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Phytochemical screening of *Andrographis paniculata* (Burm.F.) Nees Leaf and Stem Extract

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Abstract
The objective of a study is to evaluate phytochemical analyses of *Andrographis paniculata* plant extract to support the traditional claim given for as herbal medicine in Ayurveda. The plant parts are used as traditional or tribal medicine for the treatment of various illnesses such as, fever, diarrhea, hepatic, worm infestation and skin diseases. *Andrographis paniculata* belongs to the family Acanthaceae commonly known as 'King of Bitter' abundantly grows forests, plains, hill area and wetlands. *Andrographis paniculata* plant was collected from a local herbal garden, Dehradun and extract was prepared in different solvent. Phyto-chemical analysis of the plant shows the presence of alkaloids, phenols, amino acids, flavonoids, saponins, steroids, and tannins. Therefore, it assumed that its effectiveness as a medical plant is due to the presence of various phenolics, and antioxidants compounds in the plant.

Keywords: *Andrographis paniculata*, Phytochemicals, Phenolic, Terpenoids, Flavonoids, Saponins.

Introduction
Chemicals produced by plants through primary or secondary metabolism are known as phytochemicals (from the Greek word phyto, which means "plant"). They generally have biological activity in the plant host and contribute to its development or protection by activating defense mechanisms and giving the plants colour, odour, and flavor (Molyneux *et al*. 2007) [14]. The plant contains numerous bioactive components, including tannins, coumarins, diterpenoids, flavonoids, terpenoids and polyphenols, as well as quinones, anthraquinone, and other secondary metabolic products. They are responsible for generating antioxidants to scavenge toxic radicals, which is the root cause of various chronic diseases. They are a potential source of bio-molecules that can be used in the synthesis and production of phyto-medicine (plant-based drug) (Oladeji *et al*. 2019) [19] for the treatment of various diseases and disorders from centuries (Joshi *et al*., 2016, Khajuria *et al*., 2021) [10, 11].
Natural products and their derivatives have fewer adverse effects and greater efficacy than other synthetic counterparts. These plant-derived components like flavonoids, quinine, terpenoids, etc perform certain biological processes that enhance therapeutic activities such as anti-carcinogenic, anti-mutagenic, anti-inflammatory, and antioxidant properties (Batiha *et al*. 2020) [4]. Phytochemical screening is the scientific process of analyzing, examining, extracting, experimenting, and thus identifying different classes of phytoconstituents present in various parts of the plant for the discovery of drugs, the active components could be further taken for investigation and research. The procedure, known as phytochemical screening, was qualitative. The study's findings may help in the development of effective medications for a number of diseases.
Andrographis paniculata Nees is a medicinal plant belonging to the Acanthaceae family, commonly known as Kalmegh, or 'King of Bitter' in English, grows abundantly in Southeast Asia, India, and Sri Lanka. Plant is widely used in Indian traditional medicine, including Ayurveda, Unani, and Siddha, as a home cure for many illnesses. Due to its significant anti-inflammatory characteristics, this plant is widely recognized for its curative properties against upper respiratory tract infections (Liu *et al*., 2008) [13]. *Andrographis paniculata* shows a variety of biological properties, such as antibacterial, antiviral, cold and fever, anticancer, urinary tract infection, anti-diabetic, cardiovascular, immune-modulatory, and anti-hepatotoxic (Bhatnagar, 2023) [5] etc. The presence of important phytochemicals in A.paniculata makes the plant useful for treatment of different ailments and has potential of providing useful drugs of human use.

Material and Methods

Plant Materials and Extract Preparation: Plant material was identified and collected from the local herbal garden, Dehradun (Uttarakhand), India. The authentication of the plant was done and specimen was kept in the herbarium. Collected plant parts were washed with distilled water, cut into small pieces, and dried in shade for 4 weeks. Dried plant parts were ground into fine powder using an electric grinder. The 100 g of powdered sample was soaked in 500 ml of solvent in a conical flask, stirred occasionally, and macerated for 72 hours at room temperature. Maceration intends to soften and break the plant's cell wall to release the soluble phytoconstituents (Handa *et al*. 2008) [7]. It was then filtered and the supernatant was concentrated and evaporated to dryness at 50° C with a rotary evaporator under reduced pressure.

Phytochemical Analysis

The prepared extract was used to test various phyto constituents present in them. These various tests were qualitative and hence termed phytochemical screening. One gram of leaves/stem extract was dissolved in 100 ml ethanol (95%), the solution was used to preliminary phytochemical screening following standard methods based on journal articles (Harborne *et al*. 1998) [8], (Alamzed *et al*. 2013) [1], (Thusa & Mulmi, 2017) [23], and (Talukdar & Chaudhary, 2010) [24].

1. **Test for Phenolic:** A few drops of $FeCl_3$ (10 %) solution was added in a 1 ml plant extract. The formation of green, red, purple, red, or blue-black color indicated the presence of phenolic.
2. **Test for Flavonoids (Shibita's reaction test):** 1 ml plant extract solution was mixed with 3 ml of boiled water and incubated for 5 minutes. After that, it was added with 0.05 mg of Mg powder and 1 ml of concentrated HCl then it was shaken. Immediate development of a red yellow or orange color will indicate the presence of flavonoids.
   Dil. NH3 test: 5 ml of dilute NH3 solution in the extract was taken with the addition of conc. H_2SO_4. The appearance of yellow-colored precipitation indicated flavonoids.
3. **Test for alkaloids (Wagner reagent test):** Two drops of Wagner reagent was added to 2 ml of extract and mixed well. Appearance of a reddish color indicates the presence of alkaloids.
   Meyer's test: To 2 mL of extract, 1 mL of Meyer's reagent was added. The presence of pale yellow precipitate indicated the presence of alkaloids. **Dragendroff's reagent test:** 2 mL of extract was warmed with 2% H_2SO_4. Few drops of Dragendroff's reagent were added. Orange-red precipitate indicated the presence of alkaloids.
4. **Test for Phytosterol** – 1 ml of concentrated sulphuric acid was added to the 5 ml extract solution and allowed to stand for 5 minute. After shaking, formation of golden yellow color in the lower layer indicates the presence of steroids.
5. **Test for Tannins (Ferric Chloride Test):** 1 ml of plant extract solution was stirred with 5 ml of distilled water. The formation of a blue, blue-black or blue-green color or precipitation on the addition of (5%) $FeCl_3$ solution indicated the presence of tannins. The occurrence of blue-black colour showed the presence of gallic tannins and a blue-green colour indicated presence of catechol tannins.
6. **Test for saponins (Foam Test):** About 1 ml of plant extract solution was mixed with 3 ml of boiled water and shaken vigorously. If a foam was produced and it was stable for 1-2 minutes and persisted on warming, its evidence for the presence of saponin.
7. **Test for Glycosides:** 1 ml of plant extract was hydrolyzed with 5 ml hydrochloric acid for few hours on a water bath and subjected to Fehling test. 2 ml of extract was added in 2 ml of Fehling solution 1 ml of Fehling's A solution and 1 ml of Fehling's B solution), mixed well and boiled. Appearance of yellow or red color precipitate indicates the presence of reducing sugars.
8. **Test for Amino Acids:** Added few drops of 5% Ninhydrin solution in 1 ml of plant extract. The solution was heated in a water bath for 10 minutes, cool and made alkaline. Appearance of red, blue, deep blue precipitate indicates the presence of amino acids.
9. **Test for Terpenoids (Salkowski Test):** Plant Extract (5 ml) was mixed with chloroform (2 ml), and concentrated sulphuric acid (3 ml) was carefully added to form a layer. A reddish brown coloration of the inter face was formed to show positive results for the presence of terpenoids.

Results & Discussion

Phytochemical analysis

Plants have pharmacological activities attributed to the secondary metabolites which are responsible for essential bioactivities. The plant extract were screened for the presence of various secondary metabolites such as alkaloids, phenols, amino acids, flavonoids, saponins, steroids, and tannins according to common phytochemical methods. Phytochemical screening of *Andrographis paniculata* were carried with different solvent revealed the presence of some secondary metabolites as shown in the table-1.

Table 1: Phytochemical screening of plant in different solvents

Sl. No	Phytochemicals	Methanol		Ethanol		Chloroform		Petroleum ether		Aqueous	
		Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
1.	Phenols	+	+	+	+	+	+	+	+	-	-
2.	Flavonoids										
	a) Shibita's reaction test)	+	+	+	-	+	+	+	+	-	-
	b) Dil NH3 test	+	+	+	-	+	+	+	+	-	-
3.	Alkaloids										
	a) Wagner test	+	+	+	+	+	+	+	+	+	+
	b) Meyer's test	+	+	+	+	+	+	+	+	+	+
	c) Dragendroff's reagent test	+	+	+	+	+	+	+	+	+	+
4.	Phytosterol	-	+	-	-	-	-	+	+	-	-
5.	Tannins	+	+	+	+	-	-	+	+	-	-
6.	Saponins	+	+	+	+	+	+	+	+	-	-
7.	Glycosides	+	+	-	-	+	+	-	-	-	-
8.	Amino acids	+	+	+	+	+	+	+	+	+	+
9.	Terpenoids	+	+	+	+	+	+	-	-	-	-

Note: (+) means Presence, (-) means Absence of Phytochemicals

The results of the various phytochemical screening tests obtained during the experiment are shown in Table 1. In the present study presence of phenol, flavonoids, alkaloids, phytosterols, tannins, saponins, glycoside, aminoacids and terpenoids, were detected in *Andrographis paniculata* in varying concentration in all the extracts and showed potent biological activity and medicinal properties.

Methanol extract revealed the presence of maximum phytochemical such as phenol, flavonoids, alkaloids, tannins, saponins, glycoside, amino acids and terpenoids. Ethanol extract showed the presence of all bioactive molecules except phytosterols and glycoside. Chloroform extract revealed the presence of all bioactive molecules except phytosterols and tannins. Petroleum ether extract revealed the presence of phenol, flavonoids, alkaloids, phytosterols, tannins, saponins, glycoside, aminoacids and terpenoids. Aqueous extract showed the presence of alkaloids and amino acids.

The phytochemical compounds (alkaloids, phenolic, flavonoids, steroids) detected are known to have medicinal importance. The presence of several secondary metabolites in plants is influenced by several environmental factors. Phenolic and flavonoids content will be higher on intense drought stress condition (Manurung *et al* 2019) [15]. Soobrattee (2005) [22] reported that the phenolic compounds have redox properties and act as an antioxidant. Flavonoids are polyphenolic compounds exhibit several activities such as antioxidant, antibacterial, anti-inflammation, anti-allergy and anti-mutagenic. They are potent antioxidant capable of scavenging ROS due to presence of phenolic hydroxyl group (Lahare *et al*., 2020) [12]. Flavonoid caused risk reduction mainly from cardiovascular diseases and cancer (Ballard & Marostica, 2019) [3]. The presence of classes of phytochemicals as such; flavonoid, alkaloid, tannin showed cytotoxic effect (Chowdhury *et al*. 2017) [6]. The color and aroma imparting flavonoids were stated to show anticancer properties.

Alkaloids are present in several medicinal plants, and it constitutes an appreciable percentage in many available drugs, hence highly essential in disease management. Cardiac glycosides possess an effective and direct action on the cardiac system, supporting the strength of the heart and the rate of contraction when failing (Iwu *et al*., 1983) [9].

The presence of saponins, which are triterpenoid glycosides responsible for the bitter taste and as well-known for their hemolytic effect on red blood cells (Prohp *et al*., 2012) [20]. They possess cholesterol-reducing abilities and exhibit structure-dependent bioactivities (Roa *et al*., 1995) [21]. The saponins content of plants also helps in fighting pathogens and boosting the immune system. Additionally, cytotoxic qualities, as well as anti-bacterial, anti-viral properties, are credited to the presence of saponin (Bailly & Vergoten, 2020) [2]

Tannin shows an anticancer property that is perceptible from its inhibitory activity towards growth (Mazni, *et al*. 2016) [16]. The presence of terpenoids indicates that steroidal compounds could be present, which are of great use/importance in synthesizing sex hormones synthetic compounds (Okwu *et al*. 2001) [18]. The plant shows the positive test of cardiac glycoside which is beneficial for the heart. The phenolic compound, tannin, terpenoids, flavonoids possess an anti-helmintic property so the plant could be used to treat stomach problems (Nath & Yadav, 2016) [17]. Thus, potentially making *A. paniculata* leaves a great medicinal herb for large varieties of diseases (Trease *et al*. 2002) [25].

Conclusion

Plants are the reservoir of various chemical constituents and contain different biological and pharmacological properties that can be of valuable therapeutic index. Phytochemical constituents of the plant possess wide range of activities which provide protection against chronic and acute diseases. Phytochemical analysis showed rich contain of bioactive molecules in *Andrographis paniculata* due to the presence of steroid, terpenoid, flavonoid, tannin, glycoside, saponin, and alkaloid. Methanol extract and ethanol showed more bioactive constituents followed by chloroform, petroleum ether and water. Further advanced studies have conducted on these medicinal plants for characterization and structure elucidation of bioactive molecules. This study should be beneficial in pharmacological as well as industrial point of view.

Thus, this plant may be used for the production of herbal drugs for commercial purposes. Due to its various therapeutic applications, it is widely cultivated in many parts of the world, and its relevance as a medicinal plant is constantly increasing.

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Conflicts of Interest: Nil

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