding metals that are natural essential components of coenzymes which play major role in growth, photosynthesis and respiration.\textsuperscript{23}

Agricultural practice such as spraying and treatment of soils occurring throughout the processes of cultivation, and administration of fumigants during storage may result in contamination of medicinal plant/plant parts with pesticides. WHO has therefore, suggested that every nation dealing with production of medicinal plants/plant parts should have at least one central laboratory which will provide information regarding standard limits of pesticides.\textsuperscript{9} The roots of \textit{E. chinense} did show the presence of chlorinated and phosphated pesticides but were present in accordance with the standard limits of these pesticides.

The chemical nature of the active constituents present in the plant material can be identified by performing preliminary phytochemical screening of that plant material.\textsuperscript{4} The results from the phytochemical analysis revealed the presence of flavonoids, phenols, carbohydrates, alkaloids, steroids, tannins and saponins. The quantitative estimations performed in the present study depicted the presence of carbohydrates, flavonoids, phenols and tannins in major quantities while saponins and alkaloids were present in quite considerable amount. Flavonoids have shown potential antioxidant and anti-inflammatory activities which are attributed to an increased capillary permeability and have been also associated in treatment of various cardiovascular diseases.\textsuperscript{24,25} Phenols play beneficial role in active, quenching of oxygen-derived free radicals thus, neutralizing them by donating hydrogen atom or an electron to the free radicals. Therefore, they are considered as strong antioxidants and free radical scavengers with anticarcinogenic, antibacterial, anti-inflammatory activities and are also used in coronary heart disease, some types of tumors and coronary artery disease.\textsuperscript{26,27} Tannins have a strong astringent action and are reported to have antibiotic, anti-inflammatory, anti-viral and antioxidant activities.\textsuperscript{4,28,29} Alkaloids have been reported to possess wide range of therapeutic importance in the fields of cancer, malaria, pain, inflammation, parkinsonism, hypertension and number of central nervous system disorders.\textsuperscript{30} The study also included HPTLC quantification of lupinifolin in EEC. Lupinifolin is a prenylated flavanone reported to have chemopreventive (anti-tumor promoters), antimycobacterial, anti-HSV-1, antibacterial and antioxidant activities.\textsuperscript{3,31,32} Thus, lupinifolin can be used as a chemical marker for standardization of roots from \textit{E. chinense}.

The results also depicted a potent \textit{in vitro} antioxidant activity of EEC which was evident through total antioxidant capacity, reducing power assay and different free radical scavenging methods. Many studies have reported that, free radicals such as nitric oxide, hydroxyl radical and hydrogen peroxide in human body, get bound to DNA nucleotides and damages various biological systems thus, resulting in carcinogenesis, mutagenesis, and cytotoxicity. These impairments have been potentially improved by the use of plants rich in flavonoids, phenols and tannins as they neutralize the free radicals by donation of hydrogen atom, quenching of oxygen and by chelation of metals which reduces the elevated oxidative stress.\textsuperscript{10,13}

5. Conclusion

In conclusion, the present study provides various qualitative and quantitative standards of roots from \textit{E. chinense} which will benefit interested researchers as a referential source of valuable information that will certify its identity and authenticity. The evaluated parameters may help in maintaining quality and purity of the vegetable root and will prevent its adulteration with drug of same or other genus having low potency.

Conflicts of interest

All authors have none to declare.

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