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## Phytochemical Screening and Measurement of Antioxidant activity from *Andrographis paniculata*

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**ABSTRACT :** *Andrographis paniculata*, commonly known as ‘kalmegh’ or Bhuineem, is a medicinal herb belonging to the family *Acanthaceae*. The plant referred to as ‘King of Bitters’ due to its extreme bitter taste, is used as a traditional herbal medicinal for the treatment of various diseases. The aerial parts of this plant are rich in bioactive components such as alkaloids, flavonoids, tannins, terpenoids, phytosterols, proteins, etc. Andrographolide is the main diterpene lactone in leaves of *Andrographispaniculata* that exhibits multiple medicinal properties such as anti-inflammatory, anti-pyretic, antioxidative, anti-bacterial, anti-diabetic, hepatoprotective, tumor suppressing activities, etc. Extracts of different samples of *Andrographis paniculata* contain a mixture of bioactive and inactive chemical ingredients. Chemical composition of samples varies not only in different parts but also on growing region and the collection time. Therefore, efficient and effective methods of identification, extraction, standardizations, qualitative, quantitative and pharmacological testing is essential. The extraction of *Andrographispaniculata* stem and leaf powder was carried out using a soxhlet assembly method with petroleum ether and methanol as solvent. The extract was used for various qualitative phytochemical tests. The antioxidant activity was measured by calculating the absorbance of the extract using the DCPIP dye reagent. Antioxidants are gaining importance due to increase in Reactive Oxygen Species generated in our cells due to modern life style. The leaf and stem extract show noteworthy antioxidant activity which proceeds to be an important medicinal element of the plant.

**KEYWORDS:** Absorbance, antioxidant activity, bioactive, DCPIP, soxhlet extraction

### I. INTRODUCTION

*Andrographis paniculata* (Burm. f.) Nees is one of the widely distributed medicinal plants in India. It is one of the most commonly used plants in ayurvedic formulations since ancient times. It is available abundantly in south eastern Asia i.e., India, Sri Lanka, Pakistan and Indonesia. It is found in wild through out of plains of India especially in Tamil Nadu, Karnataka, Maharashtra, Orissa, Uttar Pradesh and Uttarakhand.

*Andrographis paniculata* or “Kalmegh” is an erect annual herb which is extremely bitter in taste in each and every part of the plant body. The plant is known in north-eastern India as ‘Maha-tita’, which literally means ‘King of bitters’. *Andrographis paniculata* is the main source of the bitter principle Andrographolide and called “King of bitters” due to extremely bitter and characteristic taste. Whole plant of *Andrographis paniculata* has a wide range of pharmacological activity. The genus *Andrographis* consists of 28 species of small annual herbs essentially distributed in Tropical Asia. Only a few species are of medicinal value out of which *Andrographis paniculata* (Burm. f.) Nees is the most popular. The plant is gregarious abundantly found in moist, shady waste grounds and sometimes in dry forests. Phytochemical studies of *Andrographis paniculata* has led to the isolation of various plant metabolites such as alkaloids, flavonoids, phytosterols, terpenoids, lactones, etc.

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Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. Solid material containing some of the desired compound is subjected to soxhlet extraction followed by repeated cycles. During each cycle, a portion of the non-volatile compound dissolves in the solvent. After many cycles the desired compound is concentrated in the distillation flask. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled.

After extraction the solvent is removed, typically by means of a rotary evaporator, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble, and is usually discarded.

Aim of our project is to perform Phytochemical Screening of *Andrographis paniculata* extract and to measure its antioxidative activity.

## II. MATERIALS AND METHODS

### Collection of Plant material:

Plants were collected from PDKV, Akola and medicinal plant nursery, Amravati.

### Preparation of extract:

**Materials:** Shade dried leaves and stems, petroleum ether, methanol, grinder, soxhlet extraction unit, glassware

**Method:** healthy and fresh leaves and stem of the plants were collected separately and shade dried for 3 days (Figure 1 and Figure 2). The dried samples were crushed directly without addition of solvent in a grinder to obtain leaf powder and stem powder (Figure 3 and Figure 4).

A. Stem Extract: 15 g of stem powder was subjected to extraction in petroleum ether using a soxhlet assembly (Figure 5) for 3 hours at 60°C. The extract was collected in a conical flask and stored in a refrigerator until further use. (Figure 6)

B. Leaf Extract: Stem Extract: 15 g of leaf powder was subjected to extraction in methanol using a soxhlet assembly for 4-5 hours at 65-70°C. The extract was collected in a conical flask and stored in a refrigerator until further use. (Figure 7)

### Qualitative Analysis (Phytochemical screening)

#### 1. Test for Alkaloids (Wagner's test)

Materials: Wagner's reagent (Iodine, Potassium iodide, distilled water), extracts, Glassware

Method: 1ml Wagner's reagent was added to 3ml of extract and observed.

#### 2. Test for Flavonoids (Ammonium test)

Materials: ethyl acetate, 1% ammonium solution, extracts, filter paper, boiling water bath, Glasswares.

Method: 10 ml of ethyl acetate was mixed with 3ml of extract and kept in boiling water bath for 10 min. The sample is then filtered. 1% of ammonia solution was added to 3ml of filtrate and observed.

#### 3. Test for Saponins (Foam test)

Materials: extract, distilled water, Glassware.

Method: 3 ml of distilled water was mixed with 1ml of extract and shake well.

#### 4. Test for Tannins/Phenol:

Materials: Ferric chloride solution, extract, Glassware

Method: Add 2-3 drops of 10% ferric chloride in 1 ml of extract containing 2 ml H<sub>2</sub>O and observe.

#### 5. Test for Lactones (Kedde's Test)

Materials: Kedde's reagent-2% 3, 5- dinitrobenzoic acid, Potassium hydroxide, methanol, distilled water, extract, glassware

Method: Add 1 ml of Kedde's reagent in 2 ml of extract and observe.

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## 6. Test for Phytosterols (Salkowski's Test)

Materials: Chloroform, conc H<sub>2</sub>SO<sub>4</sub>, extract, filter paper, glassware

Method: Add 10 ml chloroform to 2 ml sample and filter. Take 1ml filtrate in a test tube and add equal volume of conc. H<sub>2</sub>SO<sub>4</sub> and observe.

**Quantitative Analysis** (Anti-oxidant activity using DCPIP)

Reagent: 2,6dichlorophenol-indophenol dye solution containing 0.1mM of the dye and 0.01M KCl in 0.04M potassium phosphate buffer, pH 6.5

**Method:**

Take 0.2 ml of plant extract into test tube and add 4ml of water followed by 2ml of 2, 6- dichlorophenol-indophenol dye solution. Absorbance was recorded at 610 nm (A<sub>0</sub>) and 680 nm (A<sub>1</sub>) and the anti-oxidant activity was calculated using the following formula:

**Formula:**

$$\text{Anti-Oxidant activity (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

## III. RESULT

### Phytochemical Screening

Sr.No	Test for:	Chemical test	Stem extract (Petroleum Ether)	Leaf extract (Methanol)
1	Alkaloid	Wagner's test	+	+
2	Flavonoids	Ammonium	+	+
3	Saponnin	Foam test	-	-
4	Tannin/Phenol	Ferric chloride test	-	+
5	Lactone	Kedde's test	+	+
6	Phytosterol	Salkowski's test	-	+

Table 1: Phytochemical screening tests

### Antioxidant Test (DCPIP method)

Absorbance in nm	Optical Density (stem)	Optical Density (leaf)
610 (A <sub>0</sub> )	0.11	0.18
680 (A <sub>1</sub> )	0.09	0.13
Total Antioxidant activity	<b>18.1%</b>	<b>27.7%</b>

Table 2: Estimation of Antioxidant activity

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## IV. DISCUSSION

Qualitative analysis of this plant was carried out for stem and leaf extracts in Petroleum ether and Methanol respectively. The extracts were subjected to phytochemical analysis for the presence of alkaloids, flavonoids, saponins, tannins/phenols, lactones and phytosterols. The stem extract in petroleum ether showed presence alkaloids, flavonoids and lactones, while tannins/phenols, saponins, phytosterols were absent. The leaf extract in methanol revealed the presence of alkaloids, flavonoids, tannin/phenols, lactones and phytosterols and absence of saponins.

The presence of Flavonoids in the leaves and stem of *Andrographis paniculata* enables them to be used as antioxidant. The antioxidant activity was calculated using DCPIP method was found to be 18.1% in stem extract and 27.7% in leaf extract.

## V. CONCLUSION

The demand of *Andrographis paniculata* is greatly increased in the past few years for its overwhelming therapeutic potentials. Thus, it can be safely regarded as one of the modern remedies due to its extensive medicinal properties. However these properties need validation through the proper clinical study. Verification of the efficacy of other biological activities of *Andrographis paniculata* including anti-oxidant, anti-diabetic, anti-cancer anti-inflammatory and hepatoprotective activities on human study subjects would bring a lot of benefits for the human population. Extensive research to optimize the concentration for clinical feasibility is now greatly demanded. So the choice of *Andrographis paniculata* as an alternative resource in medical therapy requires further active research involving the study of its bioactive components and their properties.

### Tables and Figures:



**Fig 1: Shade dried stems**



**Fig2: Shade dried leaves**



**Fig 3: Stem Powder**



**Fig 4: Leaf Powder**

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**Fig 5: Soxhlet Assembly**



**Fig 6: Stem extract**



**Fig 6: Leaf extract**

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