Comparative phytochemical evaluation of crude and ethanolic extract *Andrographis paniculata* (Kalmegh)

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Abstract

The aim of this study was to evaluate the phytochemical analysis of *Andrographis paniculata*. It is a traditional medicinal plant and it is commonly known as kalmegh. *A. paniculata* have number of pharmacological properties like, anticancer, antiheptotoxicity, Anti-diabetic & anti-inflammation. The crude and ethanolic extract of the plant was analyzed. The phytochemical screening of both the extracts were positive for alkaloids, phenols, tannins, flavonoids, terpenoids and saponins. The atomic absorption spectrophotometry (AAS) was applied for estimation of organic elements namely Ca, Mg, Fe, Cu and Zn. HPLC analysis shows the presence of possible main compounds in both the prepared extract.

Keywords: Phytochemical, *Andrographis paniculata*, crude, ethanolic, AAS, HPLC

Introduction

*Andrographis paniculata* is an erect annual herb extremely bitter in taste [1]. The plant is native to tropical and sub-tropical regions of India, belongs to Acanthaceae family and has been used for centuries for the treatment of fever and many infectious diseases. *Andrographis paniculata* plays a significant role in ethnomedicine and has also been reported to have anti-angiogenetic [2], antibacterial [3], anti-cancer [4], anti-inflammatory [5, 6], antimalarial [7], antioxidant [8] and hepatoprotective activities [9].

The main chemical constituents are Andrographolide and neoandrographolide that are responsible for the therapeutics of the plant. Andrographolide, in particular, has shown cytotoxic and cytoplastic activity against cancer cells and hepatoprotective activity [10]. Various medicinal properties like anti diarrhoeal, immunostimulant have been attributed to this plant in traditional system of medicine [11, 12]. According to ancient Indian literatures, it “cools” and relieves internal heat, inflammation and pain and it is also used for detoxification 12,13. Because of its mechanism of action by enzyme activation, it has great therapeutic value and is commonly used to treat fevers and to remove toxins from the body.

Material and Methods

Plant material

*Andrographis paniculata* was collected and authenticated by Medicinal Plants Research and Development Center (MRDC) of the G.B.P.U.A.T., Pantnagar. Leaves were washed 2-3 times with distilled water and dried in shade, grinded into fine powder, and stored in close container for extraction.

Extraction method

The coarse dried powder of leaves (200g) was subjected to extraction with 2000 ml ethanol for 48 hours. The ethanol extract was collected, filtered and concentrated in vacuum under reduced pressure and dried in desiccator and stored for further analysis. The concentrated methanol extract was further subjected to phytochemical screening [14, 15].

Phytochemical testing

The obtained extracts were subjected to phytochemical testing according to standard test [16, 17].
| S. No. | Phytochemical | Procedure | Interpretation |
|-------|--------------|-----------|----------------|
| 1.    | Alkaloids    | Extract + 1 ml dilute HCl and filter | Dragendorff’s reagent | Appearance of precipitate |
| 2.    | Saponins    | 2 gm of sample + 20 ml distilled water (boil in water bath) and filtered 10 ml filtrate+ 5ml distilled water | Shake vigorously | Forming of emulsion |
| 3.    | Glycosides | Small quantity of the extract | Fehling’s test | Yellow or red color precipitate |
| 4.    | Proteins | 3 ml extract + 1 ml HNO3 (Conc.) solution is heated and cooled under tap water | Addition of 40% NaOH (to make it alkaline) | Orange ppt. |
| 5.    | Flavanoids | Extract + H₂SO₄ | Fehling’s test | Yellowish orange |
| 6.    | Terpenoids | 5 g extract + 2 ml of chloroform + concentrated H₂SO₄ (3ml), form a layer | | reddish brown Colouration of the inter face |
| 7.    | Tannins | 0.5 g of the dried powdered sample boiled in 20 ml of water in a test tube and then filtered | | few drops of 0.1% ferric chloride brownish green or a blue-black Colouration |

**HPLC analysis**

Liquid chromatography-mass spectrometry (LC-MS or HPLC-MS) is an analytical technique that combines liquid chromatography (or HPLC) physical separation capabilities with the mass analysis capabilities of mass spectrometry. MSLC-MS is a powerful technique which, in many applications, has very high sensitivity and selectivity and is therefore useful. It offers compound separation (Retention Time) and compound detection (as different adduct formation due to ESI source) by MS (providing compound/analyte molecular weight).

**Metal estimation**

**Sample preparation**

**Overnight digestion with conc. HNO₃**

Ten milliliters of concentrated HNO₃ (ultrapure 65%) was added to 1.000 g each of crude and ethanolic extract of sample and allowed to stand overnight at room temperature. The samples were then heated for 4 h at 120°C, after which the temperature was increased to 140°C. Digestion at this temperature continued until only 1ml of acid remained. The suspension was filtered into a 50ml volumetric flask after cooling and diluted to the mark. Thus, he samples each of crude and ethanolic extract of the plant *Andrographis paniculate* were prepared.²⁸,²⁹,³⁰

**Standard solution preparation**

Stock standard solutions of Ca, Mg, Fe, Zn, and Cu containing 1000ppm of each metal were prepared by dissolving weighted quantities of appropriate dried analytical grade salts in distilled water. Calibration standards of 1ppm, 2ppm, 5ppm and 10 ppm of each element were obtained by appropriate dilution of the stock solutions.

**Atomic absorption spectrometry**

In both the samples i.e., crude and ethanolic extract of *Andrographis paniculate*, Ca, Mg, Fe, Cu and Zn contents were measured using flame atomic absorption spectrometry. The elements were measured under the optimum operating conditions with an air-acetylene flame.

**Results and Discussion**

The phytochemical analysis of the crude and ethanolic extract revealed the presence of alkaloids, phenols, tannins, flavonoids and saponins. (Table 1).

| S. No. | Constituents | *A. paniculate* | *EE of A. paniculate* |
|-------|--------------|-----------------|----------------------|
| 1.    | Alkaloids    | +               | +                    |
| 2.    | Sterols      | -               | -                    |
| 3.    | Glycosides   | -               | -                    |
| 4.    | Phenols      | +               | +                    |
| 5.    | Proteins     | -               | -                    |
| 6.    | Tannins      | +               | +                    |
| 7.    | Flavonoids   | +               | +                    |
| 8.    | Reducing sugars | -               | -                    |
| 9.    | Saponins     | +               | +                    |

The atomic absorption spectrophotometric was used to estimate the different elements in both the samples. The results are presented in table 2. The crude extract had higher amount of Calcium (2410 ppm), Magnesium (1717 ppm), Copper (18.4 ppm) and Iron (7.35 ppm) as compared to ethanolic extract which had higher amount of Zinc (8.2ppm).

| Element (ppm) | Crude *A. paniculate* | Ethanol Extract of *A. paniculate* |
|--------------|-----------------------|-----------------------------------|
| Calcium      | 2410                  | 2184                              |
| Magnesium    | 1717                  | 1500                              |
| Iron         | 7.35                  | 5.62                              |
| Zinc         | 1.4                   | 8.2                               |
| Copper       | 18.4                  | 16.4                              |
The HPLC of crude and ethanolic extract revealed presence of active constituents. The results of HPLC of both i.e., the crude powder and the ethanolic extract are presented in table 3 and fig 3(a) and 3(b).

Table 3: HPLC of dried powder and ethanolic extract of *A. paniculata*

| S. No. | Parameters                                      | RESULTS |          |          |
|--------|------------------------------------------------|---------|----------|----------|
|        |                                                 | Dried Powder (μg/mg) | Liquid extract (mg/ml) |
| 1.     | Andrographolide                                 | 12.70   | 1.53     |
| 2.     | Neoandrographolide                              | 2.58    | 0.25     |
| 3.     | 14 deoxy-11,12 didehydroandrographolide         | 3.42    | 0.26     |
| 4.     | Andrograpanin                                  | 0.19    | 0.02     |

Fig 3(a): Chromatography of crude powder of *A. paniculata*

Fig 3(b): Chromatography of ethanolic extract of *A. paniculata*
Conclusion
The data obtained in the current study would be useful in the development of new modern drugs with different plant combinations which can be used potentially in the cure of many ethno-medical diseases. Therefore, the plant can be used in the treatment of various diseases. However, more detailed analysis of chemical composition of these medicinal plants is required to be done.

Andrographis paniculata is potential sources of nutrients and some essential macro, micronutrients. These can be incorporated in other foods as nutraceuticals for effective and proper metabolism as well as for the maintenance of good physiological state in man and animals.

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