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# PHYTOCHEMICAL SCREENING OF *ANDROGRAPHIS PANICULATA* LEAF EXTRACT COLLECTED FROM Dr. V. S. KRISHNA GOVERNMENT DEGREE COLLEGE CAMPUS

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## ABSTRACT

Medicinal plants possess bioactive components called phytochemicals which are administered for treating various human ailments. Phytochemicals are of two categories i.e., primary and secondary constituents. Primary constituents include proteins, chlorophyll, sugar and amino acids. Secondary constituents contain alkaloids and terpenoids. Medicinal plants have antifungal, antibacterial and anti-inflammation activities because of these phytochemicals. The aqueous extract of leaf samples of the medicinal plant *Andrographis Paniculata* is collected from agricultural lands of S.Kota. This extract is used for the phytochemical screening with an objective to check the presence or absence of the phytochemical constituents in the selected plant. The results of the phytochemical analysis of the plant leaf extract shows that the terpenoids, tannins, reducing sugars, flavonoids and alkaloids are found to be present in aforementioned medicinal plant. The key phytochemical properties identified by this study will be very helpful to pharmaceutical industries for the fabrication of the novel drugs for treating various diseases.

**KEYWORDS:** Phytochemicals, *Andrographis Paniculata*, alkaloids, flavonoids, terpenoids and FTIR.

## 1.1 INTRODUCTION

Phytochemistry is the part of chemistry that deals with the chemical processes involved in plant life. Phytochemicals are naturally occurring and biologically active chemical substances present in plants. Proteins, chlorophyll and regular sugars are the primary constituents and alkaloids, terpenoids, phyto sterols, flavonoids, glycosides, tannins and phenolic compounds are being the secondary constituents.[1] Phytochemicals guard plant cells from pollution, drought, stress and pathogenic attack.[2] Phytochemicals are synthesized in almost all parts of the plant like leaves, root, bark, stem, root, fruits, flower, seeds etc.[3,4] Phytochemicals are responsible for the colour and organoleptic properties of plant. Recent research shows that phytochemicals play a vital role in protecting humans against diseases. To extricate these compounds from plants phytochemical screening is inevitable. Phytochemical screening deals with the extraction, screening, and identification of the bioactive substances found in plants.[5,6]

*Andrographis* is a herbal plant belonging to the family Acanthaceae (**Figure 1.1**) which is usually found in agricultural lands of India. In native language it is called as Nelavemu. Literature reveals that the parts of this plant are used to treat inflammation and pain in the human body and also used as a potent antiviral and antidiabatic agent.[7-9] It is found to be a good source of phytochemicals such as terpenoids, flavonoids, alkaloids, phenolic compounds, glycosides, gums, tannins, terpenes, carbohydrates and aminoacids. This present paper deals with the phytochemical screening of the leaf extract.

**Figure 1.1: *Andrographis Paniculata* plant.**

## 1.2 MATERIALS AND METHODS

### 1.2.1 Chemicals required

The chemicals required for the phytochemical screening of the leaf extract.[9] are Mayers reagent (potassium mercuric iodide), Hager's reagent, Molisch's reagent, Benedict's reagent, Fehling's reagent, Schiffs reagent sodium nitroprusside, NaOH, ferric Chloride, benzene, $H_2SO_4$, chloroform, lead acetate, gelatin, $HNO_3$, acetic anhydride, ferric chloride, Ninhydrin reagent, copper acetate, sodium bicarbonate, hydrochloric acid, litmus papers, 2,4-DNP, Tollens reagent, iodine solution and deionized water.

### 1.2.2 Collection of *Andrographis Paniculata* leaves

Fresh leaves of *Andrographis Paniculata* plant are collected from botanical garden of Dr.V.S. Krishna Government Degree College, Visakhapatnam (**Figure 1.2**). 100 g of leaves are weighed and thoroughly cleaned with running tap water to eliminate debris on surface of leaves followed by deionized water to remove other contaminants from leaves and dried up under shade for six days i.e., until the weight of the dried leaves remains constant. These leaves are sliced into tiny pieces and made homogenized powder by using home blender. The obtained powder is stored in an air tight container for further usage.

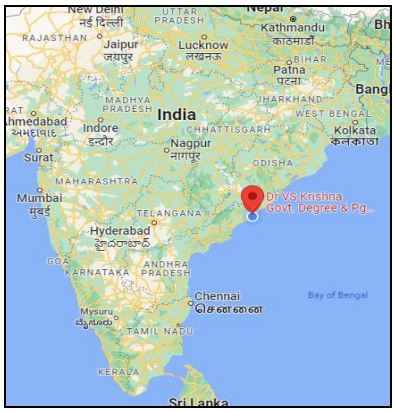


**Figure 1.2: Map showing plant collection site in India.**

### 1.2.3 Preparation of leaf Extract

250 mL deionized water is taken in 500 mL beaker to this 10 g stored powder weighed and added. The contents in the beaker boiled for 20 minutes with occasional stirring with glass rod and then cooled to attain room temperature. The cooled leaf broth is filtered 2 times with Whatman No.1 filter paper and reserved in refrigerator at 4°C. This is taken as leaf extract throughout the experiment (**Figure 1.3**).


**Figure 1.3: Image of *Andrographis Paniculata* leaf extract.**

### 1.3 Phytochemical Screening Tests

Aqueous extract of *Andrographis Paniculata* is screened to various phytochemical tests. Standard methods are used for phytochemical screening.[10]

### 1.3.1 Test for Alkaloids

**a) Mayers Test**

To 4 mL of 2% HCl, 4 mL of leaf extract is added boiled in a water bath and then filtered. 2 mL of the filtrate is treated with three drops of Mayer's reagent. Development of yellow precipitate indicates the presence of Alkaloids.

**b) Hager's Test**

To 4 mL of 2% HCl, 5 mL of leaf extract is added boiled in a water bath and then filtered. 2 mL of above filtrate is treated with 2 drops of Hager's reagent. Formation of yellow precipitate shows the presence of alkaloids.

**c) Wagner's Test**

To 4mL of 2% HCl, 5 mL of leaf extract is added boiled in a water bath and then filtered. 2 mL of above filtrate is treated with two drops of Wagener's reagent. Formation of brown colour precipitate indicates the presence of alkaloids in leaf extract.

### 1.3.2 Test for Carbohydrates

**a) Benedict's Test**

To 2 mL algal extract 5mL of distilled water is added and filtered. To the 2 mL of filtrate 2 drops of Benedict's reagent is added and heated gently for two minutes. Formation of red precipitate indicates the presence of carbohydrates (reducing sugars).

**b) Molisch's Test**

To 2 mL algal extract 5mL of distilled water is added and filtered. To the 2 mL of filtrate 2 drops of Molisch's reagent (alcoholic solution of α-naphthol solution) is added followed by the addition of concentrated sulphuric acid along the walls of the test tube. Formation of violet ring indicates the presence of carbohydrates.

**c) Fehling's Test**

To 2 mL algal extract 5mL of distilled water is added and filtered. To the 2 mL of filtrate 1mL of each Fehling

solution A and B is added and boiled in a water bath for 2 min. Formation of brown precipitate indicates the presence of carbohydrates (reducing sugars).

**1.3.3 Test for Glycosides**

**a) Modified Borntrager's Test**

5 mL of extract is treated with 2 mL of $FeCl_3$ solution and immersed in boiling water for about five minutes. The mixture is cooled and extracted with equal volumes of benzene. The benzene layer is separated and treated with ammonia solution. Formation of rose-pink colour indicates the presence of anthranol glycosides.

**b) Legal's Test**

5 mL of extract is treated with 4mL of pyridine contained 2 mL of sodium nitroprusside solution. This is neutralized with 10% NaOH. Appearance of pink colour shows the existence of glycosides.

**c) Keller-kilani test**

The crude extract (2 mL) is reacted with glacial acetic acid (2 mL) containing 1-2 drops of 2% $FeCl_3$ solution. The mixture is then transferred into another test tube already containing 2 mL concentrated $H_2SO_4$. Appearance of brown ring at the interphase confirms the presence of cardiac glycosides.

**1.3.4 Test for Saponins**

**a) Foam Test**

To 5 mL of crude extract 10 mL of distilled water is added and this solution shaken vigorously in a 50 mL conical flask for 10 minutes. Persistent foaming on shaking confirms the presence of saponins.

**1.3.5 Test for Steroids and Phytosterols**

**a) Salkowski's Test**

5mL of extract is treated with chloroform and filtered. The filtrate is treated with few drops of conc. $H_2SO_4$, shaken and allowed standing. Appearance of golden yellow colour indicates the presence of steroids.

**b) Libermann-Burchard's Test**

5mL of extract is treated with chloroform and filtered. The filtrate is treated with few drops of acetic anhydride, boiled and cooled. Conc. $H_2SO_4$ is added. Formation of reddish brown colour indicates the presence of steroid ring.

**1.3.6Test for Phenolic compounds**

**a) Ferric Chloride Test**

The extract is dissolved in 5mL of distilled water and 2-4 drops of 5% $FeCl_3$solution is added. Formation of deep green colour specifies the presence of phenolic compounds.

**b) Lead Acetate Test**

2mL of 5% lead acetate solution is added to the extract solution. Formation of yellow precipitate indicates presence of phenolic compounds.

**1.3.7Test for Tannins**

**a) Gelatin Test**

To 1% gelatin solution containing 10% NaCl 5mL diluted algal extract is added. The formation of white precipitate indicates the presence of tannins.

**1.3.8 Test for Flavonoids**

**a) Alkaline Reagent Test**

5mL of extract is treated with few drops of sodium hydroxide solution. Formation of an intense yellow colour, which becomes colourless on addition of dilute acid connotes the presence of flavonoids.

**1.3.9 Test for proteins**

The extract is treated with few drops of Conc. $HNO_3$. Formation of yellow colour suggests the presence of proteins.

**1.3.10 Test for Amino acids**

**a) Ninhydrin Test**

5mL of extract is diluted by the addition of 15mL of distilled water. To the extract, 0.25% w/v Ninhydrin reagent is added and boiled for a few minutes. Formation of blue colour denotes the presence of amino acids.

**1.3.11Test for Diterpenes**

a) Crude extract (2 mL) is dissolved in chloroform (2 mL) and then evaporated to dryness. Concentrated $H_2SO_4$ (2 mL) is added and heated for 2 minutes. The appearance of grayish coloration indicates the presence of terpenoids.

**b) Copper acetate Test**

Extract is dissolved in water and treated with 2-4 drops of copper acetate solution. Formation of emerald green colour confirms the presence of diterpenes.

**1.4 Detection of functional groups present in the leaf extract by FTIR analysis**

The FTIR data is used to identify the functional groups of the leaf extract (**Figure 1.4**). The active components are separated based on its peak values in the region of IR radiation. The spectrum shows the signals at 3329 $cm^{-1}$, 3290 $cm^{-1}$, 2860 $cm^{-1}$, 1640 $cm^{-1}$, 1556 $cm^{-1}$, 1019 $cm^{-1}$, 825 $cm^{-1}$, 683 $cm^{-1}$ and 583$cm^{-1}$ indicates the presence alcohols, phenols, Ketones, aromatic compounds, carboxylic acids, alkyl halides in the aqueous leaf extract of *Andrographis Paniculata*.

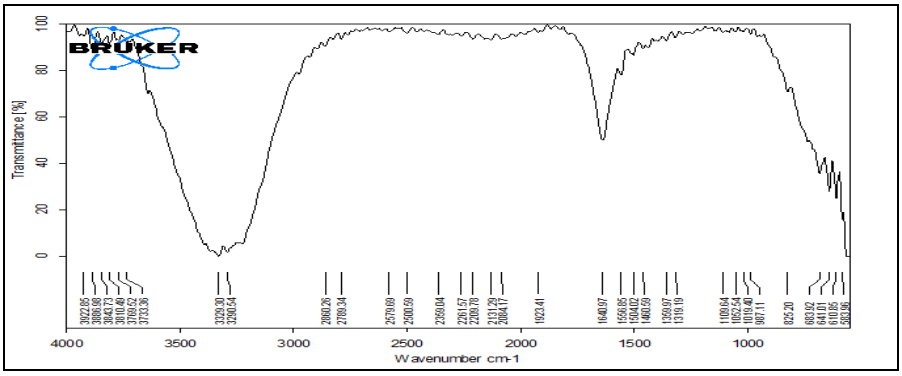


**Fig. 1.4: FTIR spectrum of *Andrographis Paniculata* leaf extract.**

### 3.5 CONCLUSIONS

The phytochemical screening and FTIR spectroscopic analysis of *Andrographis Paniculata* leaf extract confirm the presence of alkaloids, carbohydrates, glycosides, saponins, phytosterols, phenolic compounds, tannins, flavonoids, proteins, amino acids and terpenes. These are the plant secondary metabolites present in the leaf extract. The important phytochemical properties recognized by this study will be very useful in the development of new drugs for the treatment of various diseases of mankind.

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