



A phytochemical investigation on *Andrographis paniculata*

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ABSTRACT

Andrographis paniculata is a traditional medicinal plant used in Madhya Pradesh, It is commonly known as kalmegh. This plant is a richest source of bioactive constituents used in India to treat diseases, so far. Phytochemical study was made on the different plant extract. The leaves extract were prepared with selected solvents. The phytochemical screening shows the presence of alkaloids, steroids, flavonoids, tannins, triterpenoids, quinones, protein, sugars, gum. FTIR analysis shows the presence of possible compounds in the prepared extract.

Keywords: *Andrographis paniculata*, kalmegh, phytocontents, HPLC, FTIR.

INTRODUCTION

Medicinal plants have been used by human beings since time immemorial for curing health. Bioactive constituents have been reported from plant extract, this phytoextract can protect human against diseases. *Andrographis paniculata* belongs to family(Acanthaceae), native to India, is a medicinal herb with bitter taste. There are some phytochemical present in fruits and herbs which works differently[1]. *A. paniculata* have number of pharmacological properties like, anticancer, antiheptotoxicity, Anti-diabetic & anti –inflammation[2]. This plant is found in Taiwan, China having bitter taste used to treat liver disorders, bowel complaints of children, common cold and respiratory infection. Various Medicinal properties like antiarrhoeal, immunostimulant have been attributed to this plant in traditional system of medicine [3,4] . According to Indian ayurveda, *A.paniculata* “cools” and relieves internal heat, inflammation and pain and it is used for dextotification [4,5].

EXPERIMENTAL SECTION

1. Plant material:- *Andrographis paniculata* leaves were collected in month of January from residential garden and authenticated by botanist Dr. D.K Shrivastav (Department of Tissue culture, Govt. Agriculture College, Indore). Leaves were washed 2-3 times with distilled water and dried in shade, grinded into fine powder, & allow it to stored in close container for extraction.

2. Extraction method:- The coarse dried powder of leaves (200g) was subjected to extraction with 2000 ml methanol for 48 hours. The methanol 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 [6,7].



Figure1: *Andrographis paniculata* plant

3. Phytochemical testing:- The obtained extracts were subjected to phytochemical testing according to standard test [8,9].

Test for alkaloids:- Test substance shaken with few drops of 2N HCL. Aqueous layer formed, decanted and to which one or two drops of Mayer's reagent added. Formation of white precipitate indicates the presence of alkaloids.

Test for triterpenoids:- Noller's test- The substance was warmed with Tin and Thionyl chloride. Purple coloration indicates the presence of Triterpenoids.

Test for steroids:- One gram of substance was dissolved in a few drops of acetic acid, acetic anhydride, warmed and cooled under tap water and drop of sulphuric acid were added along the sides of the test tube. Presence of green color shows the positive test for steroids.

Test for flavonoids:-Shinoda's test:- To the substance in alcohol, a few magnesium turnings and few drops of concentrated HCL were added and boiled for five minutes. Red coloration shows the presence of flavonoids.

Test for tannins:- The substance mixed with basic lead acetate solution. Formation of white precipitate indicates the presence of Tannins.

Test for quinones:- To the test substance, sodium hydroxide was added. Blue green or red color indicates the presence of Quinone.

Test for protein:- To the test solution the Biuret Reagent is added. The blue reagent turns violet in the presence of protein.

Test for sugars:- The substance was mixed with equal volume of Fehling's A and B solutions, heated on water bath. Formation of red color is the indication of the presence of sugar.

Test for gum:- To the substance, add few ml of water and shake well. Formation of swells and adhesives indicates the presence of gum.

Table 2: The analysis of phytochemicals in the different plant extract of *A. paniculata*

Phytochemical	Petroleum ether	Methanol	Water
Alkaloids	-	-	+
Triterpenoids	+	-	-
Steroids	+	+	-
Flavonoids	-	+	-
Tannins	+	-	-
Quinones	-	+	-
Protein	+	+	-
Sugars	+	-	-
Gum	-	-	-

+ = Presence, - = Absence

4. Isolation of andrographenolide from crude drug :- 50 g of green plant extract is introduced in soxhlet extractor for extraction of main bioactive content that is andrographolide, concentrate the obtained extract and dissolve in ethanol pure crystals of andrographolide (2g) is obtained.

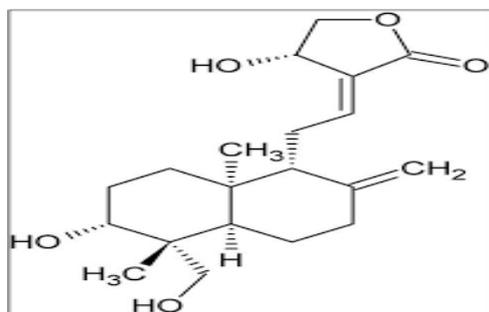
Figure 3: Crude drug of *Andrographis paniculata*

Fig 4: Molecular structure of Andrographolide (Biomarker compound)

5. HPLC analysis:-

HPLC analysis and related chromatogram of pure andrographinolide and the same compound isolated from the *Andrographis paniculata* plant is illustrated below to compare the purity of isolation which confirms the identity of Andrographolide. Their results are listed as below for their comparisons.

Mode: LC

Detector: UV, 223nm

Column: 4.6mmx 25-cm ; 5µm packing L1

Flow rate : 1.5 ml/min

Injection size : 20 µL

Sample : Standard solution A and Standard solution B.

Figure 5: Chromatogram (Standard)

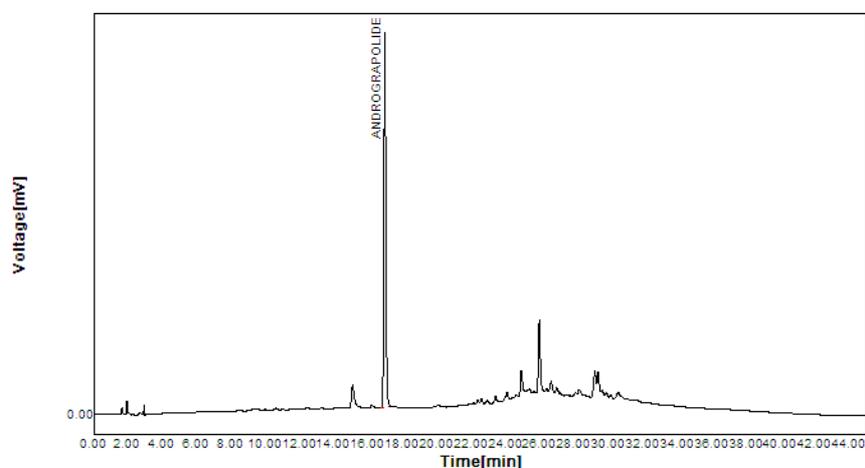


Table 6

S.no.	Name	RT[Min]	Area[Mv*s]	Height[MV]	Amt
1	Andrograp-holide	16.9500	1402.4926	177.2338	1.0000
Sum			1402.4926	177.2338	1.0000

Figure 7: Chromatogram (pure)

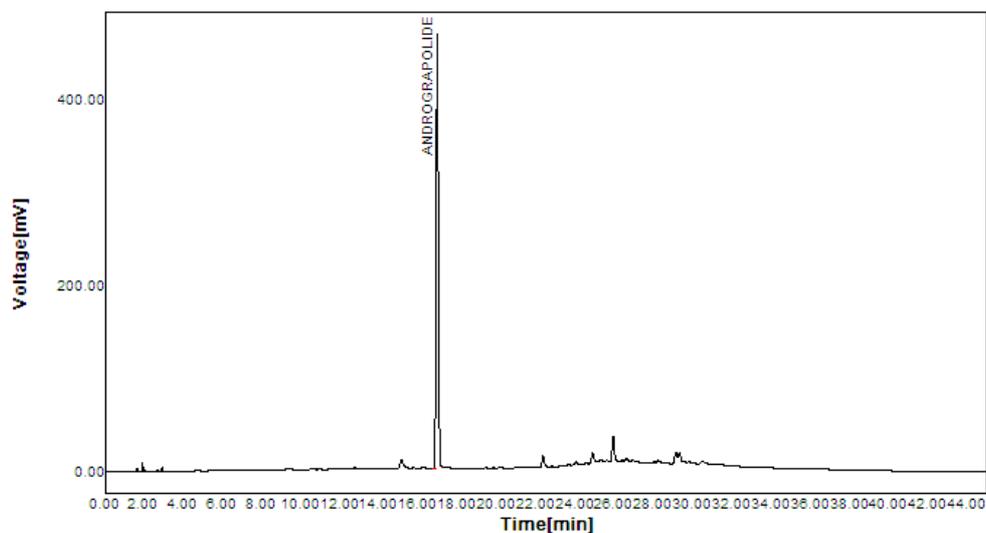


Table 8

S.no.	Name	RT[min]	Area[Mv*s]	Height[mV]	Amt[mg/ml]
	Andrographolide	16.9333	3691.0457	465.4735	2.9185
Sum			3691.0457	465.4735	2.9185

FTIR analysis of Andrographolide:-

FTIR of isolated Andrographolide is measured by model Vertex 70 Bruker, at the range 4000-400 cm^{-1} the various peaks in the spectra shows the presence of functional group in the isolated compound.

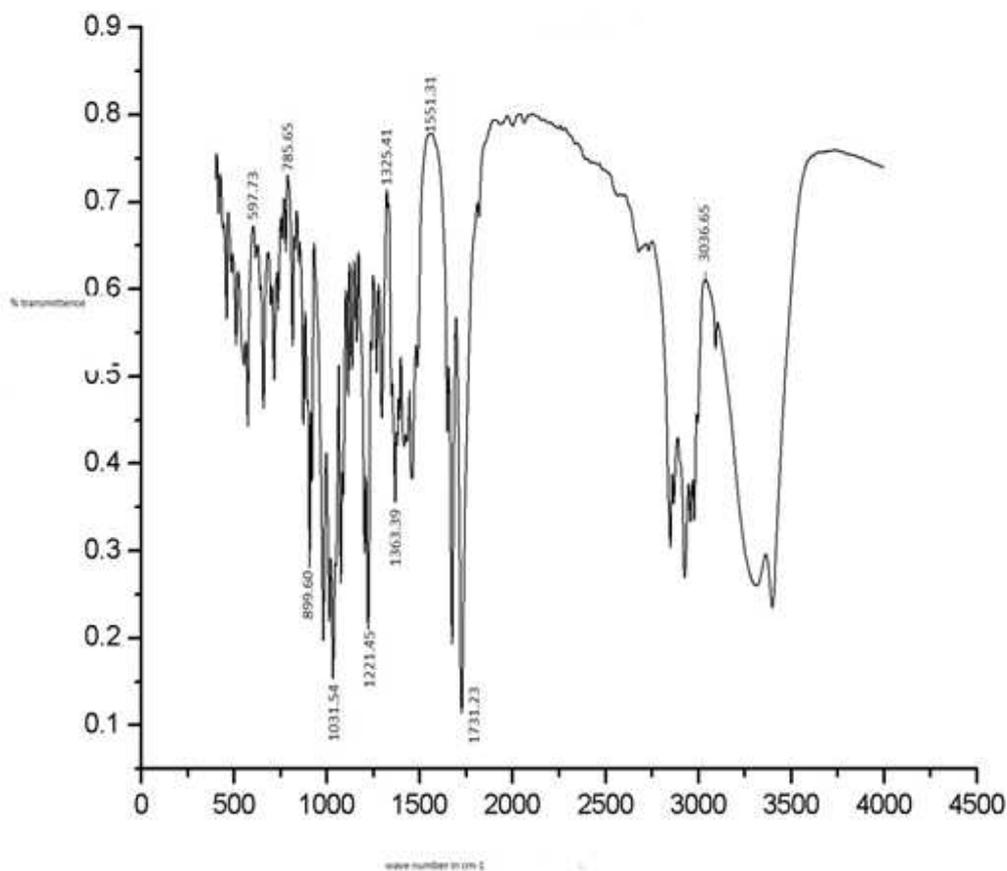


Figure 9: FTIR Spectra for isolated compound

Table 10. FTIR Results of *Andrographis paniculata*

Wave number (cm ⁻¹)	Assignments
785.65	Alkynes
899.60	Aromatics
1031.54	Alkenes
1221.45	Alcohols
1363.39	Alkanes
1731.23	Carbonyl group
3036.65	Alkanes

RESULTS

In the present investigation, preliminary phytochemical testing has been done in the different extract of *Andrographis paniculata* leaves. The extraction is done with methanol as a solvent using Soxhlet apparatus. It shows the presence and absence of some phytochemicals in the extract. Finally the compound are characterized using FTIR analysis.

DISCUSSION

Studies on medicinal plants are important to find and development of new drugs. Herbal extracts contain different bioactive contents having pharmacological properties. In the present work, it was observed that the plant *A. paniculata* are validated in their uses by the various parts of India.

The medicinal value depends on the presence of chemical substance and their role in human body. Phytochemical screening of different extract of plant shows wide range of activity against diseases. For example, Alkaloids protect

against chronic diseases [10]. The herb can be used to cure diseases and isolation and identification of active components which can lead to invention of new drug at less economic cost to patients.

CONCLUSION

The plant *A.paniculata* under study can be used as potent drugs exploiting the anticancer, anti-infection, anti-diabetes activities of the plant. This study is to aware peoples towards the application of drugs at affordable cost. Further research is needed to isolate, identify, and characterize the structure of bioactive constituents.

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