INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

STUDY OF ANDROGRAPHOLIDE CONTENT IN *ANDROGRAPHIS PANICULATA* FROM DIFFERENT FOREST TYPES OF MADHYA PRADESH, INDIA

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Article Received on: 09/11/18 Approved for publication: 15/01/19

DOI: 10.7897/2230-8407.100385

ABSTRACT

This study aimed to develop and validate the content of andrographolide content in *Andrographis paniculata* from different forest types of Madhya Pradesh India. Total six samples of *Andrographis paniculata* were collected from three forest types of Madhya Pradesh. Sample were analysed through HPLC chromatographic method. HPLC instrument that was used for the estimation of andrographolide were of the following features, HPLC- grade waters, Pump - 515 Isocratic pump, Injector - Rheodyne injector with a 20-microlitre loop, Detector - UV Vis detector, Software - Data ace software, Column - Thermo C-18 column (4.6 x 250mm, 5µ particle size), sample size (20µl). Highest percentage of Andrographolide in this plant were from Bamhni forests accounts to 0.658%, followed by Barhi 0.459%, Amarvada 0.124%, Panpatha 0.101%, Amarpur 0.071%, and Kurrai 0.061%. There was considerable variation in the percentage of andrographolide from one site to another site of different forest types of Madhya Pradesh. Considerable variation in the content of andrographolide was studied from one forest to another forest.

Keywords: *Andrographis paniculata*, Andrographolide, Forest types, HPLC-Method.

INTRODUCTION

India having wide range biodiversity of medicinal plants found in uncultivated and forest areas. In this present world the demand of medicinal plants its organization and globalization increased many folds. *Andrographis paniculata* an important medicinal plant belongs to family Acanthaceae. The leaves, roots even whole plant of *Andrographis paniculata* are to be used since centuries in European and Asian countries to cure many health problems. The plants are used to cure leprosy, liver diseases, torpid liver, diabetes, headache, inflammation, carbuncle, mumps, pneumonia, gonorrhea, scabies, boils, skin eruptions, and chronic and seasonal fever for its high blood purifying properties [1,3,5].

Species of this genus varied in different reports, which comprises either nineteen [2,10] twenty-eight [4,8] forty [3] and forty-four [9] species. The genotypic differences were found out high yielding germplasms. It grows in hill slopes, waste ground, farms, moist habitat, seashores, and roadsides and wastelands are preferable for their well development [7, 8]. This plant grows richly in India, Sri Lanka, Pakistan and Indonesia, while it is cultivated in India, China, Thailand, Barbados, Bahamas, Hong Kong, and the tropical areas in America and also in southwestern Nigeria [6, 8].

The medicinal value of this plant is due to the presence of active ingredients *viz* andrographolide and neoandrographolide which are derivatives of diterpenoids. The content of these active ingredients in plant varies with in plant parts and with the geographical distribution [6]. It is used both in all system of medicines for possess immunological, antibacterial, anti-inflammatory, antithrombotic and hepato-protective properties. Andrographolide is an interesting pharmacophore with anticancer and immuno modulatory activities and hence has the potential to be developed as an anticancer chemotherapeutic agent as well [10].

MATERIALS AND METHODS

Plant material

Andrographis paniculata was collected from different forest types of Madhya Pradesh. Samples were collected for comparative study of andrographolide content. Plant material at different sites were collected washed through tap water and dried in shade for 15 days followed by grinding to form powder of it. The fine powdered plant samples were used in methanol solvent to estimate andrographolide content by HPLC method.

HPLC Instrument

HPLC instrument that was used for the estimation of andrographolide were of the following features, HPLC- grade waters, Pump - 515 Isocratic pump, Injector - Rheodyne injector with a 20-microlitre loop, Detector - UV Vis detector, Software - Data ace software, Column - Thermo C-18 column (4.6 x 250mm, 5µ particle size), sample size (20µl). Isocratic elution was carried out with methanol at a flow rate (1ml/min). The detection was performed with wavelength (230 nm) and column temperature was ambient (30^0C). Class VP software was used for integration and calibration. Evaluation was via peak areas with linear regression.

Preparation of herbal extract

Fresh aerial part of the plant sample collected from different forest types of Madhya Pradesh. Reflux 1g dried powder along

with 50 ml of methanol was kept in soxhlet for one hour. After one hour the refluxing load was subjected to Rota-vapor at 60 RPM and heated at 60^0C. Filter & subject the marc for another two cycles of refluxes (1h. each) with methanol (50 ml) combine with the filtrate. Evaporate under vacuum to dryness Dissolve the residue 10 mg in methanol (10ml). Filter, Inject the solution in HPLC with the help of 20 µl fixed loop injector and percent content of andrographolide were estimated by counting the area of andrographolide peak in HPLC chromatogram in all samples.

RESULTS

Table 1: Collection of *Andrographis paniculata* from different forest types of Madhya Pradesh

Forest types	District	Forest range	Sample code	Percent of andrographolide
5A/C3 Southern Dry Mixed Deciduous Forest	Dindori	Amarpur	SAP-28	0.071
	East Mandla	Bamhni	SAP-3	**0.658**
5B/C1c Dry Peninsular Sal Forest	Katni	Barhi	SAP-5	0.459
	Umaria	Panpatha	SAP-6	0.101
5A/C1b Dry Teak Forest	Seoni	Kurrai	SAP-26	0.061
	East Chhindwara	Amarvada	SAP-25	0.124

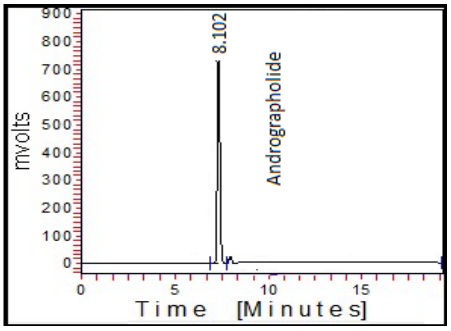
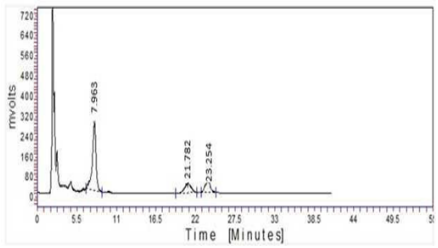
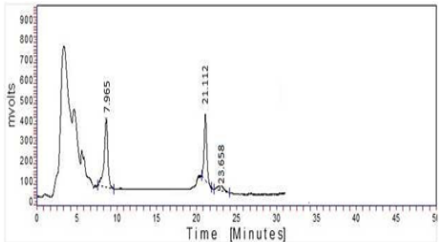


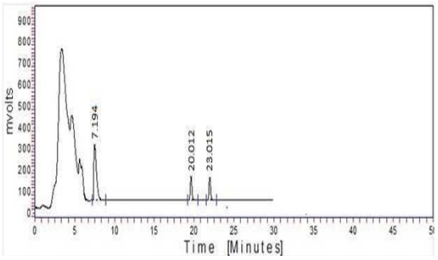
Figure 1: Standard of andrographolide prepared by HPLC method



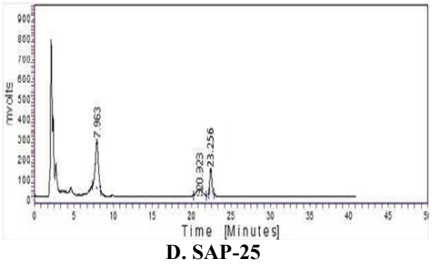
A: SAP-3



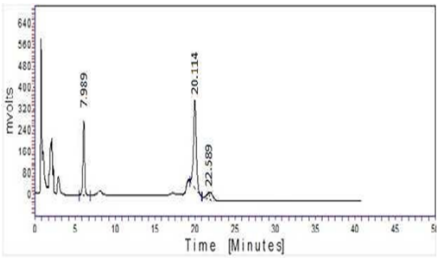
B: SAP-5



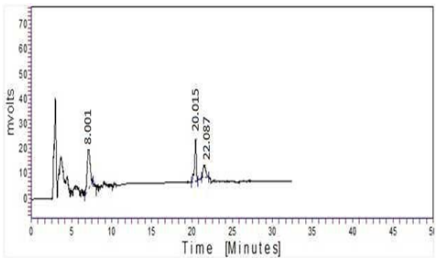
C: SAP-6



D. SAP-25



E: SAP-26



F: SAP-28

Figure 2 (A, B, C, D, E and F): Andrographolide peaks of six different samples prepared by HPLC method

Table 2: Retention time, peak area of above figures from HPLC methods

Content of *Andrographis paniculata*	Sample code	Retention time	Peak area
Andrographolide	SAP-3	7.963	3309.81
	SAP-5	7.965	2309.31
	SAP-6	7.194	509.81
	SAP-25	7.963	624.16
	SAP-26	7.989	309.12
	SAP-28	8.001	359.14

DISCUSSION

The data pertaining to the percent concentration of andrographolide in plant samples of different forest types of Madhya Pradesh were presented in Table 1. The data showed significant variation in the concentration of andrographolide. Six different samples were analysed from various forest types of Madhya Pradesh. Samples were analysed through HPLC chromatographic method. Highest percentage of Andrographolide in this plant were from Bamhni forest type that accounts to 0.658%, followed by Barhi 0.459%, Amarvada 0.124%, Panpatha 0.101%, Amarpur 0.071%, and Kurrai 0.061%. There was significant variation in the percentage of andrographolide from one site to another site of different forest types of Madhya Pradesh.

CONCLUSION

The developed HPLC method used for the determination of andrographolide content in *Andrographis paniculata* herb samples. The method developed is stastically validated, simple and sensitive. Extensive work has been done on this plant but variation of andrographolide content from this plant in different forest areas of Madhyapradesh was not reported.

AKNOWLEDGMENT

Authors are grateful to Dr. Dharmendra Kumar Verma, Director SFRI, Jabalpur and Prof. Anjna Sharma, Head, Department of Bioscience, R. D. University, Jabalpur for their kind guidance, supervision and encouragement.

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Cite this article as:

Anil Kushram and Sajad Ahmad Mir. Study of andrographolide content in *Andrographis paniculata* from different forest types of Madhya Pradesh, India. Int. Res. J. Pharm. 2019;10(3):88-90 http://dx.doi.org/10.7897/2230-8407.100385

Source of support: Nil, Conflict of interest: None Declared