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Phytochemical Screening Studies on the Leaves and Stem of *Andrographis neesiana* Wight - An Endemic Medicinal Plant from India

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Abstract: The present communication deals with the phytochemical screening studies of leaves and stem of the medicinal plant *Andrographis neesiana. Andrographis neesiana* is an endemic medicinal plant belonging to the family Acanthaceae. It has been used in traditional medicine to treat antifungal and aphrodisiac. Phytochemical screening of any medicinal importance from *Andrographis neesiana*. Qualitative analysis of the plant parts the presence of various components of therapeutic importance including tannins, saponins, phenolic compounds, glycosides, flavonoids, gums and mucilases, steroids and triterpenoids and absence of alkaloids, carbohydrates, protein and amino acids have been reported here in this herb for the first time. The experiment carried out in the medicinal plant leaves and stem. The results are discussed with the available literature.

Key words: *Andrographis neesiana* Wight · Acanthaceae · Phytochemical analysis · Medicinal plant

INTRODUCTION

Medicinal plants are used as herbs or traditional medicines for different types of ailments since ancient times. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, food supplements, nutraceuticals, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [1]. Modern medicine has evolved from folk medicine and traditional system only after thorough chemical and pharmaceutical screening. The use of plants as medicine was practiced by our ancestors, a process which must have started by trial and error [2]. Initially, in order to survive and then for civilization to develop, people needed to learn what plants were useful for medicines, foods, fuels and fibres and how such plants resources could be mined or managed for human benefits [3]. Medicinal plants help in alleviating human suffering and are widely used for subsistence home remedies and trade [4]. India is known for its rich diversity of medicinal world [5]. Nearly 70 percent of the world population is dependant on the traditional medicines for primary health care. The knowledge of medicinal plants has been accumulated during the course of many centuries based on different medicinal systems such as Ayurvedha, Unani and Siddha. In India it is reported that traditional healers used 2500 plant species and that 100 species of plants served as regular sources of medicine [6].

Species of *Andrographis* Wallich ex Nees (Acanthaceae) are used in the Indian systems of medicine namely Siddha, Ayurvedha and Unani [7]. The genus exhibits antipyretic properties [8]. This genus consists of 40 species distributed in Tropical Asia [9]. About 21 species are distributed in India [10] and all of them available in Tamilnadu [11]. Among the 21 species 18 species are reported to be endemic to India [12]. *Andrographis neesiana* Wight (Acanthaceae) is an endemic medicinal herb [12] found in wild in Kotagiri of Nilgiri district, Tamil Nadu. The Nilgiris, the so called blue mountains, is one of the richest hill district with its plant diversity, culture and ethnic groups of Tamil Nadu in Southern Peninsular India. It lies between 11°12' and 11°43' N and 76°14' to 77°1'E.

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The inhabitants of an area utilize plant wealth for food, medicine and many other purposes. Many of the food and medicinal plant contain a variety of chemical substances such as alkaloids, tannins, steroids, flavonoids, glycosides, saponins and exalates etc. Thus phytochemical screening of such plants is an important aspect for the scientific verification of folklore claim with regard to the utility of plants. Phytochemical are non-nutritive plant chemicals that have protective or illness preventive properties. Plant produces these chemicals to product itself but recent research demonstrates that many phytochemicals can protect human against diseases. The role of plants in maintaining health is well documented [13]. The practice of medicine both past and present often involves the prescription of specific foods or their potent derivatives, to treat wide spectrum of diseases [14]. *Andrographis neesiana* has been used in the treatment of aphrodisiac and antifungal activity [7,15]. Chalcone and flavone were isolated from the whole plant extract [16]. There is no earlier report on phytochemical studies of this useful plant. The present investigation was undertaken to study the phytochemical parameter on the leaves and stem of *Andrographis neesiana.*

MATERIALS AND METHODS

Plant materials are collected from the Kotagiri, Nilgiri district of Tamilnadu. This plant was identified and confirmed with the authentic. A voucher specimen was deposited (No. CA/36/2009) in the Department of Botany, Government Arts College (Autonomous), Salem, Tamilnadu. Fresh leaves and stem were washed several times under running tap water followed by surface sterilization by Mercuric chloride (0.01%). The leaves and stem were air dried in the laboratory at room temperature (30±2°C) for 15 days. While the leaves and stem samples were dried at 50°C for 24 hours in an oven. These plant materials were grinded to powder and used for the further analysis. Dried and powdered leaves and stem samples were tested for the availability of phytochemicals like, tannins, steroids, phenolic compounds, saponins, flavonoids and triterpenoids following standard methods [17, 18].

Phytochemical Analysis

Test for Alkaloids: Five ml of extract was added to 2 ml of hydrochloric acid. To this acidic medium, 1 ml of Dragendroff's reagent was added. An orange or red precipitate forms immediately indicates the presence of alkaloids.

Test for Steroids: Two ml of acetic acid anhydride was added to 0.5 g ethanolic extract of each sample (leaf extract and stem extract) with 2 ml H_2SO_4 The colour changes from violate to blue or green indicating the presence of steroids.

Test for Flavonoids: Five ml of dilute ammonia solution was added to a portion of aqueous filtrate of leaf extract and stem extract followed by addition of Conc. H_2SO_4. A yellow colouration observed in each extract indicates the presence of flavonoids. The yellow colour disappears on standing.

Test for Triterpenoids: Five ml of each extract (leaf extract and stem extract) was mixed in 2 ml of chlorofom and concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface was formed to show positive result for the presence of terpenoid.

Test for Tannins: About 0.5 g of the dried powdered samples were boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% feric chloride was added and observed for brownish green or a blue-back coloration.

Test for Carbohydrates: Five ml of Barfoed's reagent was added to the extracts and boiled on a water bath. The appearance of red precipitate indicated the presence of monosaccharides.

Test for Glycosides: Five ml of extract was dissolved in pyridine and freshly prepared sodium nitroprusside solution was added. The formation of pink to red colour indicated the presence of glycosides.

Test for Gums and Mucilages: About 10 ml of extract was slowly added to 25 ml of absolute alcohol under constant stirring. The appearance of precipitation indicated the presence of gums and mucilages.

Test for Saponins: About 2 g of the powdered sample (leaf and stem) was boiled in 20 ml of distilled water in a water bath and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Test for Protein and Amino Acids: Five ml of extract, 2 drops of freshly prepared 0.2 per cent ninhydrin reagent was added and heated. The appearance of blue colour indicated the presence of proteins, peptides or amino acids.

Test for Phenolic Compounds: Five ml of extract was treated with 1 drop of $FeCl_3$. Intense blue to violet colour will denote the presence of phenolic compounds.

RESULTS AND DISCUSSION

The present study carried out on the leaf and stem samples revealed the presence of medicinally bioactive constituents. The phytochemcial characters of *Andrographis neesiana* investigated are presented in Tables 1 and 2. Phytochemical test for ethanol, methanol, acetone, chloroform and petroleum ether extracts of the drug carried out. The phytochemical analysis of the various extracts from the leaf and stem sample of *Andrographis neesiana* showed the presence of phytochemical constituents such as tannins, saponins, phenolic compounds, flavonoids, gums and mucilases, steroids, glycosides and triterpenoids. The presence of these secondary metabolites suggests that the plant might be of medicinal importance and industrial. The phytochemical constituents like alkaloids, carbohydrates, protein and amino acids were absent in leaf and stem sample of *Andrographis neesiana* (Table 1).

Table 2 shows quantitative estimation of the percentage phytochemical constituents of *A.neesiana. A.neesiana* contained the highest percentage yield of flavonoids (16.45%) in petroleum ether leaf extract. The content of gums and mucilages was found high (15.83%) in methanol leaf extract. *A.neesiana* contained the lowest yield of phenolic compounds (0.07%) but the highest yield of tannin (5.36%). Triterpenoids were obtained in the plant but the yields recorded were minimal (0.24-0.12%). Saponin high yield 3.40% and lowest yield was found in 1.05%. Steroids were obtained in the plant but the yields recorded (0.26-0.14%). The content of glycosides was found in *A.neesiana* (0.26-0.14%). The phytochemical investigation and quantitative estimation of the percentage yields of chemical constituents of the plant studied that the leaves and stem were rich in flavonoids, saponins, tannins, gums and mucilages.

Table 1: Qualitative analysis of the phytochemicals in the leaves and stem of Andrographis neesiana

	Ethanol extract		Methanol extract		Acetone extract		Chloroform extract		Petroleum ether extract	
Phytochemicals	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
Flavonoids	+	+	+	+	+	+	+	+	+	+
Gums and Mucilages	+	+	+	+	+	+	+	+	+	+
Alkaloids	–	–	–	–	–	–	–	–	–	–
Carbohydrates	–	–	–	–	–	–	–	–	–	–
Glycosides	+	–	–	+	+	–	+	–	–	+
Protein and Amino acids	–	–	–	–	–	–	–	–	–	–
Steroids	+	+	–	–	–	+	–	–	–	–
Triterpenoids	+	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+	+	+	+
Phenolic Compounds	+	+	+	+	+	+	+	+	+	+

(+) - Positive (-) - Negative

Table 2: Phytochemicals composition percentage of *Andrographis neesiana*

	Ethanol extract		Methanol extract		Acetone extract		Chloroform extract		Petroleum ether extract	
Phytochemicals	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
Flavonoids (%)	12.46±0.11	4.37±0.4	13.42±0.32	5.34±0.20	15.41±0.30	2.30± 0.33	14.40±0.22	3.29± 0.16	16.45±0.12	5.26± 0.27
Gums and Mucilages (%)	12.80±0.10	6.74±0.13	15.83±0.40	11.77±0.14	13.76±0.20	8.68± 0.11	14.84±0.33	10.70±0.10	14.71±0.22	5.66± 0.01
Alkaloids (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Carbohydrates (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Glycosides (%)	0.26±0.14	0.00	0.00	0.20±12.00	0.24±0.50	0.00	0.21±0.30	0.00	0.00	0.14± 0.26
Protein and Amino acids (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Steroids (%)	0.47±0.11	0.33±0.05	0.00	0.00	0.00	0.30± 0.16	0.00	0.00	0.00	0.00
Triterpenoids (%)	0.18±0.3	0.09±0.18	0.20±0.01	0.11±0.08	0.14±0.22	0.09± 0.50	0.21± 0.22	0.10± 0.33	0.24±0.41	0.12± 0.20
Tannins (%)	3.11±0.17	2.08±0.32	5.36±0.41	1.40±0.08	3.66±0.10	1.10± 0.70	2.17±0.40	1.04± 0.51	2.19±0.20	1.45± 0.60
Saponins (%)	2.17±0.60	1.05±0.10	2.08±0.22	1.22±0.66	3.40±0.80	1.85± 0.04	2.01±0.14	1.27± 0.56	2.71± 0.33	1.17± 0.45
Phenolic Compounds (%)	0.30± 0.14	0.07±23.00	0.80±0.20	0.20±0.41	0.35±0.21	0.19± 0.11	0.60±0.40	0.21± 0.10	0.85±0.19	0.30± 0.44

They were known to show medicinal properties as well as exhibiting physiological activity [19]. Saponin has the activity of precipitating and colligating red blood cells. Saponin also foams in aqueous solutions, bitterness and hemolytic activity, flavonoid on the other hand, are effective water soluble anti-oxidants activity which prevent oxidative cell damage, have potent anti-cancer activity. The plant products over synthetic compound in the treatment of diseases are needed, because it does not have a deleterious effect in higher plants and animals including man. The urge in research on new drugs from natural sources is now moving out of the herablists shop, away from the core texts into the drug research laboratories [20].

The medicinal properties of plants are perhaps due to the presence of various secondary metabolites which are the non-nutritive plant compounds. These classes (such as alkaloid, tannin and flavonoid) of compounds are known to have medicinal activity against several pathogens and therefore could suggest the use traditionally for the treatment of various diseases [21]. The phytochemicals are known to have antimicrobial activity [22,23]. Tannin and flavonoid are thought to be responsible for antidiarrheal activity [24]. Usman and Osuji [21] reported that tannin has been widely used topically to sprains, bruises and superficial wounds as such. The phytochemical analysis revealed the presence of the tannins, saponins, phenolic compounds, flavonoids, gums and mucillases, triterpenoids and steroids respectively. It can be suggested that *Andrographis neesiana* are not only interesting source of medicinal activities but also potential source of phytochemicals. These findings can from the basis of further studies to isolate, identify, characterize and elucidate the structure of the bioactive compounds, to find new therapeutic principles. Thus the phytochemical screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery.

The phytochemical screening carried out on this plant shows that *A.neesiana* from Tamilnadu, India, rich in secondary metabolites which could be explored as potential drug leads and phytomedicine.

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