



Preliminary Phytochemical Screening of *Elytraria acaulis* Roots

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Authors' contributions

This work was carried out in collaboration among all authors. Author SS designed the study, wrote the protocol. Author RN supervised the study. Author AS managed the analyses of the study. Author AK wrote the first draft of the manuscript. Author VL managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The main objectives of this study were to qualitatively evaluate the profile of phytochemical constituents of *Elytraria acaulis* root (EAR) extracts. The dried powder of the roots of the plant was extracted with three different solvents namely petroleum ether, methanol and water, and subjected to various phytochemical tests and UV-VIS spectrophotometry to ascertain the principal constituents contained in the extracts. The result revealed the presence of alkaloids, flavonoids, tannins, glycosides with various other phytoconstituents in different extracts of *Elytraria acaulis* roots (EAR). We suggest that various extracts of *Elytraria acaulis* roots (EAR) might have potential chemical constituents that could be used in the future for the development of novel therapeutic agent.

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1. INTRODUCTION

There exists a plethora of knowledge, information, and benefits of herbal drugs in our ancient literature of Ayurvedic (Traditional Indian Medicine), Siddha, and Unani medicine. Most of the people around the world particularly in developing countries are not being able to afford pharmaceutical drugs, depends on plant based traditional medicines, to sustain their primary health care needs. Herbs are thus serving as an effective alternative for the harmful and debilitating effects of synthetic drugs. *Elytraria acaulis* is one such important medicinal plant that is being used the world over in the traditional system of medicines. Medicinal plants are being used since ages for the cure of various ailments. Herbal medicine is still the mainstay of about 75-80% of the whole population, and most of the traditional therapy involves the use of plant extract and its active constituents. In the Indian system, a large number of medicinal plants have been used for many centuries for treating various diseases. WHO (World Health Organization) estimates that 80% of the world population relies on plants for their primary health care.

Elytraria acaulis belongs to the family *Acanthaceae* locally called Patharchatta in Hindi, is a widely distributed, stemless perennial herb with an unbranched flowering stem. *Elytraria Acaulis* is widely distributed in tropical Africa and Peninsular India [1]. The phytochemical evaluation of *Elytraria acaulis* whole plant extracts (Ethanol, Methanol, Petroleum ether, Aqueous, Acetone) showed the presence of glycosides, saponins, phytosterols, phenolic compounds, flavonoids, and tannins [2]. Methanolic extract of the *Elytraria acaulis* leaves exhibited the presence of alkaloids, amino acids, carbohydrates, flavonoids, glycosides, phenol, protein, saponins, steroids, and tannins [3]. The methanolic extract of *Elytraria acaulis* plant revealed the presence of various phytochemicals namely, total phenols, flavonoids, and tannins [4].

Elytraria acualis is used in medicine by some ethnic people of India in treating skin diseases, ringworm [5], leucorrhoea [6]. The roots of *Elytraria acualis* have been used as various medications mainly in asthma, leucorrhoea, snake bite, piles [7].

Methanolic and ether extracts of *Elytraria acaulis* roots showed significant antibacterial activity against both Gram-positive and Gram-negative bacteria [8]. Although *Elytraria acualis* are commonly used in traditional medicine, a literature survey showed a lack of studies on the phytoconstituents from root extracts as well as spectroscopic analysis of UV-VIS scan of the root extracts. The present study aims to evaluate the various phytoconstituents present in the *Elytraria acaulis* roots (EAR) combined with their spectral data.

2. MATERIALS AND METHODS

The dried powder of the roots of the plant was extracted with petroleum ether, methanol, and water respectively and subjected to various phytochemical tests to ascertain the principle constituents contained in the extract. Different qualitative tests namely Molisch's test, alkaloid test, frothing test, FeCl₃ test, alkali test, Salkowski's test were employed to determine the various phytoconstituents from the *Elytraria acaulis* roots (EAR).

2.1 Collection and Identification of Plant Materials

The fresh roots of *Elytraria acaulis* were collected in the month of July-August from Firozabad district, Uttar Pradesh, India. The plant species were taxonomically identified and authenticated by the Department of Botany, Rashtrasant Tukadoji Maharaj Nagpur University, Maharashtra, India and also by the Central Institute of Medicinal and Aromatic Plants, (CIMAP), Lucknow, Uttar Pradesh, India with reference authentication number/herbarium accession number 1007 and 8333, respectively.

2.2 Extraction

The plant's roots were powdered after being dried under shade. The powder was passed through sieve plate no. 20 to collect the fine powder. This powder was used for the preparation of *Elytraria acaulis* methanolic, petroleum ether, and aqueous extracts (a total of six extracts). Two methods, maceration for cold extraction and Soxhlet for hot extraction were used.

2.3 Phytochemical Screening

Freshly prepared *Elytraria acaulis* root extracts (EAR) were subjected to different qualitative tests [9].

2.3.1 Test for carbohydrates (Molisch's test)

Approximately 500 mg of crude extracts were dissolved in 5 ml of distilled water each and later filtered. A few drops of Molisch's reagent (α -naphthol 10% (w/v) in 90% ethanol) were added to these filtrates. Then 1 ml of concentrated H_2SO_4 was poured carefully along the side of the test tube. Two minutes later, 5 ml of distilled water was added. A positive test, indicating the presence of carbohydrates, was confirmed with the formation of dull violet or red color at the interphase of the two layers.

2.3.2 Tests for alkaloids

Aqueous HCl (5 ml, 1% v/v) was used to dissolve 50 mg of each extract separately and later filtered, and then the filtrates were divided into 2 ml aliquots. Subsequently, Mayer's, and Hager's reagents were used to test the filtrates for the presence of alkaloids.

2.3.2.1 Mayer's test

One or two drops of 0.35 mol/l Mayer's reagent (potassium mercuric iodide solution, 1.36 g mercuric chloride and 5 g of potassium iodide, dissolved in 100 ml distilled water) were added to 2 ml of each filtrate along the side of the test tube. A positive test, demonstrating the presence of alkaloids, was indicated by a creamy white precipitate.

2.3.2.2 Hager's test

The extracts were treated with diluted hydrochloric acid and thereafter filtered. The filtrate is then added with the saturated picric acid; the presence of alkaloids is detected as a yellow colour precipitate.

2.3.3 $FeCl_3$ test for tannins

500 mg of extracts were dissolved in 20 ml of distilled water and boiled followed by the addition of a few drops of 0.1% $FeCl_3$. The appearance of blue-black colour indicates the presence of tannins.

2.3.4 Test for Anthraquinones (Borntrager's test)

500 mg of extract was mixed with 2 ml of benzene and filtered after shaking. 10 ml of 1% ammonia was added to the filtrate. The mixture was shaken for the appearance of violet color at the lower phase, indicating the presence of anthraquinones.

2.3.5 Test for glycosides

2.3.5.1 Keller-killiani test

1 g of extract was dissolved in 4 ml of glacial acetic acid with few drops of ferric chloride. After that 2 ml of concentrated sulfuric acid (H_2SO_4) was added through the wall of the test tube. The presence of glycosides is indicated by the presence of a brown ring.

2.3.6 Frothing test for saponin

1 g of extracts was dissolved in 10 ml of methanol for making stock solutions. These stock solutions were diluted to 0.5 mg/ml by the addition of 20 ml of distilled water. Test tubes containing the dilution were then shaken for 15 min. The formation of foam on the top of the test tubes indicates the presence of saponin.

2.3.7 Alkali test for flavonoids

0.5 g of extract was treated with few drops of 5% sodium hydroxide (NaOH) solution forming intense yellow colour and the color is lost in the presence of dilute HCl confirming the presence of flavonoids.

2.3.8 Test for steroids

0.5 g of extract was dissolved in 2 ml of chloroform, thereafter 2 ml of concentrate sulfuric acid (H_2SO_4) was added to it, and a presence of green to bluish colour indicates the steroids.

2.3.9 Test for phenols

0.5 g of extract was dissolved in 3-4 ml of ferric chloride ($FeCl_3$) solution. The formation of bluish-black colour indicates the presence of phenols in the extract.

2.3.10 Detection of protein and amino acid

2.3.10.1 Ninhydrin's test

The extracts were treated with Ninhydrin's reagent. The formation of purple colour with extract shows the presence of protein.

2.3.10.2 Biuret's test

An equal volume of 5% sodium hydroxide solution and 1% copper sulfate solution was added to the extract. The formation of a violet colour indicates the presence of amino acids.

2.3.11 Detection of phytosterols (Salkowski test)

200 mg of dry extract was shaken with 1ml of chloroform solution then a few drops of concentrated sulfuric acid were added by the side of the test tube. The formation of a brown ring indicates the presence of phytosterols.

2.4 UV-VIS Spectrophotometry Studies

After extraction, the dry sample of all six extracts i.e. hot and cold was dissolved in 50 % DMSO. UV spectra were recorded using UV-VIS double beam spectrophotometer (Systronics AU-2701) at a wavelength ranging from 200 nm to 1100 nm.

3. RESULTS

3.1 Phytochemical Screening

Phytochemical screening of the EAR showed the presence of several primary and secondary metabolites or phytoconstituents. In the phytochemical screening, all the extracts were shown to have different compositions. Both methanolic and aqueous extracts of EAR showed the presence of most of the phytoconstituents like carbohydrates, alkaloids, saponins, tannins, flavonoids, glycosides, phenols, protein, amino acids, and phytosterols. However, both methanolic and aqueous extracts of EAR failed to show the presence of anthraquinones and steroids. Ether extracts of EAR failed to show most of the constituents present in the methanolic and aqueous extracts except steroids.

3.2 UV-VIS Analysis

The UV-VIS analysis was performed for the identification of phytoconstituents present in all six extracts (hot and cold) of *Elytraria acaulis*. The qualitative UV-VIS profile of all extracts of *Elytraria acaulis* was observed at the wavelength ranging from 200 nm to 1100 nm. The profile peaks were observed at 200 to 600 nm (Fig. 1a-1f). The highest absorption of peaks of UV-VIS

spectroscopic analysis was observed at an interval of 10 wavelength (nm) range.

The absorption peaks (wavelength (nm)) in ether cold extract (E_{c1}), ether hot extract (E_{H2}), methanol cold extract (M_{c1}), methanol hot extract (M_{H2}), aqueous cold extract (W_{c1}), and aqueous hot extract (W_{H2}) at 460-451, 450-441, 410-401, 400-391, 420-411, and 440-341 with absorption of 10, 3.34, 2.88, 2.60, 3.30 and 3.64 were observed. These absorption bands are characteristic of phenol, tannins, glycosides, saponins, phytosterols, proteins, and amino acids, and the absorption bands characteristic for flavonoids are in the range 200-260nm, with the absorption as high as in the range of 5-10.

4. DISCUSSION

Phytochemicals are naturally present in medicinal plants, leaves, vegetables, stems, bark, and roots that provide protection from various diseases. The primary objective of this study was to evaluate the presence of various phytoconstituents in the *Elytraria acaulis* roots (EAR) combined with their spectral data. Common solvents used in extraction are polar solvents (water, alcohol), and nonpolar solvent (ether). In general, extraction procedures incorporated were maceration and Soxhlet extraction. The choice of an appropriate extraction method normally depends on the nature of the plant material, solvent used, pH of the solvent, temperature, and solvent to sample ratio [10]. Water is the most polar solvent and is used in the extraction of a wide range of polar compounds and it dissolves a wide range of substances, alcohol could extract polar secondary metabolites while ether is a nonpolar solvent and is useful in the extraction of compounds such as alkaloids, terpenoids, and fatty acids [11,12]. Maceration is suitable for thermolabile plant materials, whereas Soxhlet extraction, also known as continuous hot extraction is applied to plant materials that are heat stable [9].

Various extracts of *Elytraria acaulis* roots (EAR) revealed the presence of carbohydrates, alkaloids, saponins, tannins, flavonoids and phytosterols. Methanol and distilled water extracts exhibited nine positive preliminary phytochemical tests against eleven tests. Phenol, tannins, glycosides, saponins, flavonoids, alkaloids, carbohydrates, phytosterols, protein and amino acid were positive with methanol and distilled water extract and steroid, alkaloids,

carbohydrates, and phytosterols were positive with petroleum ether extract. Collectively methanol and distilled water extract showed more positive results against the preliminary phytochemical tests compared to ether extract.

There are always some differences, advantages and disadvantages when extracting the phytoconstituents from different parts of the plants like root, stem, bark, leaves and flowers. The difference lies in the presence of different active ingredients in different parts of the plant. One part of the plant could have medicinal value whereas other part may have some toxic principle. Also, depending on the type of plant, the phytoconstituents may vary in root, seed, flower, leaves. Roots can be a source of variety of phytochemicals of medicinal importance.

Phenolic compounds were found only in methanol and distilled water extracts. The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites. Phenolic compounds from plants have potent antioxidant properties that can be utilized in the prevention of various oxidative stress associated diseases such as cancer [13]. The redox properties of phenolic compounds allow them to act as antioxidants, their hydroxyl groups facilitate free radical scavenging activity, and thereby the total phenolic concentration could be used as a base for rapid screening of antioxidant activity [14]. Flavonoids were found in methanol and distilled water extract. Flavonoids are potent water-soluble antioxidant and free radical scavenger, which prevent oxidative cell damage and also have strong anticancer activity [15]. It also helps in managing diabetes-induced oxidative stress [16]. Tannins were found only in methanol and distilled water. Tannins have remarkable astringent properties [17].

Steroid was detected only in petroleum ether extracts. Steroids are extremely important compounds mainly due to their association with compounds such as sex hormones. Saponins are known to cause damages in the membrane of some monogenean parasites causing vacuolization and disintegration of tegument [18]. Saponins also play a role in the suppression of inflammation [19]. Alkaloids were detected in all three extracts, whereas anthraquinones were absent in all three extracts. Anthraquinone was also found to be absent in all the extracts studied [20]. However, alcoholic extracts (methanolic and ethanolic) of *Elytraria acaulis* roots showed the presence of the various phytoconstituents along with quinones [21].

Alkaloids are a very diverse class of plant secondary metabolite with numerous biological activities such as antiseptic, antimicrobial, anticholinergic, diuretic, sympathomimetic, antihypertensive, and analgesic [22]. It also has the ability to intercalate with the protein synthesis of the parasites [23]. All three extracts of EAR revealed the presence of alkaloids. The methanolic extract of dried whole plant of *Elytraria acaulis* revealed two pyrazole alkaloids withasomnine and 4'-hydroxywithasomnine [24]. Withasomnine, a pyrazole alkaloid with sleep-inducing properties has been first isolated in 1966 [25], is used as somniferous drugs [26]. Phytoconstituents namely, phenols, flavonoids and tannins and terpenoids possess antioxidant activity [27].

UV-VIS spectroscopy is a simple, cost-effective and rapid test for detecting phytocomponents. UV-VIS spectroscopy is a reliable and sensitive method for the detection of biomolecular composition [28].

Table 1. Extractive values of *Elytraria acaulis* roots

	Solvent used in extraction		
	Petroleum Ether	Methanol	Aqueous
Temperature maintained	Room Temperature		
Method of extraction	Maceration (Cold Extraction)		
% Yield	0.662%	10.53%	27.34%
Temperature maintained	40-50°C		
Method of extraction	Soxhlet extraction (Hot Extraction)		
% Yield	0.679%	18.10%	32.03%

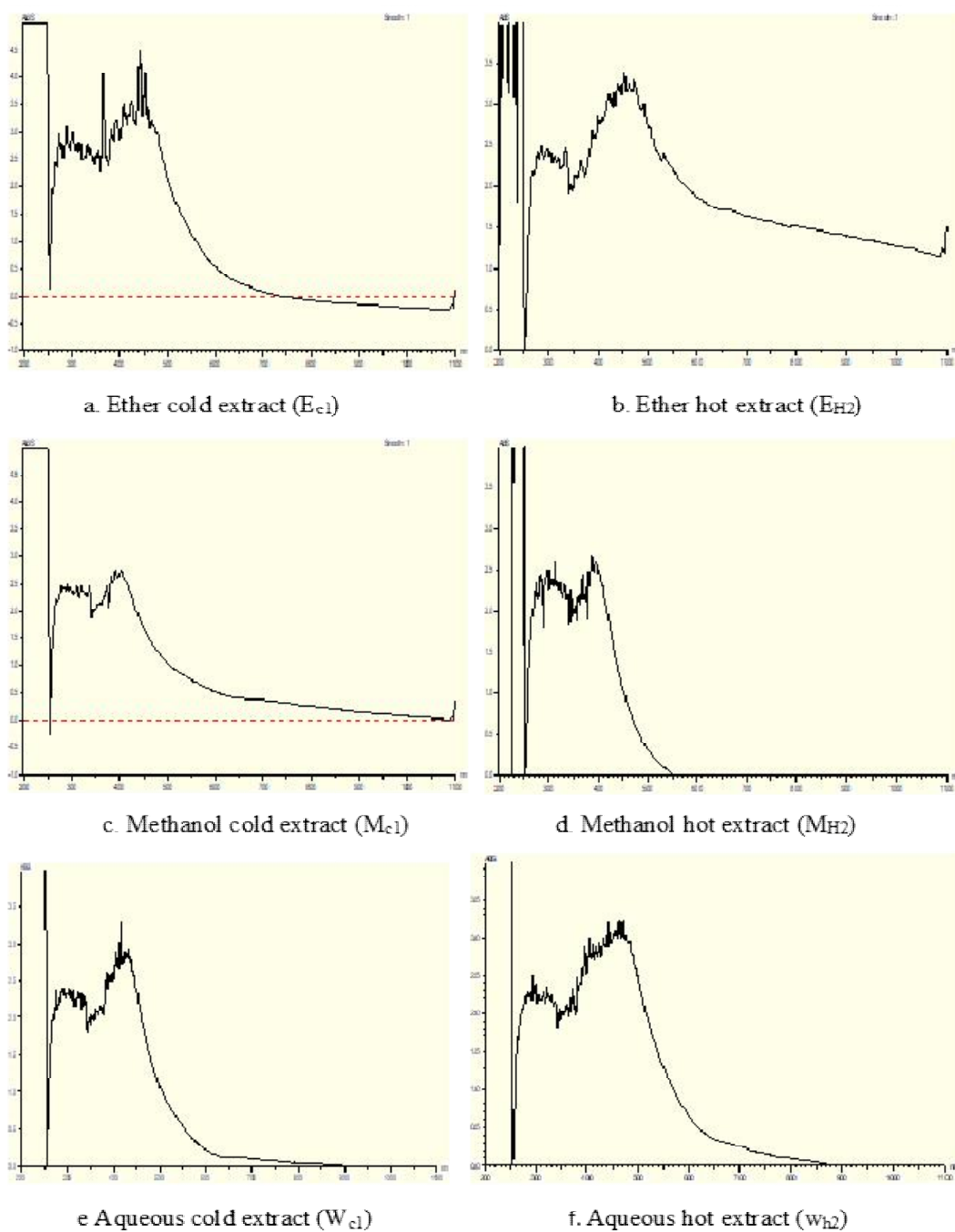


Fig. 1 a-f. UV-VIS scan of the *Elytraria acaulis* root extracts (200-1100 nm)

Table 2. Phytochemical screening of *Elytraria acaulis* roots extract

Phytoconstituents	test name	Ether extract		Methanolic extract		Aqueous extract	
		Cold Extract (E _{c1})	Hot Extract (E _{H2})	Cold Extract (W _{c1})	Hot Extract (W _{H2})	Cold Extract (W _{c1})	Hot Extract (W _{H2})
Tannins	FeCl ₃ test	-	-	+	+	+	+
Anthraquinones	Borntrager's test	-	-	-	-	-	-
Glycosides	Keller-Killiani test	-	-	+	+	+	+
Saponins	Frothing test	-	-	+	+	+	+
Flavonoids	Alkali test	-	-	+	+	+	+
Steroids	Sulfuric acid test	+	+	-	-	-	-
Phenols	FeCl ₃ test	-	-	+	+	+	+
Alkaloids	Mayer's test	+	+	+	+	+	+
	Hager's test	+	+	+	+	+	+
Carbohydrates	Molisch's test	+	+	+	+	+	+
Protein and amino acid	Ninhydrin's test	-	-	+	+	+	+
	Biuret's test						
Phytosterols	Salkowski test	+	+	+	+	+	+

+: presence of specific phytoconstituents; -: absence of specific phytoconstituents

The UV-VIS spectral characteristics could prove to be a useful approach for distinguishing among species of the genus *Elytraria*. UV-VIS spectroscopy along with FTIR is generally used to identify functional groups [29]. However, using advanced techniques namely LCMS, QTOF, and GCMS will help in ascertaining the actual phytoconstituents present in EAR in further studies. Chemoprofiling of *Elytraria acaulis* showed the presence of alkaloids, tannins, flavonoids, steroids, volatile oils, and carbohydrates in both aerial and underground parts of a plant, while proteins, glycosides and coumarins are present in underground parts while these are absent in aerial plant.

5. CONCLUSION

Analysis of the *Elytraria acaulis* plant roots of all three extracts revealed the presence of phytochemicals such as phenol, tannins, glycosides, saponins, flavonoids, alkaloids, carbohydrates, phytosterols, protein and amino acid. The spectral data would be helpful in taxonomic identification as well as quality control of EAR. This study suggests that the identified phytochemical compounds may be the bioactive ingredients and this plant could prove to be a precious reservoir of bioactive ingredients of significant medicinal value. However, the amounts of active ingredients are always low and its extraction and isolation is always time-consuming and expensive.

CONSENT

It's not applicable.

ETHICAL APPROVAL

It's not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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