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Preliminary phytochemical analysis of different parts of *Achyranthes aspera* L. from Kathgodam, Nainital district

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Abstract
Phytochemicals represent significant resources for addressing diverse health concerns. This study aimed to conduct a preliminary phytochemical screening of sequential extracts (chloroform, methanol, distilled water) obtained from the roots, stem, and leaves of *Achyranthes aspera* Linn. (Family: Amaranthaceae) using standard phytochemical screening methods. Our findings indicate that the extracts from various parts of *A. aspera* contain carbohydrates, proteins, amino acids, alkaloids, flavonoids, phenolics, tannins, steroids, terpenoids, glycosides, and saponins. These plants show potential for use in herbal medicine because of the abundance of these compounds.

Keywords: *Achyranthes aspera*, phytochemical screening

Introduction
Medicinal plants represent reservoirs of potentially bioactive compounds suitable for pharmaceutical development. Secondary metabolites such as alkaloids, flavonoids, phenols, quinones, tannins, and terpenoids derived from these plants are globally utilized for the treatment of various ailments. The quality and therapeutic efficacy of medicinal and aromatic plants are intricately linked to their secondary metabolite content, which in turn is influenced by environmental factors [1]. *Achyranthes aspera* L. (family Amaranthaceae), widely distributed in India and other tropical Asian countries, is known to contain numerous phytochemicals including alkaloids, flavonoids, tannins, terpenoids, saponins, glycosides, and steroids [2]. Traditional uses of this plant include applying powdered leaves to stop bleeding, treating sprains and headaches, and using root decoctions for constipation, venereal diseases, and malaria. Burned leaves are administered topically to boils, while a leaf paste is applied to wounds as a hemostatic agent. Ingestion of leaf juice is practiced for the treatment of gonorrhea, while a decoction of the roots is employed to prevent miscarriages. The present study aims to assess the phytochemical screening of *Achyranthes aspera* from Kathgodam [3-6].

Materials and methods
Collection of plant samples
The whole plants of *Achyranthes aspera* were collected from Kathgodam, Nainital District Uttarakhand India, in May 2019. The parts of the plants that were selected for phytochemical analysis were properly washed with tap water and then rinsed in distilled water. The rinsed parts were then air-dried under shade. The dried parts of each plant were crushed to obtain powder and stored in an airtight container protected from sunlight until use.

Extraction
The plant extracts were obtained through the homogenization process, wherein 10 grams of tissue were dissolved in 100 mL of solvent and vigorously agitated for 5 to 10 minutes before being allowed to stand for 24 hours at room temperature. Following this period, the extract was filtered. Different extracts were produced using a series of solvents arranged in increasing polarity from non-polar (chloroform) to polar (methanol and distilled water). These extracts were subsequently preserved in air-tight containers at 4°C for future experimental investigations [7].

Qualitative assessment of phytochemicals
To identify chemical constituents, a preliminary phytochemical screening was conducted on various extracts using established standard procedures [7-14].

Test for Carbohydrates
Benedict's test
This test involves adding five mL of Benedict's reagent to two mL of the extract and incubating the mixture in a water bath, maintained at 100ºC, for 2 minutes. Change in the color from blue to green, yellow, orange, or even red indicated the presence of sugar.

Tests for proteins and amino acids
Xanthoproteic test
For the Xanthoproteic test, treat 1 mL of the plant extract with 2 – 3 drops of nitric acid (concentrated). The presence of protein is confirmed by the appearance of a yellow color in the test tube.

Ninhydrin test
To conduct the Ninhydrin test, 2 mL of plant extract was mixed with two drops of Ninhydrin solution. The presence of amino acids was confirmed by the appearance of a purple color.

Test for alkaloids
For an alkaloid test, the extracts were dissolved in dilute HCL, individually, and then the following tests were performed on them:

Wagner's test
In the laboratory experiment, 1 mL of plant extract was utilized, and Wagner's reagent was gently introduced along the inner wall of the test tube. The formation of a reddish-

brown precipitate was indicative of the presence of alkaloids.

Test for Phenol
Lead acetate test
To test the presence of phenol, the plant extract was combined with 0.5 mL of a lead acetate solution (1% w/ v). The occurrence of a white precipitate was indicative of the presence of phenol.

Test for Tannin
Ferric chloride test
For the examination of tannins, plant extract (2mL) was added to the 1 mL of a ferric chloride solution (5% w/v). The positive test for tannins was ensured by the emergence of a green-black color.

Test for Flavonoid
Lead acetate test
For the evaluation of flavonoids, a 2 mL portion of plant extract was combined with 3 to 4 drops of a solution containing 10% lead acetate. The emergence of a yellow precipitate signified the existence of flavonoids.

Test for Saponin
Foam test
The method employed was the foam test. In a test tube, 2 mL volume of the plant extract was combined with 4 mL of distilled water. After thorough shaking, the presence of foam indicated the presence of saponin

Test for steroids
Salkowski's test
One milliliter of the extract was taken in a test tube. Subsequently, chloroform and concentrated sulphuric acid, 2 mL each, were gently added down along the inner wall of the test tube. The upper layer exhibited a reddish hue, while the lower layer displayed a yellow color accompanied by a green fluorescence, thus confirming the presence of steroids.

Test for glycosides
Keller Killiani test
In the Keller Killiani test, 2 mL of the extract was added to an equal amount of glacial acetic acid. Following this, two drops of a 5% ferric chloride solution and 1 mL of concentrated sulphuric acid were carefully introduced. The appearance of a reddish-brown color at the junction and a bluish hue in the upper layers conclusively confirmed the presence of glycosides.

Test for terpenes
To ascertain the presence of terpenes, 2 mL each of the chloroform, concentrated sulphuric acid and the plant extract were mixed. The appearance of a reddish-brown ring served as a positive confirmation of the presence of terpenes.

Results and discussion
A preliminary phytochemical screening was conducted to gather information about the presence of therapeutically important phytochemicals in the plant extracts. The important phytochemicals namely carbohydrates, proteins, amino- acids, alkaloids, saponins, terpenes, tannins, phenols, flavonoids, glycosides, and steroids, were detected in this study (Table1). Solvent-wise analysis revealed that an equal number of the phytochemicals were detected in methanol and aqueous extracts followed by the chloroform extract. Carbohydrates, proteins, alkaloids, saponins, terpenes, tannins, phenols, flavonoids, glycosides, and steroids, were detected in all three solvents except tannins, and saponin which were absent in chloroform extract. Amino acids were only present in methanol extract. Sample-wise analysis revealed that an equal number of phytochemicals were present in all parts. Many researchers have previously reported similar phytochemical screening of primary and secondary metabolites. They reported that *A. aspera* contains carbohydrates, proteins, alkaloids, saponins, terpenoids, tannins, and flavonoids in methanol extract [15-16]. Phytochemical screening of *A. aspera* root showed the presence of alkaloids, carbohydrates, phytosterol, tannin, flavonoids, and diterpenes in the chloroform extract. Alkaloids, glycosides, saponin, phenol, tannin, flavonoids, protein, and diterpenes in ethanol extract [12]. Kumar & Mishra reported alkaloids, phenolics, saponins, phenols, and flavonoids in chloroform extract. Alkaloids, carbohydrates, saponins, amino acids, and flavonoids were reported in methanol extract. Alkaloids, saponins, amino acids, and flavonoids have been observed in water extract from the whole plant of *Achyranthes aspera* [17]. Singh *et al*. have reported alkaloids, flavonoids, and steroids reducing sugar, glycosides, and terpenoids from the methanolic extract of the root [18]. Abhaykumar reported chloroform extract of leaves contained carbohydrates, glycosides, and tannin. Methanol extract contains carbohydrates, steroids, phenolic compounds, glycosides, tannins, and alkaloids [19]. According to Priya *et al*. aqueous extract contained the key phytochemical groups' phenolic compounds, saponins, flavonoids, alkaloids, and tannins, whereas methanol extract demonstrated the presence of carbohydrates, phenolic compounds, oil, lipids, saponins, flavonoids, and tannins [20]. Dhale & Bhoi, reported ethanolic, methanolic, and water extracts of leaves, stem, and root of *A. aspera* contained protein, alkaloids, tannin and phenolic compounds, steroids, saponin glycosides, and reducing sugar [21]. Similarly, the whole plant of *A. aspera* is reported to contain carbohydrates, terpenoids, flavonoids, phytosterols, phenolic compounds, tannins, saponins, and alkaloids [22].
The present findings on these extracts are in agreement with previous workers. Each phytochemical exhibits some biological activity; For example, Carbohydrates provide numerous benefits in drug screening, including an abundance, high density of functional groups, cheap cost, and variety of molecular structures. Carbohydrates have also been utilized for the production of vaccines, such as antimicrobial and antioxidant vaccines based on carbohydrates [23]. Flavonoids defend plants from biotic and abiotic challenges by acting as UV filters, signal molecules, allopathic substances, phytoalexins, detoxifying agents, and antimicrobial defense compounds. Flavonoids are involved in cold hardiness, drought resistance, plant heat acclimatization, and chilling tolerance [24]. Most of the antioxidant properties in plants or plant products are due to phenolics, the most prevalent class of phytochemicals. Phenolics have a wide range of biochemical properties, including gene expression alteration, antioxidant, and antimutagenic effects [25]. Tannins have long been used to protect inflammatory mouth surfaces as well as to cure

catarrh, cuts, hemorrhoids, and diarrhea [26]. Alkaloids play an important role in plant defense against herbivores and diseases. Alkaloids are pharmaceutically significant; historic and current usage of alkaloids ranges from 25 to 75 percent in medications, emphasizing their high therapeutic potential [27]. Plant saponins assist humans in fighting fungal infections, bacteria, and viruses, improve the efficacy of some vaccinations, and eliminate specific types of tumour cells, notably lung, and blood malignancies. These chemicals acted as natural antibiotics, boosting the body in fighting illnesses and microbial invasion [28]. Terpenoids have been discovered to be beneficial in the prevention and treatment of a variety of disorders, including cancer. Terpenoids have antihyperglycemic, antibacterial, antifungal, anti-allergenic, antiviral, antispasmodic, antiparasitic, anti-inflammatory, and immunomodulatory effects [29]. Plant steroids are recognized to be crucial for their cardiotonic effects; they also have insecticidal and antimicrobial properties. Because of their extensive biological activity, they are frequently employed in medicine [26]. Glycosides are non-volatile and odorless, and they act as defense mechanisms against various microbes, insects, and herbivores [30]. Plants include phytochemicals that have been isolated and are primarily employed to treat certain types of health-related problems, as well as in the production of nutritional supplements and minerals. Each phytochemical has distinct biological activity, which may boost the likelihood of discovering new substances such as antibiotics against infections [31].

Table 1: Result of the qualitative phytochemical analysis of different parts of *Achyranthes aspera* L.

S. No.	Phytochemicals	Chl			MeOH			D. W		
		R	S	L	R	S	L	R	S	L
1.	Carbohydrates	+	+	+	+	+	+	+	+	+
2.	Proteins	+	+	+	+	+	+	+	+	+
3.	Amino-acids	-	-	-	+	+	+	-	-	-
4.	Alkaloid	+	+	+	+	+	+	+	+	+
5.	Phenols	+	+	+	+	+	+	+	+	+
6.	Tannins	-	-	-	-	+	+	+	+	+
7.	Flavonoids	+	+	+	+	+	+	+	+	+
8.	Saponin	-	-	-	+	-	-	+	+	+
9.	Steroids	+	+	+	+	-	-	-	+	+
10.	Terpenes	+	+	+	+	+	+	+	+	+
11.	Glycosides	+	+	+	+	+	+	-	+	-

Chl: Chloroform extract; MeOH: Methanol extract; D.W.: Distilled water extract; R: Root; S: Stem; L: Leaves; +: presence, -: absence

Conclusion

Medicinal plants comprise a substantial category of economically significant flora, serving as fundamental reservoirs of raw materials essential for pharmaceutical purposes. *Achyranthes aspera* stands out as a notable source of novel bioactive compounds, exhibiting diverse properties such as anti-inflammatory, anticancer, antiviral, antibacterial, cardioprotective, and anticoagulant activities. In contemporary contexts, plant-derived materials persist in playing a pivotal role in primary healthcare, serving as therapeutic interventions, particularly in developing regions. Moreover, they represent a promising alternative source of pharmacological agents.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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