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## Determination of phytochemicals present in ethanolic extracts of *Andrographis paniculata*

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### Abstract

The study was conducted to analyse the phytochemical constituent of *Andrographis paniculata*. A 60% ethanolic extract was prepared and the different phytochemicals were qualitatively analysed by standard protocol. A total of ten numbers of phytochemicals were tested, out of which the extract was positive for alkaloid, phenolic compounds, steroids, terpenoids, saponins, tannins, flavonoids and negative for glycosides, amino acids and proteins. Analysis was done on the basis of colour change and precipitate formation. Presence of different important phytochemicals indicates the importance of the plant as herbal medicine.

**Keywords:** *Andrographis paniculata*, ethanolic extracts, phytochemicals, antiviral, immunostimulant

### Introduction

*Andrographis paniculata* (AP) is popularly known as Kalmegh or "King of Bitters" belongs to family *Acanthaceae*. The plant has been used since ages in Asian continent to treat different digestive issues and infections of upper respiratory tract, pyrexia, various viral infection, sore throat, and a variety of other diseases. The plant grows in abundance in Asian countries and in India and it is one of the most commonly used medicinal plants in Ayurvedic systems of medicines. The naturally occurring biologically active substances which are produced by plants for their protection are known as phytochemicals or phytonutrients. The proteins, chlorophyll, regular sugars are the primary constituents of phytochemicals and alkaloids, terpenoids, phyto sterols, flavonoids, glycosides, tannins and phenolic compounds are the secondary constituents (Li *et al.*, 2007) [14]. Major phytochemicals of *A. paniculata* are diterpenoids, flavonoids and polyphenols (Churiyah *et al.*, 2014) [5]. Andrographolide (AD) is the major compound in terms of bioactive properties and abundance which is present in *A. paniculata*. Studies have revealed that different parts of the plants possess antiviral, antimicrobial (Shao *et al.*, 2012; Arifullah *et al.*, 2013; Hua *et al.*, 2015) [22, 3, 9], act as hepatoprotective (Kunwar *et al.*, 2010) [13] and has anti-parasitic effects (Roy *et al.*, 2010; Mishra *et al.*, 2011; Zaid *et al.*, 2015) [20, 15, 25]. *A. paniculata* is one of the major components of *Nilavembu Kudineer Chooranam*, a herbal preparation consisting of nine plants which was reported to be effective in the prevention and treatment of chikungunya and dengue viral fever and therefore it was approved for use by the Government of Tamil Nadu (Kavinilavan *et al.*, 2017) [10]. The plant is found to be a good source of phytochemicals with extensive variety of restorative applications. In this study an endeavor was made to perform qualitative assay of the phytochemicals of the ethanolic extract of *Andrographis paniculata*.

### Materials and Methods

#### Preparation of plant extract

The plant extract was prepared with commercially available plant powder of *Andrographis paniculata* (Amritha Naturmed's Herbal products Hyderabad). A 60% ethanolic extract was prepared and further downstream processing was done following the procedure of Colney, 2018 [6].

#### Phytochemical Screening Test

Phytochemical screening was conducted to analyze the bioactive components present in the ethanolic extracts using standard methods (Shaikh and Patil, 2020) [21].

**Test for Alkaloids**

**Hager's test:** 2 ml of 60% ethanolic extract was taken in a glass test tube and few drops of Hager's reagent were added to the extract. A yellow precipitate was formed which indicates the presence of alkaloid.

**Test for glycosides**

**Keller Killiani test:** To 1ml of crude extracts, 100µl of acetic acid and 100µl of 2% ferric chloride was added was added dropwise and mixed. Later, 1 mL of concentrated H<sub>2</sub>SO<sub>4</sub>, was added carefully along the walls of the test tube. The development of brownish colour ring at the junction of two liquids indicated the presence of glycosides.

**Test for phenolic compounds**

1ml of extract was added in a test tube and 3 drops of 5% ferric chloride was added to it. Development of deep blue or dark green is a positive test.

**Test for steroids**

**Salkowski test:** 2 ml of test extract was added to a test tube and chloroform was added. Concentrated Sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) was added slowly along the walls of a test tube; formation of red colour indicates the presence of steroids.

**Test for terpenoids:** The plant extract was taken in a tube and 3 drops of concentrated Sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) was added. Development of greenish blue colour indicates positive reaction.

**Test for Saponins:** 1 ml of crude extract was taken in a tube and 0.5 ml of distilled water was added and mixed properly. Formation of foam which is stable for 15minutes indicates the presence of saponins.

**Test for tannins:** For detection of tannins 0.5 gram of dried plant extract was boiled in 20 ml water and filtered. Few drops of 0.1% ferric chloride was added to the filtrate. Development of brownish green or blue black colour indicates positive reaction.

**Test for amino acid**

**Ninhydrin test:** For this test 2 drops of 0.2% freshly prepared ninhydrin solution was added slowly to 1ml of plant extract. Development of purple colour shows the presence of amino acid.

**Test for protein:** Biuret test was performed for detection of proteins. 300 µl of 10% Sodium hydroxide (NaOH) and 300µl of 0.1% Copper sulphate (CuSO<sub>4</sub>) was added to 1 ml of crude extract. Violet or pink colour formation indicates the presence of protein.

**Test for flavonoids:** 2-3 drops of sodium hydroxide were added to 2ml of extract. Initially there was development of deep yellow colour. Then when dilute HCL was added the solution should become colourless incase of positive reaction. Result and discussion

Plant extracts were screened for the presence of major groups of secondary metabolites such as alkaloids, phenolic compounds, saponins, terpenoids, glycosides, steroids, amino acid and proteins, according to conventional methods. The results of phytochemical tests carried out for *Andrographis*

*paniculata* ethanolic extract are depicted in Table 1.

**Table 1:** Phytochemical analysis of ethanolic extract of *Andrographis paniculata*

Sl.no.	Components	Presence (+ve) /Absence (-ve)
1	Alkaloids	Present
2.	Glycosides	Absent
3.	Phenolic compounds	Present
4.	Steroids	Present
5.	Terpenoids	Present
6.	Saponins	Present
7.	Tannins	Present
8.	Amino acids	Negative
9.	Protein	Negative
10.	Flavonoids	Positive

Phytochemical analysis of ethanolic extracts *A. paniculata* showed positive for seven major phytochemicals like alkaloids, phenolic compounds, steroids, terpenoids, saponins, tannins and flavanoids whereas glycosides, amino acid and protein was not detected. This finding was at par with Nagajothi *et al.*, 2018 <sup>[16]</sup> where he compared phytochemicals present in different extracts of *A. paniculata* and found that the ethanolic extract was negative for the presence of glycosides, amino acid and protein. The performed tests were qualitative and based on visual observation of the change in color or precipitation reaction after the addition of specific reagent. But in another study performed by Pandey *et al.*, 2019 <sup>[17]</sup> it was reported that the presence of glycosides in extracts of methanolic, petroleum ether, acetone and chloroform. The probable reason for this might be due to different solvents used for extraction (Polash *et al.*, 2017) <sup>[18]</sup>. Alkaloids, phenols, flavonoids, terpenoids and saponins are important constituents of the ethanolic extract of *A. paniculata* which have proven to be important for antiviral activity (Arbab *et al.*, 2017) <sup>[2]</sup>. Diterpenoids lactones are the most common terpenoids available in *A. paniculata* and they are isolated from aerial parts of plants and their roots. Andrographolide is the most predominant diterpenoids and it is bitter in taste and brown in colour (Siripong *et al.*, 1992) <sup>[24]</sup>. The presence of alkaloids is very crucial as it has many pharmacological importance, as it can act as antiprotozoal, has cytotoxic activity, antidiabetic properties (Zhang and Tan, 2000) <sup>[26]</sup>, as antimalarial, analgesic, immunostimulant as well as anti-inflammatory properties (Appiah *et al.*, 2017; Churiyah *et al.*, 2015) <sup>[1, 5]</sup>. Flavonoids present in the extract are known to inhibit tumorous growth and provide protection against various gastrointestinal infections. Presence of flavonoids are important as it act as a proof that the plant can be utilized in the field of herbal medicine. Tannin compounds present in *A. paniculata* plant extracts can selectively inhibit the replication of Human Immuno deficiency Virus as well as it is used as a diuretic (Hossain *et al.*, 2014) <sup>[8]</sup>. Alkaloids and flavonoids in plant extracts of *A. paniculata* are water-soluble antioxidants that act as free radicals and prevent oxidative cell damage (Sheeja *et al.*, 2006) <sup>[23]</sup>. In a study conducted by Colney (2018) <sup>[6]</sup> it was proven that 60% ethanolic extract of *A. paniculata* has no adverse effect on hatchability when embryos were injected with 60mg of plant extract along with HVT vaccine by *in ovo* route on 18<sup>th</sup> day of embryonation and high HI titre was observed against Newcastle disease vaccine (14 days after hatch). This study points out the immunoregulation effect of *A. paniculata* that it could

possibly counter the TH1 skewing effect of HVT. Other beneficial properties of *A. paniculata* plant extracts are that they have strong effect against cancer (Kumar *et al.*, 2004)<sup>[12]</sup> and possess anti rheumatic (Hidalgo *et al.*, 2013)<sup>[7]</sup> effects.

### Conclusion

From the studies carried out, it could be concluded that *A. paniculata* is an important medicinal plant and was traditionally used to treat fever, colds and various infections. All available phytonutrients act synergistically and have beneficial effects in the treatment of many diseases from fever to cancer. In India, a vast population of people depends on ayurvedic medicine for treatment and *A. paniculata* is one of the most beneficial plant. It is an important ingredient in several herbal preparations due to its antitumor (Ram, 2001)<sup>[19]</sup>, antiviral (Calabrese *et al.*, 2000)<sup>[4]</sup> and immunostimulatory effects (Kavinilavan *et al.*, 2017)<sup>[11]</sup> both in humans and in animals. Due to rapid increase in antimicrobial resistance peoples are now turning to alternative medicine and phytochemical analysis of plant extract of *A. paniculata* can provide many insight into the credibility of the plant as enthomedicine and will help in new drug development. The plant extract may also be used as an adjuvant in vaccination of poultry which will help in immunomodulation and enhance vaccine efficacy.

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