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Research Article

# STUDIES ON STANDARDISATION OF *ANDROGRAPHIS PANICULATA* NEES AND IDENTIFICATION BY HPTLC USING ANDROGRAPHOLIDE AS MARKER COMPOUND

**ARJUN SINGH*[1], A K MEENA[2], SUDEEP [1], MEENA[3], P. PANT[1], M M PADHI[1]**

[1]Central Council for Research in Ayurvedic Sciences, Janakpuri, Delhi-110058, [2]National Institute of Ayurvedic Pharmaceutical Research, Patiala – 47001, Punjab, [3]Doon College of Engineering & Technology, Sunderpur, Saharanpur, India.
Email: arjunccras@gmail.com; ajaysheera@yahoo.com

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**ABSTRACT**

The present communication attempts to evaluate the physicochemical and preliminary phytochemical studies on the extract (Hydro-alcoholic) *of Andrographis paniculata* Nees*. (Acanthaceae).* It is widely found and cultivated in tropical and subtropical Asia, south-east Asia and India. It is wide range of pharmacological effects and some of them extremely beneficial such as anti-inflammatory, anti-diabetes, antidiarrhoeal**,** antiviral, antimalarial, hepatoprotective, anticancer, antihuman immunodeficiency virus (HIV), immune stimulatory and antisnakebite activity. In Physico-chemical parameters studies LOD was found to be 4.54 %, Total ash content was 28.57 % and acid-insoluble was 5.45%. The water-soluble and alcohol soluble extractive values were found to be 88.27, 55.47% respectively. Other parameters like density, solvent residue were also analysed. The levels of toxic heavy metals and microbial contamination was indicated in such herbal drugs was in permissible limit as per WHO specification. The data indicated suggest that there is requirement of in process improvement to provide better quality for consumer health in order to be competitive in international markets.

**Keywords**: *Andrographis paniculata* Nees., Toxic metals, Physicochemical studies.

## INTRODUCTION

*Andrographis paniculata* Nees. is a slender upright annual varying in height from 30 to 100 cm (1 to 3 feet), with a square stem and "lanceolate" leaves (i.e., shaped like a lance, sharp at the ends and curved in the middle). *Andrographis paniculata Nees.* is an erect annual herb extremely bitter in taste in all parts of the plant body. The plant is known in north-eastern India as *Maha-tita*, literally "king of bitters", and known by various vernacular names like Chiorta in Assamese, Oli-kiryata in Marathi, Kālmegh in Bengali, Bhuinimba in Oriya, Green chirayta, creat, andrographis, India Echinacea in English, Kariyatu in Gujarati, Kālamegha, Bhūnimba in Sanskrit[1], Kirayat in Hindi, Nilavembu in Tamil, Nelaberu in Kannada, Nelavemaa or Nelavepu meaning "Neem of the ground" in Telugu. Nilavembu in Malayalam.

Since ancient times, *Andrographis paniculata Nees.* is used in traditional Siddha and Ayurvedic[2] systems of medicine as well as in tribal medicine in India and some other countries for multiple clinical applications. As an Ayurveda herb it is known as *Kalmegh* or *Kalamegha*, meaning "dark cloud". It is also known as *Bhui-neem*, meaning "neem of the ground", since the plant, though being a small annual herb, has a similar strong bitter taste as that of the large Neem tree (*Azadirachta indica*). The genus *Andrographis* consists of 28 species of small annual shrubs essentially distributed in tropical Asia. Only a few species are medicinal, of which *Andrographis paniculata Nees.* is the most popular.

Hydro-alcoholic extract of *Andrographis paniculata Nees.* is traditionally used as a medicine to treat different diseases in India, China and Southeast Asia including Malaysia. The leaves and roots have traditionally been used over the centuries in Asia and Europe as a folklore medicine for a wide variety of ailments or as herbal supplements for health promotion. In traditional Chinese medicine, it is widely used to get rid of body heat, as in fevers and to dispel toxins from the body. In Scandinavian countries, it is commonly used to prevent and treat common cold[3]**.** Previous studies have explicitly revealed that *Andrographis paniculata Nees.* has a wide range of pharmacological effects and some of them extremely beneficial such as anti-inflammatory[4], anti-diabetes[5], antidiarrhoeal[6]**,** antiviral[7], antimalarial[8], hepatoprotective[9], anticancer[10], antihuman immunodeficiency virus (HIV)[11], immune stimulatory[12], and antisnakebite activity[13]. Diterpenoids and flavonoids are the main chemical constituents of *A. paniculata* which are believed to be responsible for the most biological activities of this plant[14]. Andrographolide is the major constituent extracted from the leaves of the plant which is a bicyclic diterpenoid lactone. Bitter diterpenoid lactones, especially 14-Deoxy-11-dehydroandrographolide, 14-Deoxy-11-oxoandrographolide, 5-Hydroxy-7, 8, 2', 3'-Tetramethoxy flavones, Andrographolide, Neoandrographolide, Paniculide-A, Paniculide-B, Paniculide-C, have been isolated from the whole plant and leaves. Andrographine, Panicoline, Diterpene dimers Flavonoids available in the roots. The main active constituents and marker compounds are considered to be the andrographolides and andrographis extracts are often standardized to these compounds[15].

Important actions according to Ayurveda of *Andrographis paniculata* Nees*. (*Kalmegh*)* balances both Kapha and Pitta Doshas; so it can be used in all health problems originating from aggravation of Pitta or Kapha or both**.** *Andrographis paniculata* Nees*.* ignites the digestive fire in stomach, stimulates perspiration and removes toxins from the body via sweat. *Andrographis paniculata* Nees*.* works well against parasites and microbes, stimulates expulsion of bile from liver. Andrographis paniculata Nees. is indicated in all disorders of liver. *Andrographis paniculata* Nees*.* is useful in skin diseases. The Tamils have been using Nilavempu - as it is called in Tamil - for centuries. In Siddha medicine, *Andrographis paniculata* Nees*.* is used widely to treat fevers like chikenguinea, swine-flu, typhoid etc[16].

## MATERIALS AND METHODS

### Extract material

The extract (Hydro: Alcoholic-60:40) of *Andrographis paniculata* Nees was procure from GMP certified firm. Sample was studied for physiochemical evaluation and identification.

### Methods for Physicochemical Parameter

### Moisture content

4 g of the sample was taken and heated in an oven at 105°C for 5 hour in a previously weighed 100 ml beaker. It was cooled in desiccators and weighed. The procedure was repeated till constant weight is obtained. The percentage of loss in weight of the sample was calculated.

Deterioration time of the plant material depends upon the amount of water present in plant material. If the water content is high, the plant can be easily deteriorated due to fungal attack. The loss on drying at 105°C of *Andrographis paniculata* extract was found to be 4.54% w/w.

**Determination of Total ash**

2 g of the sample was taken accurately in a previously ignited and tarred Silica dish. The material was spread evenly and ignited in a muffle furnace by gradually increasing the temperature to 600$^{o}$C until it is white, indicating the absence of carbon. The crucible was cooled in desiccators and allowed to stand for 30 minutes and weighed.

Total ash value of plant material indicated the amount of minerals and earthy materials attached to the plant material. Analytical results showed total ash value of *Andrographis paniculata* extract was 28.57 % w/w.

**Determination of Acid insoluble ash**

To the dish containing the total ash, 25 ml of 20 % Hydrochloric acid was added covered with a watch glass and boiled gently for 5 minutes. The watch was rinsed with a hot water and added to the crucible. The residue was washed with the hot water till the washings were neutral to the litmus. The insoluble material was collected and again placed in a same crucible and again ignited for 6 hr. to constant weight. The residue was cooled a desiccators for 30 minutes and weighed.

Percentage of acid insoluble as was calculated. The amount of acid-insoluble siliceous matter present in the *Andrographis paniculata* extract was 5.45% w/w.

**Determination of Water soluble extractive value**

4 g of the sample was taken in a glass stoppered flask.100 ml of distilled water was added. The flasks were shaken occasionally for 6 hours and then allowed to stand for 18 hours. The extract was filtered and 25 ml of the filtrate was pipette out in a pre-weighed 100 ml beaker and evaporated to dryness on a water bath. It was kept in a hot air oven for 5 hr at l05°C, cooled in desiccators for 30 minutes and weighed. The procedure was repeated till constant weight.

The water-soluble extractive value indicated the presence of sugar, acids and inorganic compounds. The water soluble extractive value in the *Andrographis paniculata* extract sample was found to be 88.27% w/w.

**Determination of Alcohol soluble extractive Value**

Same procedure as for the water soluble extractive value was followed. Instead of water, rectified spirit was taken as a solvent.

The alcohol soluble extractive values indicated the presence of polar constituents like phenols, alkaloids, steroids, glycosides, flavonoids and secondary metabolites present in the plant sample. The alcohol soluble extractive value was found to be 55.47% w/w in the *Andrographis paniculata* extract.

**Determination of pH Value**

10% aqueous solution of sample was prepared and used for determining the pH value by pH meter. The pH value of *Andrographis paniculata* extract was found to be 7.33.

HPTLC analysis was performed on HPTLC apparatus (CAMAG, Swetzerland) and Aluminium plate pre-coated with silica gel 60 $F_{254}$ of 0.2 mm thickness (Merck, Germany) was used for HPTLC analysis.

**Analytical Procedure**

2 g each of extract was soaked overnight in 20 ml of 90% ethanol. The solution was continuously stirred for 6 hrs and kept for next 18 hrs. Next day filtered the samples, dried and Made 10% solution. The solution was applied as 10 mm band together with a separate band of standard on Merck Aluminium plate pre-coated with silica gel 60 $F_{254}$ of 0.2 mm thickness. The plates were developed in Toluene: Ethylacetate: Acetic acid (4.0: 6.0: 0.5). The plate was dried and visualized under UV 254 & 366 nm. The plate was then dipped in Anisaldehyde-Sulphuric acid, heated at 105° C till the colour of the resolved bands appeared and visualized under white light. Marker compound andrographolide was used for the conformity of the samples used for the analysis.

**RESULTS AND DISCUSSION**

Hydro-alcoholic extract of the *Andrographis paniculata* Nees. was collected and analysed for the various standardisation parameters. Preliminary phytochemical results showed the presence or absence of certain phytochemical in the drug. Phytochemical test revealed the presence, Alkaloid, triterpene, saponins, flavonoids, polysaccharides, Steroid and Tannin results are given in Table1.

**Table 1: Qualitative analysis of the phytochemical in the extracts of *Andrographis paniculata Nees.***

| S. No. | Phytochemicals Constituents | Test performed | Result |
|---|---|---|---|
| 1. | Alkaloid | Dragendorff's test | +ve |
| 2. | Flavonoids | Shinoda test | +ve |
| 3. | Steroid | Liebermann-Burchard reagent | +ve |
| 4. | Tannin | Neutral $FeCl_3$ | +ve |
| 5. | Glycoside/Sugar | Molisch's test | +ve |
| 6. | Terpenoid | Noller's test | +ve |
| 7. | Saponin | NaOH solution | +ve |

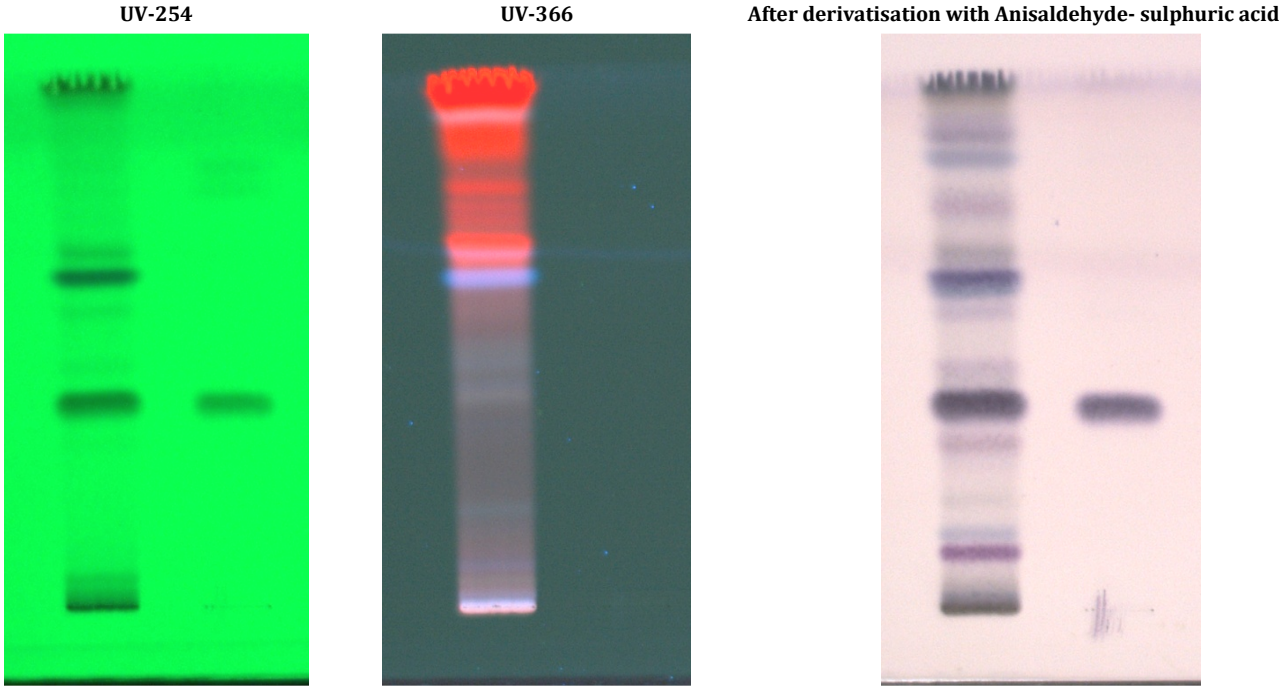
The presence of three heavy metals namely Arsenic, Cadmium, Lead and microbial contamination were analysed in the sample and the results are shown in Table 2. The concentration of all the heavy metals and microbial contamination were below the WHO/FDA permissible limits[17,18].

Physico-chemical parameters of the *Andrographis paniculata* Nees. (Hydro-alcoholic extract) are tabulated in Table 2. The pH value of 10% w/v aqueous solution is 7.33. Deterioration time of the plant material depends upon the amount of water present in plant material. If the water content is high, the plant can be easily deteriorated due to fungus. The loss on drying at 105°C was found to be 4.54 %. Total ash value of plant material indicated the amount of minerals and earthy materials attached to the plant material. Analytical results showed total ash content was 28.57 %. The amount of acid-insoluble /siliceous matter present in the plant was 5.45%. The water-soluble extractive value was found to be 88.27; it is indicating the presence of sugar, acids, inorganic compounds and other water soluble matter. The amount of alcohol soluble extractive values was 55.47%; it indicated the presence of polar constituents like phenols, alkaloids, steroids, glycosides, flavonoids. The results of other parameters like density, solvent residue, assay etc are given in Table 2.

Thin layer chromatographic technique was used to separate the chemical compounds present in the drug. Various solvent systems were checked to separate the maximum number of chemical compounds in the drug. HPTLC of the ethanolic solution developed in the mobile phase of Toluene: Ethyl acetate: Acetic Acid (4.0: 6.0: 0.5) (Figure. 1) and on observation under UV 254 nm 09 bands of sample at $R_f$ value 0.31, 0.38, 0.45, 0.55, 0.61, 0.66, 0.77, 0.82, 0.96 and 01 band of standard $R_f$ 0.38, (all bands are dark green colour) ; under UV 366 nm 09 bands of sample at $R_f$ 0.07, 0.18, 0.39, 0.46, 0.55, 0.60, 0.67, 0.73, 0.77 and no visible band of standard and after derivatisation with Anisaldehyde-sulphuric acid under white light 10 bands of sample at $R_f$ 0.11, 0.15, 0.32, 0.38, 0.44, 0.55, 0.66, 0.74, 0.83, 0.88 and one band of standard at $R_f$ 0.38 were observed.

**Table 2: Physico-chemical parameters of *Andrographis paniculata Nees.***

| S. No. | Parameters | Results |
|---|---|---|
| 1. | Description | Brown Colour Dry Powder |
| 2. | Extraction Medium | Ethanol : Water (40 : 60) |
| 3. | Loss on drying at 105 $^0$C | 4.54 % w/w |
| 4. | Total Ash | 28.57 % w/w |
| 5. | Acid-insoluble ash | 5.45 % w/w |
| 6. | Water-soluble extractive | 88.27 % w/w |
| 7. | Alcohol-soluble extractive | 55.47 % w/w |
| 8. | pH (10 % w/v aqueous suspension) | 7.33 |
| 9. | Passing through 40 mesh size sieve | 95.28 % w/w |
| 10. | Bulk density | 0.68gm/ml |
| 11. | Tap density | 1.04 gm/ml |
| 12. | Solvent residue (Ethanol) | <500 ppm |
| 13. | **Heavy metals** | |
| a) | Lead | < 5 ppm |
| b) | Cadmium | <1 ppm |
| c) | Arsenic | <2 ppm |
| 14. | **Microbial contamination** | |
| | Test for E.coli/g | Absent |
| | Test for Salemonella/g | Absent |
| | Test for S. aureus/g | Absent |
| | Total viable count/g | 300cfu/gm |
| | Total fungal count/g | <10 cfu/gm |
| | Total enterobacteriaceae/g | Absent |




**Solvent System: Toluene :Ethyl acetate :Acetic Acid (4.0:6.0:0.5)**

**Fig. 1: HPTLC Profile *Andrographis paniculata* Nees.**

## CONCLUSION

Preliminary phytochemical as well as various aspects of the *Andrographis paniculata* Nees. extract (Hydro: Alcoholic-60:40) was studied and described along with, physico-chemical, toxic/heavy metal, microbial contaminants and HPTLC studies to authenticate the adulteration for quality control of herbal drugs. HPTLC profile together with marker compound may economically verify the identity of this plant material in any formulation. *Andrographis paniculata* Nees. extract exhibits a set of diagnostic characters, which will help to identify the drug in dried condition and in other formulations.

It has been concluded from this study that estimation of heavy metals and microbial contamination is highly essential for raw drugs or plant parts used for the preparation of compound formulation drugs. The periodic assessment is essential for quality assurance and safer use of herbal drugs.

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