



Research Article

Qualitative Phytochemical Screening of the Methanolic Extracts of *Actinostemma tenerum*, *Adenostemma lavenia* and *Hydrocharis dubia*

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Abstract

Introduction: Wetland plants are often rich in bioactive compounds, yet many species remain scientifically unexplored. The present study focuses on the qualitative phytochemical screening of *Actinostemma tenerum*, *Adenostemma lavenia* and *Hydrocharis dubia* collected from Loktak Lake, a significant Ramsar site.

Methods: Standard phytochemical tests were carried out to identify the major secondary metabolites present in these species.

Results and Discussion: The analysis showed the presence of important phytochemicals such as flavonoids, tannins, terpenoids, phenols, carbohydrates, glycosides, quinones, coumarins, and saponins, though their distribution varied among the plants. Among the three, *Adenostemma lavenia* exhibited relatively higher phytochemical diversity. The occurrence of these compounds indicates possible antioxidant, antimicrobial, and anti-inflammatory properties. Terpenoids, Coumarins and Quinone are highly present in leaves extract of *Actinostemma tenerum*. Flavonoids, Terpenoids, Coumarins and Quinone are highly present in the leaves extract of *Adenostemma lavenia*. Coumarins and quinones are highly present in *Hydrocharis dubia*.

Conclusion: Overall, the study provides preliminary insight into the chemical composition of these wetland plants and highlights their potential medicinal value. Further detailed investigations are required to confirm their biological activities and therapeutic applications.

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1. INTRODUCTION

Wetland plants such as *Actinostemma tenerum*, *Adenostemma lavenia* and *Hydrocharis dubia* have been utilised for centuries in traditional medicine in our state. *Actinostemma tenerum* Griff., an annual vine of the Cucurbitaceae family, is widely distributed in Asian countries, e.g., Korea, China, Japan, and India [1, 2]. This wild vine has been traditionally utilised as a folk remedy for arthritis and diuretic diseases [3]. Meanwhile, it also exhibits multiple medical benefits, such as anti-tumour activity [4]. In addition, as it is rich in saponins [5], polysaccharides, and phospholipids [6], the whole plant is a potential supplement for the food and pharmaceutical industries. Therefore, it is an important economic plant. *Hydrocharis dubia* L., commonly named as frog-bit are perennial, predominantly floating-leaved, freshwater aquatics which are included in the monocotyledonous plant family Hydrocharitaceae. *Hydrocharis dubia* is used for its leaves. *Adenostemma lavenia* belongs to the Asteraceae family. It is an annual or perennial herb that grows wild, commonly known as sticky daisies and widely distributed in tropical regions of Asia, Africa, and America [7]. These plants are rich sources of phytochemicals and secondary metabolites responsible for their therapeutic effects. Phytochemicals such as alkaloids, flavonoids, phenolics, and terpenoids contribute to the pharmacological properties of these plants. Different phytoconstituents vary depending on various elements, including environmental factors and geographic factors. The available literature review so far indicates very limited data on the phytochemical profile of *Actinostemma tenerum*, *Adenostemma lavenia* and *Hydrocharis dubia* found in the North-Eastern states of India, including Manipur. This study aims to characterise and compare the bioactive constituents of the methanolic extract of leaves and seeds of *Actinostemma tenerum*, leaves of *Adenostemma lavenia* and leaves of *Hydrocharis dubia*.

2. MATERIALS AND METHODS

Plant Material Collection and Preparation

Fresh samples of *Actinostemma tenerum* (leaves and seeds), *Adenostemma lavenia* (leaves) and *Hydrocharis dubia* (leaves) were collected from the Thanga area, Loktak Lake (24°53'06" N latitude and 93°82'29" E longitude), Manipur. Plant samples were identified using authentic literature, floras and online

sources like POWO (<https://powo.science.kew.org/>) and the voucher specimens were deposited at Dhanamanjuri Herbarium (DMH), Department of Botany, Dhanamanjuri University, Manipur. Plant parts were washed, air-dried at 40°C, and pulverised into fine powders using a mechanical grinder.

Processing of Plant Materials

The collected plant parts were thoroughly washed and shade-dried until all surface moisture was removed, after which they were cleaned. The dried plant materials were then ground into fine powders using a mechanical grinder. The powdered samples were carefully stored in airtight containers with proper labelling for further analysis.

Preparation of Extract

The plant powder samples (50 g each for leaf samples and 30g for seeds) were extracted using 70% methanol via cold maceration for 72 hours with intermittent shaking. Extracts were filtered through Whatman No. 1 filter paper and concentrated using a rotary evaporator at 40°C. The extracts were then kept in the Lyophilizer at -80°C for 7 days. The crude extracts were stored at 4°C for further analysis.

Percentage Yield of the extracts

The extraction yield (%) was calculated by dividing the weight of the crude extract obtained after extraction by the initial weight of the plant powder and multiplying the value by 100. The resultant extract was weighed, and the extractive value was determined as follows:

$$\text{Percentage of yield (\%)} = \frac{\text{Weight of extract}}{\text{Weight of dry plant powder}} \times 100$$

Qualitative Phytochemical Screening

The qualitative screening of phytochemicals present in the plant crude extracts was carried out using standard methods Table.1. The presence of alkaloids, flavonoids, quinones, carbohydrates, phenols, tannins, saponins, steroids, cardiac glycosides, proteins and amino acids, coumarins and terpenoids in the plant extracts was determined [8-11].

Table 1: Methods for qualitative phytochemical screening of crude plant extracts

Sl. No.	Phytoconstituents	Test	Observation
1.	Alkaloids (Mayor's Test)	2ml extract + a few drops of Mayor's reagent	White/creamy precipitate
2.	Tannins (Ferric chloride Test)	2ml extract +2-3 drops FeCl ₃ (5%)	Blue -gallic tannins; Green, black -catecholic tannins
3.	Flavonoids (Alkaline reagent test)	1ml extract + Sodium hydroxide solution	Yellow/red colouration
4.	Terpenoids (Salkowski test)	2ml extract + 2ml (CHCl ₃) + 2ml conc.H ₂ SO ₄	Reddish brown colouration
5.	Saponins (Foam Test)	5ml extract + 20ml distilled H ₂ O + heat	Froth appears
6.	Steroids (H ₂ SO ₄ Test)	1ml extract + 12-15 drops of H ₂ SO ₄	Slightly red colouration
7.	Carbohydrates (Fehling Test)	Dil. HCl was hydrolysed with 2ml of extract and neutralised with alkali and heated with Fehling's Solution A & B.	Red ppt
8.	Cardiac Glycosides (Keller Killani Test)	2ml Glacial acetic acid containing a drop of FeCl ₃ , treated with the extract	Brown colour ring
9.	Proteins and amino acids (Ninhydrin Test)	To the 2 ml test solution, the ninhydrin solution was added and then boiled. Formation of blue colour indicates the presence of an amino acid. Again, 2 ml test solution, 0.2%	Violet colour

		ninhydrin solution was treated with amino acids and protein, then boiled.	
10.	Phenol (Ferric chloride test)	2ml plant extracts + 5 ml distilled water + few drops of 5% ferric chloride	Bluish black
11.	Coumarins	2ml extract + 3ml NaOH (10%)	Yellow coloration
12.	Quinone	1ml extract + 1ml conc. H ₂ SO ₄	Reddish colouration

3. RESULTS AND DISCUSSION

Yields percentage (%)

The percentage yield of extracts obtained using aqueous hydro-alcoholic extraction showed noticeable variation among the samples. The yield percentage ranged from 18% to 22% (Table 2). The highest yield percentage was obtained from the leaves of *Hydrocharis dubia* (22%), followed by seeds of *Actinostemma tenerum* (20%), leaves of *Adenostemma lavenia* (19%) and the lowest yield percentage was exhibited by leaves of *Actinostemma tenerum* (18%).

Table 2: Yield percentages of plant extracts

Sl. No.	Plant species	Parts used	Extraction type	Yield percentage (W/W)
1	<i>Actinostemma tenerum</i>	Leaves	Aqueous Hydro-alcoholic	18%
2	<i>Actinostemma tenerum</i>	Seed	Aqueous Hydro-alcoholic	20%
3	<i>Adenostemma lavenia</i>	Leaves	Aqueous Hydro-alcoholic	19%
4	<i>Hydrocharis dubia</i>	Leaves	Aqueous Hydro-alcoholic	22%

Qualitative phytochemical screening

This study reveals that *Actinostemma tenerum* (leaves and seeds), *Adenostemma lavenia* (leaves) and *Hydrocharis dubia* (leaves) are rich in diverse phytochemicals and represent promising sources of natural antioxidants. A wide range of bioactive compounds, including flavonoids, tannins, terpenoids, phenols, carbohydrates, glycosides, quinones, coumarins, and saponins, were identified in these plants, highlighting their potential value in developing plant-based therapeutic agents (Table 3). Terpenoids, Coumarins and Quinone are highly present in leaves extract of *Actinostemma tenerum*. Flavonoids, Terpenoids, Coumarins and Quinone are highly present in the leaves extract of *Adenostemma lavenia*. Coumarins and quinones are highly present in *Hydrocharis dubia*.

Numerous phytochemicals present in medicinal plants are known to play significant roles in disease prevention and health promotion. Tannins are polyphenolic compounds known for their astringent nature and are effective in wound healing, reducing inflammation, and treating diarrhoea and mucosal disorders due to their ability to precipitate proteins and form protective layers on tissues [12, 13]. Flavonoids are potent water-soluble antioxidants and free-radical scavengers that help reduce oxidative stress, protect against cardiovascular diseases, and exhibit anti-inflammatory, antimicrobial, and antidiabetic activities [14, 15]. Terpenoids have been widely reported for their analgesic, anti-inflammatory, anticancer, antimicrobial, and immunomodulatory properties, and they play an important role in protecting the body against chronic diseases, including cancer and metabolic disorders [16, 17].

Saponins possess surface-active properties and are known for their antimicrobial, anti-inflammatory, cholesterol-lowering, and immune-stimulating effects; they also exhibit haemolytic activity and contribute to cardiovascular protection [18, 19]. Carbohydrates, apart from serving as primary energy sources, play crucial roles in cellular communication, immune response modulation, and structural integrity of cells and tissues [20]. Cardiac glycosides are well recognised for their therapeutic application in the treatment of heart failure, as they enhance cardiac contractility and regulate heart rhythm [21].

Phenolic compounds exhibit strong antioxidant properties by neutralising free radicals and reducing oxidative damage, thereby contributing to anti-inflammatory, anticancer, and cardioprotective activities [22, 23]. Coumarins are reported to possess anticoagulant, antimicrobial, anti-inflammatory, and anticancer properties and are also useful in improving blood circulation and vascular health [24]. Quinones are known for their antimicrobial, antiparasitic, and anticancer activities, mainly due to their ability to generate reactive oxygen species and interfere with microbial and cancer cell metabolism [25]. The presence of these phytochemicals collectively suggests that plant extracts rich in such secondary metabolites may possess significant physiological and therapeutic potential.

Table 3: Phytochemical constituents present in crude extracts of *Actinostemma tenerum* (leaves and seeds), *Adenostemma lavenia* (leaves) and *Hydrocharis dubia* (leaves)

Sl. No.	Name of Phytochemical compounds	<i>Actinostemma tenerum</i>		<i>Adenostemma lavenia</i>	<i>Hydrocharis dubia</i>
		Leaves	Seeds	Leaves	Leaves
1.	Alkaloids (Mayer's Test)	-	-	-	-
2.	Tannins (Ferric chloride Test)	+	+	+	+
3.	Flavonoids (Alkaline reagent test)	+	+	++	+
4.	Terpenoids (Salkowski test)	++	-	++	+
5.	Saponins (Foam Test)	+	-	+	-
6.	Steroids (H ₂ SO ₄ Test)	-	-	-	-
7.	Carbohydrates (Fehling Test)	+	-	+	+
8.	Cardiac Glycosides (Keller Killani Test)	+	+	+	+
9.	Proteins and amino acids (Ninhydrin Test)	-	-	-	-
10.	Phenol (Ferric chloride test)	+	+	+	+
11.	Coumarins	++	+	++	++
12.	Quinone	++	+	++	++

+=Present, - = Absent, ++ =highly present

4. CONCLUSION

The study confirms that *Actinostemma tenerum*, *Adenostemma lavenia* and *Hydrocharis dubia* contain significant classes of secondary metabolites, including alkaloids, flavonoids, tannins, saponins, phenolics, glycosides, and terpenoids. Among the three, *Adenostemma lavenia* exhibited comparatively richer phytochemical diversity, indicating its higher therapeutic potential.

The presence of these bioactive constituents scientifically supports the medicinal value of wetland plant species and highlights their importance as potential sources of novel natural drugs. The findings serve as a baseline for further phytochemical, pharmacological, and toxicological studies aimed at isolating and characterising bioactive compounds.

Future research involving quantitative analysis and biological activity assays is recommended to establish their efficacy and safety for therapeutic applications.

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