Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(3): 168-171
Received: 24-03-2018
Accepted: 25-04-2018

Seema Sharma
Department of Forest Products, University of Horticulture and Forestry, Solan, Himachal Pradesh India

Yash Pal Sharma
Department of Forest Products, University of Horticulture and Forestry, Solan, Himachal Pradesh India

Chitra Bhardwaj
Department of Forest Products, University of Horticulture and Forestry, Solan, Himachal Pradesh India

Correspondence
Seema Sharma
Department of forest products, university of horticulture and forestry, Solan, Himachal Pradesh India

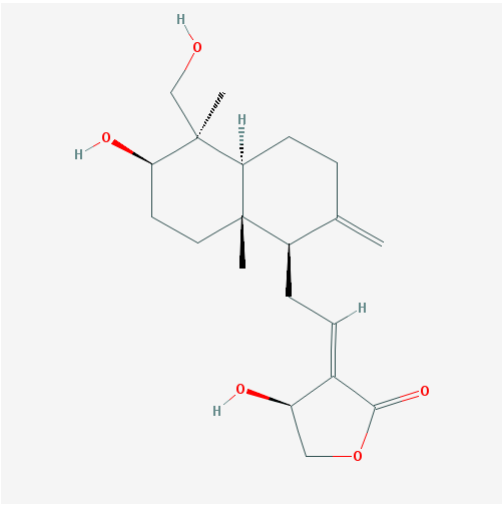
HPLC quantification of andrographolide in different parts of *Andrographis paniculata* (Burm.f.) Wall. *ex* Nees

Seema Sharma, Yash Pal Sharma and Chitra Bhardwaj

Abstract
Andrographis paniculata, a member of family Acanthaceae commonly found in plains of India, Pakistan and Sri Lanka and is an important medicinal plant, which is used in diseases like fevers, jaundice, colic dysentery and dyspepsia and also act as hepatoprotective, bitter tonic, stomachic, immunostimulant, antidote, antimalarial agent etc. The medicinal value of the plant is mainly due to active components as andrographolide, deoxy-andrographolide, neoandrographolide *etc.* present in the species. In the present study andrographolide, a major diterpenoid in *Andrographis paniculata* was quantified in different parts of plant using HPLC. The leaves were found to contain the highest content of andrographolide. The content ranged from 0.054 – 4.686%. The other parts studied were stem, flowering tops and roots. The andrographolide was detected in all four parts of the plant.

Keywords: Andrographolide, *Andrographis paniculata*, quantification, HPLC

1. Introduction
Andrographis paniculata (Burm.f.) Wall. *ex* Nees, member of family Acanthaceae, commonly known as kalmegh, also known in different languages as 'kirayat' in Hindi, 'kalamegha' in Sanskrit, 'King of Bitters' and 'Indian Echinacea' in English (Kumar *et al.,* 2012) [1]. It is an annual herb, native of South India and Srilanka (Raina *et al.*, 2013) [9] and grows in abundance in Asian countries like India, Pakistan, Java, Malaysia and Indonesia. In India, found wild through the plains of India mainly in states of Madhya Pradesh, Chhattisgarh, Orissa, Maharashtra, Assam, West Bengal, Uttar Pradesh, Uttrakhand, Tamil Nadu, Karnataka and Kerala (Prajapati *et al*., 2003) [7].
Plant is erect, up to 30 cm – 100 cm high, stem acutely quadrangular and profusely branched. Leaves are simple, opposite, lanceolate with acute apex and short petiole. Inflorescence is terminal, axillary and panicle. Flower violet to white in colour and fruit capsule with numerous seeds (Prajapati *et al*., 2003; Sareer *et al*., 2014) [7, 11]. Plant is reported by different researchers be used in the treatment of cold, cough, fevers and jaundice (Hemdari & Rao, 1984), cholera (Tripathi & Tripathi, 1991) [12], used as immunostimulant (Puri *et al*., 1993) [8], as antidote for bite of cobra (Salleh, 1991) [10], diabetes (Salleh, I991) [10], dysentry (Basak *et al*., 1999) [2], diarrhoea (Huang, 1993) [5], dyspepsia (Basak *et al*., 1999) [2] and high blood pressure.



Chemical structure of Andrographolide (source: Pub chem. CID 5318517)

In the present study andrographolide, a major diterpenoid in *Andrographis paniculata* was quantified in different parts (leaves, flowering tops, stems and roots) of plant using HPLC.

2. Experimental

2.1 Extraction, Isolation and Purification of Andrographolide

Field grown plants of *Andrographis paniculata* were harvested during flowering stage, dried under shade for 7-8 days and then coarsely powdered and further dried in the oven for 24 hours at 35-40°C. The prepared plant material was used for extraction of andrographolide. The air dried powdered material (450 gm) was then extracted with methanol in a soxhlet apparatus for 12 hours on a boiling water bath. After extraction, solvent was distilled off from the extract and sticky greenish mass was dried under vaccum.

The dried extract was then thoroughly decanted with petroleum ether (60-80˚C) for 2-3 times. Decantation discarded and residue was refluxed with hexane 2-3 times, each for half an hour. The filtrate again discarded and residue dried. The residue pre-extracted with hexane was then dissolved in methanol and filtered. The methanol from the filtrate was distilled off for removal of methanol and the residue obtained was dried under vacuum. This dried residue was then repeatedly decanted with benzene for the removal of coloured substances and thereafter the residue was dissolved in ethyl alcohol: water mixture (1:1). The ethyl alcohol was the evaporated by heating and the aqueous part containing bitter compounds was repeatedly partitioned with ethyl acetate, each time ethyl acetate fraction was collected and aqueous layer was discarded. From ethyl acetate fraction, solvent was completely removed by distillation resulting in creamish to light green coloured mass, andrographolide was further purified by crystallization in chloroform and adding methanol into it dropwise. The dissolved mixture was allowed to stand overnight and white crystals of andrographolide were obtained. Repeated crystallization was done to obtain purified andrographolide. Purity of the obtained crystals was ascertained by TLC and HPLC.

2.2 Plant material

Plants of *Andrographis paniculata* were obtained from experimental farm (30˚51'35.85" North and 77˚10'22.66" East) during the month of September - October. Different parts of plants *i.e.* flowering tops, roots, leaves, stems were separated and dried well in open and dry place.

2.3 Preparation of material for extraction

Well dried different parts were grinded mechanically and sieved by mesh size 600 microns sieve to form the uniform particle size of the plant material.

2.4 Determination of andrographolide in plant material

2 gram samples of flowering tops, roots, leaves and stem were extracted by using methanol solvent, sonication assisted method and 32 minutes extraction duration. After extraction, solvent from each sample was distilled off and the residue was completely dried. The residue was dried to a constant weight. The extracted material was then subjected to HPLC analysis for quantification of andrographolide.

The dilution of the samples was done using mobile phase (Methanol: Water: 65: 35, v/v), centrifuged at 3500 rpm and then filtrated through 0.2µm membrane prior to injection in the HPLC system.

2.5 HPLC Instrumentation and conditions

2.5.1 Instrumentation

The HPLC method development and validation was done by using Waters binary HPLC unit with Waters HPLC pump 515 and dual λ absorbance detector 2487. Empower II software and Sunfire C-18 (4.6 x 250mm, 0.005mm) column.

2.5.2 Chromatographic conditions

UV detection was employed at 223 nm, isocratic elution was used at a flow rate of 1ml/min. and injection volume was set to 20 µl. The total run time of standard was 27 min. with retention time of andrographolide was 5.6 ± 0.088 min. (Mean ± standard deviation of triplicate analysis)

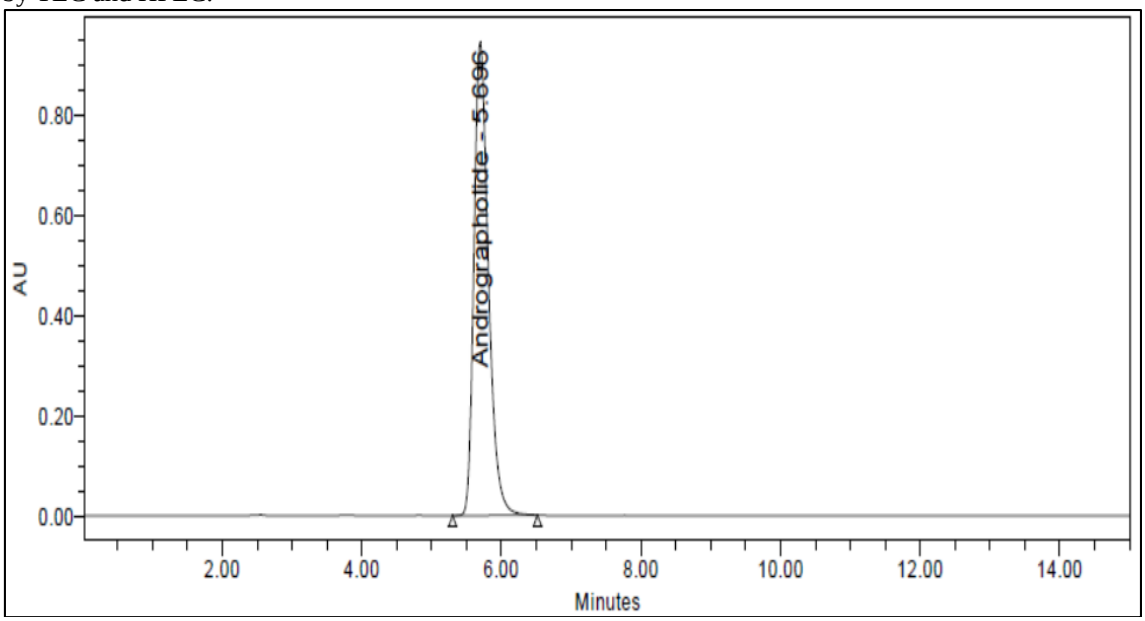


Fig 1: HPLC chromatogram of standard compound andrographolide (250µg/ml)

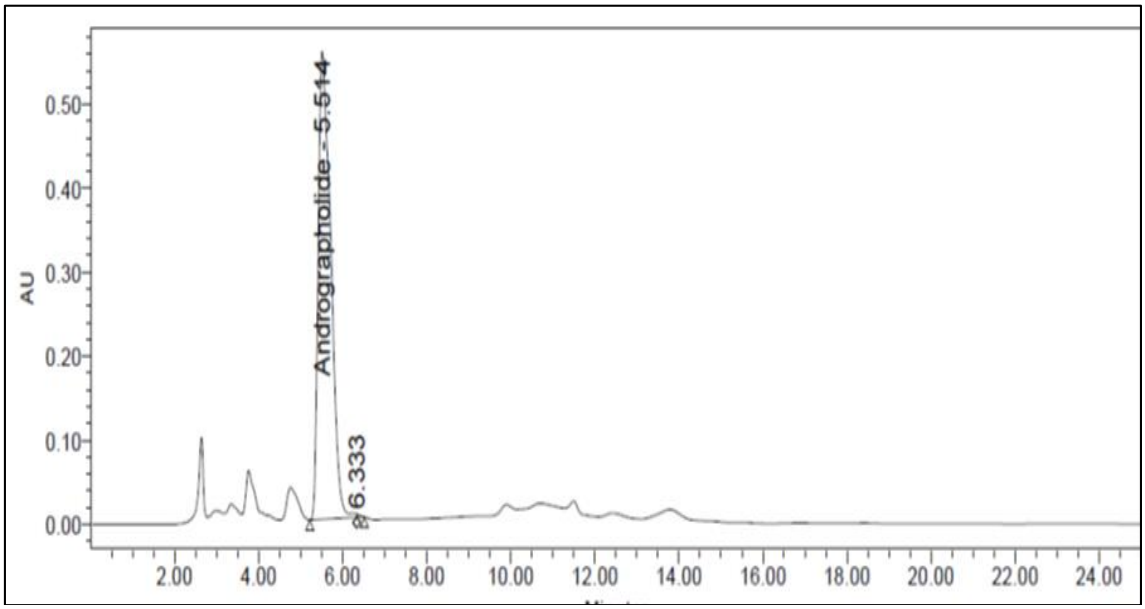


Fig 2: HPLC chromatogram of leaves sample of *Andrographis paniculata* (2gm/500ml)

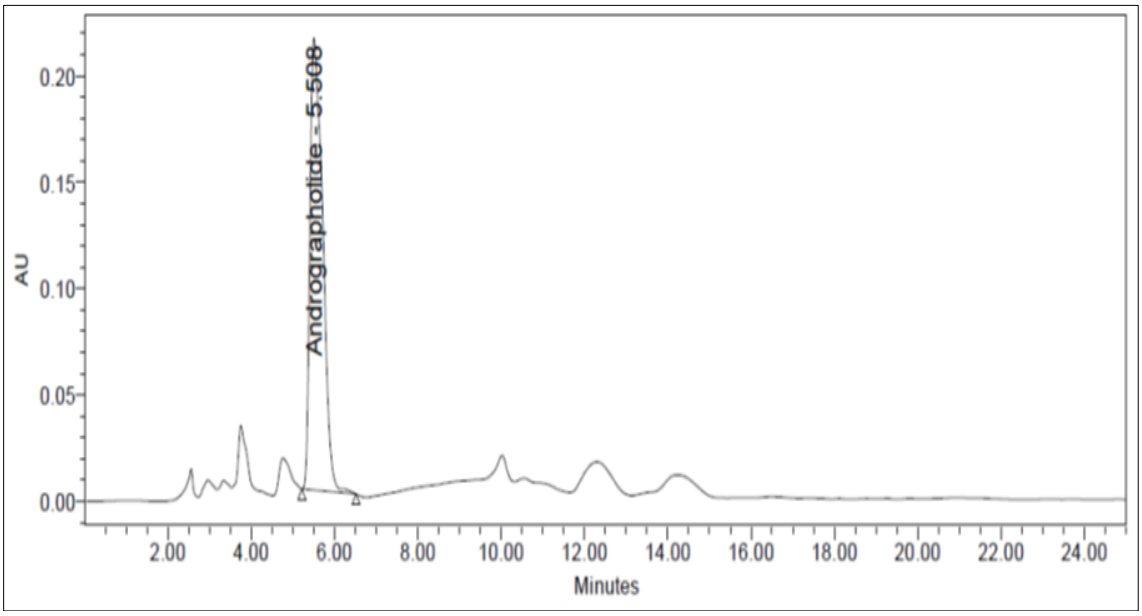


Fig 3: HPLC chromatogram of flowering tops sample of *Andrographis paniculata* (2gm/500ml)

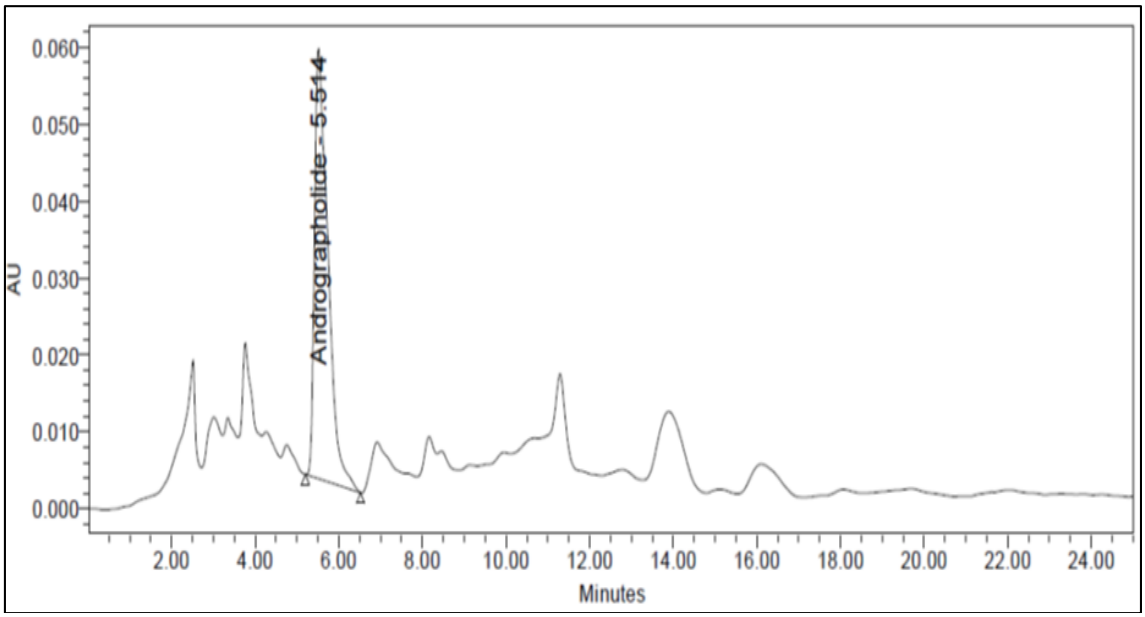
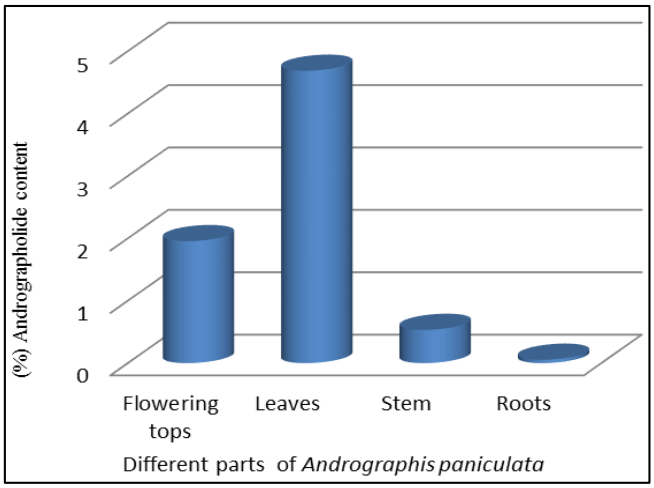


Fig 4: HPLC chromatogram of stem samples of *Andrographis paniculata* (2gm/500ml)

Table 1: Andrographolide content in different parts of *Andrographis*

R No.	Plant Part	Andrographolide Content (%)
1	Flowering tops	1.955 ±0.007
2	Leaves	4.686 ±0.065
3	Stem	0.533 ±0.005
4	Roots	0.054 ±0.031



2.6 Statistical Analysis
Statistical comparison was performed OP-STAT software and was considered statistically significant.

3 Results and Discussion
The results of the study aimed at the determination of the andrographolide content in different parts of *Andrographis paniculata*. The andrographolide content in different parts of the plants found in present study was given in Table 1. The HPLC chromatograms of standard andrographolide and sample of parts are given in Figures 1-5. Highest (4.686 %) andrographolide content was reported in leaves, which was followed by flower tops (1.955 %), stem (0.533 %) and in roots (0.054 %) lowest andrographolide content was found. Similar studies based on *A. paniculata* parts (leaves, stem, capsules, roots) have also been done by Gajbhiye & Khristi (2010) and Pandey & Mandal (2010). Highest content of andrographolide was reported in leaves by them as recorded in our study also. Variation in percentage of andrographolide may be due to differences in sample source (location and time of collection) as our samples were collected from mid-hill conditions of Himachal Pradesh. Differences may also be due to sample preparation method for HPLC quantification but the trend in andrographolide content in different parts is similar.

4. References

1. Kumar A, Dora J, Singh A, Tripathi R. A review on king of bitter (kalmegh). Int. J Res. Phar. Chem 2012; 2:116-124.
2. Basak A, Copper S, Roberge AG, Banik UK, Chreten M, Seidh NG. Inhibition of protein convertases-1, -7 and furin by diterpenes of *Andrographics paniculata* and succinoyl esters. 1999; 338:107-113.
3. Gajbhiye NA, Khristi S. Distribution pattern of andrographolide and total lactones in different parts of Kalmegh plant. Ind. J Hort. 2010; 67:591-593.
4. Hemadri K, Rao SS. Jaundice: Tribal medicine. Anc. Sc. Life. 1984; 4:209-212.
5. Huang KC. The pharmacology of Chinese herbs. Boca Raton. CRC Press. Joseph SM (2014). Scientfic aspects of the therapeutic use of *Andrographis paniculata* (kalmegh): A review. Int. J Pharm. Sc. Rev. Res. 1993; 27:10-16.
6. Pandey AK, Mandal AK. Variation in morphological characteristics and andrographolide content in *Andrographis paniculata* (Burm. f.) Nees of central India. Iran. J Ener. Env. 2010; 1:165-169.
7. Prajapati ND, Purohit SS, Sharma AK, Kumar T. A handbook of medicinal plants. Jodhpur: Agrobios, 2003.
8. Puri A, Saxena R, Saxena RP, Saxena KC, Srivastava V, Tandon JS. Immunostimulant agents from Andrographis paniculata. J Nat. Prod. 1993; 56:995-999.
9. Raina AP, Kumar A, Pareek SK, Sharma SK. Evaluation studies on Kalmegh (*Andrographis paniculata* Nees). Acta Hort. 2013; 972:117-120.
10. Salleh MS. The use of *Andrographis paniculata* extract for treatment of diabetes mellitus (Kegunaan ekstrak hempedu bumi (*Andrographis paniculata*) untuk rawatan diabetes mellitus) Bachelor of Science Project Report, Department of Biology, UPM, Malaysia, 1991.
11. Sareer O, Ahmad S, Umar S. *Andrographis paniculata*: a critical appraisal of extraction, isolation and quantification of andrographolide and other active constituents. Nat. Prod. Res. 2014; 28:2081-101.
12. Tripathi GS, Tripathi YB. Chloretic action of andrographolide obtained from *Andrographic paniculata* from rats. Phytoth. Res. 1991; 5:176-178.