Qualitative and Quantitative Phytochemical Screening of root extracts from *Arundinaria suberecta* Munro

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Abstract: *Medicinal plants are a rich source of bioactive phytochemicals with various therapeutic potential. Arundinaria suberecta Munro, is a traditional medicinal plant available in Sikkim which is used to treat kidney stones. In this current study, qualitative and quantitative phytochemical screening of Arundinaria suberecta Munro root extract was investigated using varying solvents. Qualitative analysis showed the existence of alkaloids, flavonoids, phenols, saponins, steroids, proteins, carbohydrates, and glycosides, where ethyl acetate extract revealing the significant level of bioactive components. The total phenols (6 mg GAE/g), flavonoids (7.12 mg/g), saponins (51 mg/g), and terpenoids (11 mg/g), were estimated quantitively indicating their biological activity such as antioxidant, antimicrobial, and anti-inflammatory. The current findings highlight that Arundinaria suberecta Munro is a viable source for therapeutic active chemicals. Future research should focus on extracting and characterizing these bioactive components to further investigate their potential therapeutic applications in drug development.*

Keywords: *Arundinaria suberecta Munro, phytochemical analysis, flavonoids, Total phenol.*

1. Introduction

Since time immemorial, medicinal plants have been used to cure various diseases and these trends have been passed to human communities through generation. The potential of medicinal plants for curing various alignments depends on the phytochemical constituents. Due to the lesser side effects and easy accessibility, approximately 80% of the world population currently depends on the medicinal plants. About 70% of pharmaceutical medications on the market today are derived from plant-based compounds, highlighting the significant contribution of

traditional medicinal in the advancement of the human healthcare system and sustainable alternatives to synthetic drugs[1]. Despite their vast diversity, only a small portion of the estimated 250,000–500,000 plant species have been explored for their chemical, biological and pharmacological evaluation[2]. The medicinal potential of plants depends on their broad spectrum of phytochemicals, such as alkaloids, flavonoids, tannins, terpenoids, saponins, essential oils, and phenolic compounds. These secondary metabolites provide a numerous pharmacological property, including antimicrobial, antioxidant, anti-inflammatory and anticancer activities[3]. For example, alkaloids exhibit antimicrobial and analgesic activities, flavonoids as antioxidant and anti-inflammatory effects, and saponins contribute immunological modulation and cholesterol regulations[4]. Therefore, it is important to investigate herbal plants to confirm their traditional application and further identification and isolation of bioactive components that could help in the development of novel therapeutic drugs.

Arundinaria suberecta Munro, (*Drepanostachyum khasianum* Munro), is a traditional medicinal plant belonging to the Poaceae family. This species thrives in subtropical environments which is predominantly found in Assam, the Eastern Himalayas, Myanmar, Nepal, and Sikkim. Traditionally, local healers in Sikkim used it for treating kidney stones, highlighting its therapeutic potential. It is a perennial, tufted bamboo characterized by short, thick rhizomes, with stems growing between 250–400 cm in height and approximately 12 mm in diameter[5], [6], [7], [8], [9]. To address this demand, the current study examined the qualitative and quantitative screening of the valued medicinal plant, *Arundinaria suberecta* Munro root for the first time.

Table 1: Scientific classifications[10]

Kingdom	Plantae
Phylum	Streptophyta
Class	Equisetopsida
Subclass	Magnoliidae
Order	Poales
Family	Poaceae
Genus	*Arundinaria*
Species	*Arundinaria suberecta* Munro
Synonyms	*Drepanostachyum khasianum (*Munro*)*, *Arundinaria khasiana* Munro, *Chimonobambusa khasiana (*Munro*)*, *Drepanostachyum falcatum subsp. khasianum* (Munro), *Drepanostachyum suberectum* (Munro), *Sinarundinaria suberecta* (Munro)

2. Materials and methods

2.1 Materials

The root of *Arundinaria suberecta* Munro was locally collected from Uttarey West Sikkim during the months of September and October 2022, under the authorities guidance from Forest

and Environment Department, Government of Sikkim. The voucher specimens were submitted and authenticated by Regional Ayurveda Research Institute Tadong, Gangtok, Sikkim, with accession no. RARI 5498.

2.2 Preparation of root extracts

The roots were dried under shade at room temperature and grounded into fine powder using mechanical grinder. 100 gm of root powder was subjected to successive Soxhlet extraction with petroleum ether, chloroform, ethyl acetate and methanol. The whatmann filter paper was used to separate the sample residue from the solution. Then the resulting extract was concentrated to dryness using rota vapor at 40°C. The extracts were sealed and were kept in 4°C for further use.

2.3 Qualitative analysis of phytochemicals

Screening and identification of bioactive phytochemicals in the *A. suberecta* Munro under study was carried out in the extract using the standard protocol as described by Trease and Evans, Harbone and Sofowara[11], [12]

2.3.1 Test for phenols*:* 2 mL of distilled water was added to 1 mL plant extract followed by 10% $FeCl_3$, formation of blue-green color confirms the existence of phenols.

2.3.2 Test for Carbohydrates: In a test tube containing 0.5 mL of extract solution, few drops of Molisch's reagent along with few drops of conc. HCl were added. A ring at the interface with a brown color confirms the existence of carbohydrates.

2.3.3 Test for Flavonoids: 1 mL of plant extract, 2N NaOH was mixed, formation of yellow color indicated the existence of flavonoids.

2.3.4 Test for Saponins: 5 mL of distilled water was mixed with 1.5 mL of plant extract, the mixture was shaken vigorously, formation of form indicates the existence of saponins.

2.3.5 Test for Alkaloids: 2 ml filtrate plant extract was mixed with 0.1% HCl and Mayer's reagent. A pale yellow or creamy precipitate shows the existence of alkaloids.

2.3.6 Test for Tannins: A few drops of $FeCl_3$ solution were mixed with 1 mL of extract. A blue-black or brownish-green color formation indicates the existence of tannins.

2.3.7 Test for Steroids: 1 mL of plant extract was mixed with few drops of concentrated H_2SO_4, formation of a red color in the lower layer indicates the existence of steroids.

2.3.8 Test for Terpenoids: 2 mL extract was added to $CHCl_3$ followed by 1 mL concentrated H_2SO_4, at the interface, a reddish-brown color suggested the existence of terpenoid.

2.3.9 Test for Amino acids: A few drops of the Ninhydrin reagent were mixed in a test tube containing 1mL extract. A purple color formation indicates the existence of amino acids.

2.3.10 Test for Anthraquinones: Formation of red precipitate by adding 2% HCl dropwise to the plant extract indicated the existence of anthraquinones.

2.3.11 Test for Proteins: Formation of yellow color when a few drops of concentrated HNO_3, were mixed with 1 mL of the plant extract, indicates the presence of proteins.

2.2 Quantitative determination of phytochemicals

2.2.1 Determination of total phenol content: The total phenolic content was evaluated by Folin-Ciocalteu method with slight modifications[13]. In test tubes, Folin-Ciocalteu reagent (0.25 mL) and 7.5% (w/v) Na_2CO_3 solution (1.25 mL) were mixed. After adding 100 µL of methanolic extract, the mixture was allowed to stand for 30 min at room temperature and the absorbance at 765 nm was measured with a spectrophotometer. The results were established using the linear regression equation from the standard curve on a gallic acid (R^2=0.99) and were represented as milligrams of gallic acid equivalent per gram of extract (GAE/g).

2.2.2 Determination of total flavonoid content: One gram of plant sample was extracted using 100 mL of 80% methanolic solution at room temperature and it was filtered into a pre-weighed beaker, dried and weighed. The weight of the extracted dry material was used to calculate the percentage of flavonoids[14] using the equation

$$Flavonoid\ content\ \% = \frac{Weight\ of\ dried\ extract}{Weight\ of\ plant\ sample} * 100 \quad (1)$$

2.2.3 Determination of total saponin content: Two grams of the plant sample were mixed with 20% ethanol (20 mL) and heated at 55°C 4 hr with continuous stirring. The mixture was filtered, and leftover residue was re-extracted. The resulting extract was concentrated to 10 mL, transferred into a separating funnel and mixed with 20 mL of diethyl ether. The solution was mixed thoroughly, and the aqueous part (containing the saponins) was collected, discarding diethyl ether layer. This process was replicated three times, and the aqueous layers were collected. Further, 30 mL of n-butanol was mixed, and the n-butanol extract was washed twice with 5% NaCl solution. The resulting solution was dried in a pre-weighed beaker and the total saponin content was evaluated on the weight difference of the beaker before and after drying[3].

$$Total\ saponin\ content\ \% = \frac{Weight\ of\ dried\ saponin\ extract}{Weight\ of\ plant\ sample} * 100 \quad (2)$$

2.2.4 Determination of total Terpenoids*:* One grams of plant sample were extracted in methanol for 24 hr. After filtering, liquid was extracted using petroleum ether and was then considered to contain the total terpenoids[15].

3. Results and Discussion

3.1 Yield of Extract

The root extracts (Petroleum ether, chloroform, ethyl acetate and methanol) of *A. suberecta* Munro were prepared using the solvent extraction method. The crude extract yield was determined by measuring the dry weight of the extract. Among the solvents used, ethyl acetate showed the maximum yield of 8.1%, followed by methanol (7.3%) and chloroform (6.7%). The petroleum ether showed the lowest yield percentage (4.85), possibly due to its low polarity

and limited ability to extract the bioactive compound. The extraction yields are summarized in Table 1, while Figure 1 illustrates the yield percentage of each extract in relation to the solvent used.

3.2 Qualitative screening of Phytochemicals

Essential phytochemicals are a range of primary and secondary plant metabolites that are found in medicinal plants and herbs. Phytochemicals, including alkaloids, flavonoids, and phenolic compounds, exhibit diverse bioactivities that contribute to disease prevention and therapeutic applications. Flavonoids act as potent antioxidants and anti-inflammatory agents, supporting cardiovascular health, neuroprotection, and immune modulation[16]. Alkaloids demonstrate antioxidant, anti-inflammatory, and cardioprotective properties, with notable roles in analgesia, antiarrhythmic therapy, and anticancer treatment[17]. Phenolic compounds, widely recognized for their antioxidant capacity, also contribute to cardiovascular protection and bone health by mitigating oxidative stress and enhancing cellular function[18]. The synergistic effects of these bioactive compounds highlight their potential in developing novel therapeutics for managing chronic diseases and improving overall health. As shown in Table 1, the phytochemical profile from the preliminary analysis showed the different extracts are enhanced with a variety of vital phytochemicals, including alkaloids, phenolics, steroids, proteins, and oil. The phytochemical screening of *A. suberecta* extracts in different solvents confirmed the existence of various bioactive compounds. The chloroform, ethyl acetate and methanol extracts showed the presence of alkaloids and flavonoids but were absent in petroleum ether. Tannins and anthraquinones were absent in all tested extracts. Saponin and steroids were detected in all the extracts where terpenoids were only observed in petroleum ether. The phenols, proteins and carbohydrates were present in ethyl acetate and chloroform extract. Petroleum ether, chloroform, and ethyl acetate extracts showed the presence of glycosides. Based on these results, ethyl acetate extract is a suitable solvent for the extraction of bioactive compounds as it has a broad range of phytochemicals. These results are consistent with earlier research highlighting the medicinal potential of phytochemicals found in medicinal plants.

Table 1: Yield of Crude Extracts from *A. suberecta* Munro Root.

Solvent	Initial weight of the sample (gm)	Final weight of the sample (gm)	Weight of crude extract (gm)	Crude extract %
Petroleum ether	100	95.2	4.8	4.8
Chloroform	100	93.3	6.7	6.7
Ethyl acetate	100	91.9	8.1	8.1
Methanol	100	92.7	7.3	7.3

Table 2: Qualitative Estimation of Phytochemicals present in *A. suberecta* Munro.

Phytochemicals	Pet. ether extract	Chloroform extract	Ethyl acetate extract	Methanol extract
Alkaloid	—	+	++	+

Flavonoid	—	+	+	+
Tannins	—	—	—	—
Saponins	+	+	++	+
Terpenoids	+	—	—	—
Phenols	—	+	++	++
Anthraquinone	—	—	—	—
Steroids	+	+	++	+
Proteins	—	+	++	—
Carbohydrates	+	+	++	—
Glycosides	+	+	++	—

++: Strong, +: Present, —: Absent.

Table 3: Quantitative Estimation of Phytochemicals in *A. suberecta* Munro.

Total Phenolic Content (mg GAEg^{-1})	Flavonoids Content (mgg^{-1})	Saponins Content (mgg^{-1})	Terpenoids Content (mgg^{-1})
6	7.12	51	11

3.3 Quantitative Estimation

Phenolic, flavonoid, alkaloids, saponins, terpenoids and steroids are naturally occurring substances present in different parts of plants, with strong antibacterial, antidiabetic, antiaging, antioxidant and anticancer activities. Table 2 represents the quantitative analysis of phytochemicals present in the root extract. The total phenolic and flavonoid content was found to be 6 mg GAE/g and 7.12 mg/g respectively in the extract widely recognized for its

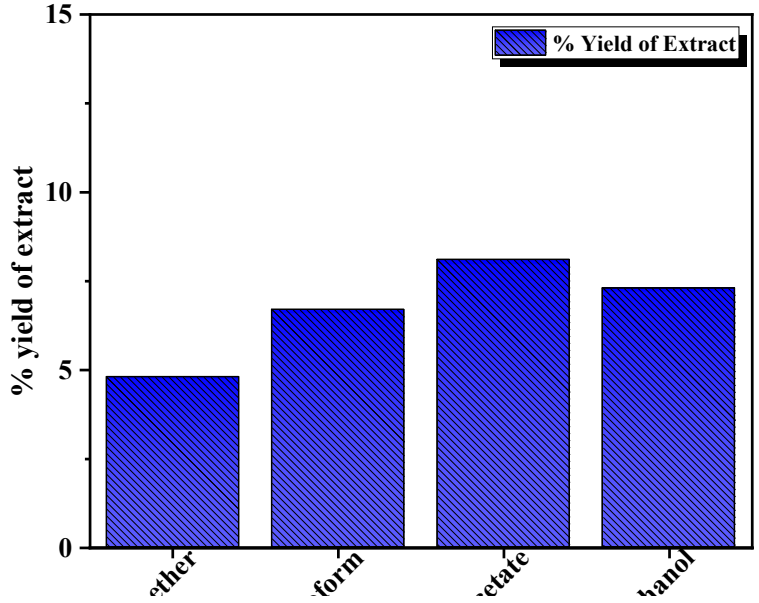

Figure 1: Percentage yield of *A. suberecta* Munro root extracts obtained using different solvents

antioxidant and antimicrobial properties[18], [19]. Saponins were determined as the predominant phytochemicals in the root extract, with a concentration of 51 mg/g, highlighting their potential medicinal advantages, including antimicrobial and anti-inflammatory effects[20]. The terpenoid concentration was measured at 11 mg/g, indicating their significance in several biological processes[2]. The varying solubility of phytochemicals in different solvents emphasizes the necessity of adopting suitable methods for extraction to maximize the resulting number of bioactive compounds. The high concentration of bioactive components in ethyl acetate and methanol extracts demonstrates their potential for isolating strong therapeutic molecules. These findings show that *A. suberecta* Munro could be a valuable source of natural antioxidants and antibacterial compounds, assisting in the discovery of plant-based medications. These findings reveal that *A. suberecta* Munro contains a high concentration of bioactive compounds, specifically saponins, which may contribute to its medicinal benefits. Future studies should focus on isolating specific compounds, investigating their biological mechanisms of action, and evaluating their therapeutic efficacy by meticulous pharmacological investigations.

4. Conclusion

This study investigates the phytochemical profile and bioactive properties of *Arundinaria suberecta* Munro root extract, highlighting its medicinal value. The qualitative screening revealed the presence of various phytochemicals, including alkaloids, flavonoids, saponins, steroids, phenols, proteins, carbohydrates, and glycosides. providing a promising medium for phytochemical extraction. Ethyl acetate extract has been found to have the most diverse range of bioactive compounds, thus providing a promising medium for phytochemical extraction. The quantitative study revealed high quantities of phenols (6 mg GAE/g), flavonoids (7.12 mg/g), saponins (51 mg/g), and terpenoids (11 mg/g), supporting their antioxidant, antibacterial, and anti-inflammatory properties. The high saponin content suggests possible therapeutic applications like immune regulation and antibacterial properties. Overall, the findings suggests that *A. suberecta* has an abundant source of bioactive chemicals with potential therapeutic applications. Future research should focus on isolating and characterizing these phytochemicals in order to further comprehend their therapeutic potential in drugs development.

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Declaration of interest

The authors declare that they have no competing interest

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