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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 cytostatic activity against cancer cells and hepatoprotective activity^[10]. Various Medicinal properties like antidiarrhoeal, 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 dessicator 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	Dragandroff'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 Shaken vigorously	Frothing will be mixed with 3 drops of olive oil and shaken vigorously	formation of emulsion
3.	Glycosides	Small quantity of the extract	Fehling's test	yellow or red color precipitate
4.	Proteins	Small quantity of the extract + 5 ml of water 3 ml extract + 1 ml HNO ₃ (Conc.) solution is heated and cooled under tap water	Addition of 40% NaOH (to make it alkaline)	Orange ppt.
5.	Flavanoids	Extract + H ₂ SO ₄		yellowish orange
6.	Terpenoids	5 g extract + 2 ml of chloroform + concentrated H ₂ SO ₄ (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, the samples each of crude and ethanolic extract of the plant *Andrographis paniculata* were prepared [18, 19, 20].

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 paniculata*, 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).

Table 1: Phytochemical analysis

S. No.	Constituents	<i>A. paniculate</i>	<i>EE of A. paniculate</i>
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).

Table 2: Concentration of different elements in crude powder of *A. paniculate* and ethanolic extract of *A. paniculate*

Element (ppm)	Crude <i>A. paniculate</i>	Ethanolic Extract of <i>A. paniculate</i>
Calcium	2410	2184
Magnesium	1717	1500
Iron	7.35	5.62
Zinc	1.4	8.2
Copper	18.4	16.4

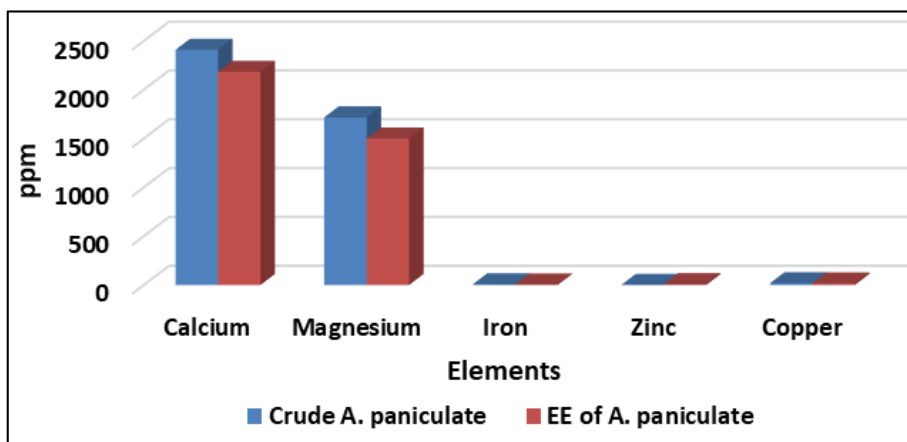


Fig 1: Amount of minerals in crude and ethanolic extract of *A. paniculate*

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

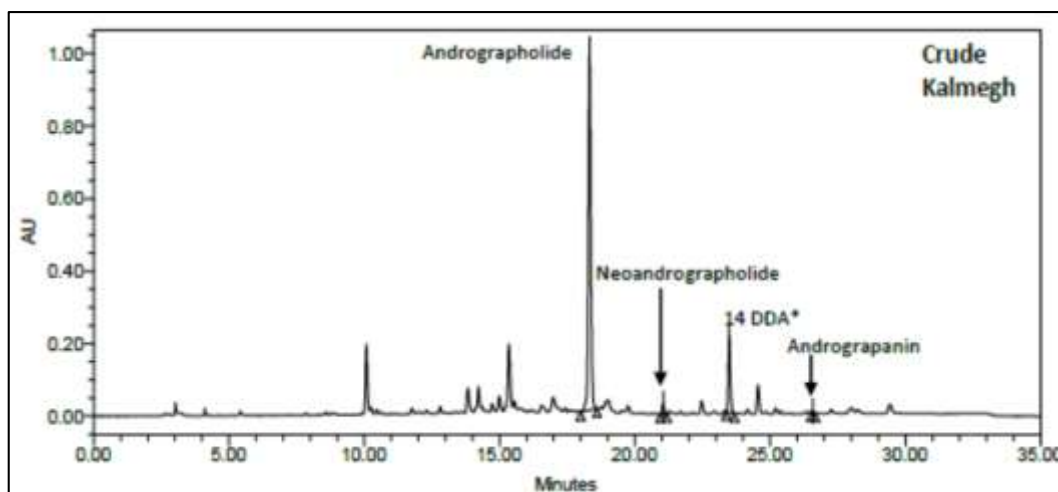


Fig 3(a): Chromatography of crude powder of *A. paniculata*

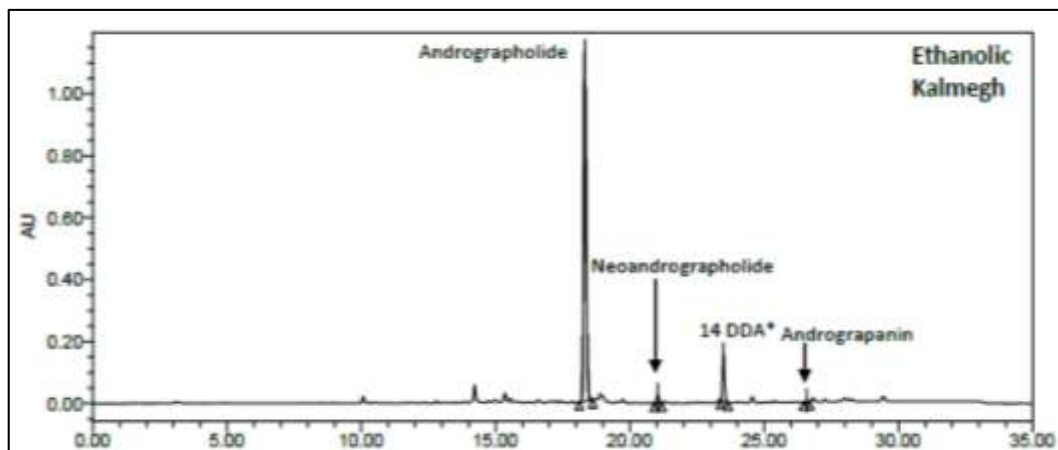


Fig 3(b): chromatography of ethanolic extract of *A. paniculata*

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-medicinal 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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