The Pharma Innovation Journal 2019; 8(4): 140-144

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(4): 140-144

www.thepharmajournal.com
Received: 06-02-2019
Accepted: 10-03-2019

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Variation in andrographolide content among different accessions of *Andrographis paniculata*

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Abstract
Andrographis paniculata (Burm. f.) Wall. ex Nees. commonly known as Kalmegh is used both in Ayurvedic and Unani system of medicines for a number of ailments related to digestion, liver (hepatic), fever, malaria and sore throat. It has an imperative place in the Indian Pharmacopoeia and is being prominently used in at least 26 Ayurvedic formulations. It is a hardy and erect plant which grows mainly as under shrub in tropical, moist deciduous forest. It is widely distributed southwards through Thailand and Peninsular Malaysia to Indonesia and in India it is found in the states of Madhya Pradesh, Chhattisgarh, Orissa, Jharkhand, Maharashtra, Assam, Bihar, West Bengal, Uttar Pradesh, Andhra Pradesh, Tamil Nadu, Karnataka and Kerala. Andrographolide, is the major constituent extracted from the plant and exhibits several biological activities. The quality of the herb depends on andrographolide content. The andrographolide content was determined by HPTLC. The average andrographolide content varied from 1.38 to 3.12 % on dry weight basis. The differences in andrographolide content among Kalmegh collected from different locations were statistically significant. The result indicated that the andrographolide being secondary metabolite may be influenced by the environmental, seasonal factors and soil characteristics. The results indicated that Kalmegh populations with highest andrographolide content may be potential source of quality raw material and ultimately more efficacious drugs.

Keywords: andrographolide, *Andrographis paniculata*, Accessions, HPTLC

Introduction
The history of herbs is as long as the history of mankind. Herbs are plants valued for their medicinal and aromatic properties and are often grown and harvested for these unique properties. Medicinal plants play an important role in disease management and livelihoods of people worldwide. There are about 15,000 to 20,000 plant species reported to have medicinal value with 30% considered as endemic to India. Among these 7,000-8,000 are reported to be used in unregulated informal systems of medicine and 1,200-2,000 in the regulated AYUSH [1]. Their active phytoconstituents are mainly responsible for these potential medicinal effects. In recent years, the growing demand for medicinal plants has accelerated over exploitation of valuable resources by unscientific and destructive manner without considering supportability and quality issues. Distinctive ecological factors, for example, temperature, humidity, altitude, rainfall and genetic makeup affect the qualitative and quantitative nature of secondary metabolites present in plants.

Andrographis paniculata (Burm. f.) Wall. ex Nees, commonly known as "king of bitters", traditionally known as 'Kalmegh', green chirayta is an annual herb widely used in tropical Asia. It is distributed southwards through Thailand and Peninsular Malaysia to Indonesia and in India it is found in the states of Madhya Pradesh, Chhattisgarh, Orissa, Jharkhand, Maharashtra, Assam, Bihar, West Bengal, Uttar Pradesh, Andhra Pradesh, Tamil Nadu, Karnataka and Kerala [2]. The plant holds an imperative place in the Indian Pharmacopoeia and is being prominently used in at least 26 Ayurvedic formulas [3]. Panchang (stem, leaf, flowers, seed and root) of the plant is being used in various formulation of Indian system of medicine. The leaves and aerial parts of the plant are used in Indian traditional medicine for the treatment of fever, malaria and sore throat [4]. Clinical information shows the adequacy of the plant for the treatment and prevention of the common cold, tonsillitis and diarrhea. The plant is additionally announced powerful against intestinal sickness [5]. The whole plant has variety of therapeutic values. It has immunosuppressive and alexipharine properties and is useful in wounds, ulcers, leprosy, sore throat, tonsillitis, osteodynea, menstrual and post partum haematometra, hypertension etc [6]. Decoction of the plant is a blood purifier and is used to cure liver disorder, jaundice and dermatological disease [7].

A. paniculata has been phytochemically investigated for a number of bioactive compounds including andrographolide, neoandrographolide, panaculoside, flavonoids, andrographonin, panicalin, apigenin-7, 4'-di-O-methyl ether. The plant contains diterpenoids: 14-deoxy-11-oxo-andrographolide; 14-deoxy-11, 12-dehydroandrographolide; 14-deoxyandrographolide; neoandrographolide and andrographolide [8]. The major bioactive constituent 'Andrographolide' constitutes a group of diterpene lactones mainly found in leaves whereas stems contain the compound in traces [9]. In accordance with bare minimum standard of acceptability specified in pharmacopoeia, herbal industries prefer to receive Kalmegh (aerial parts) with not less than 40% leaves and total andrographolides content not less than 1.8% w/w. For leaves the industry expects not less than 2.8% w/w of total andrographolides which would contain at least 2.5% w/w of pure andrographolide.

The concentration of these active ingredients varies within plant parts and with the geographical distributions of the species. The andrographolide being secondary metabolites are often influenced by the environmental, seasonal factors and its distribution in between leaves and whole plant. It has been reported that there is wide variation in the andrographolide content in leaves and whole plant. There is also significant variation in andrographolide content in Kalmegh collected from different geographical areas [10]. Phytochemical marker compound (andrographolide) showed quantitative variations among the plants of different locations [11]. Chemical composition of plants varies not only in different parts but also with respect to other factors like growing regions, agroclimatic conditions, genetic makeup etc. [12, 13] The study therefore was aimed to calculate the percentage of andrographolide among different accessions of *A. paniculata* collected from different locations.

Materials and Methods

Plant Material

Germplasm of *Andrographis paniculata* (Kalmegh) was collected from Jharkhand, Madhya Pradesh, Uttar Pradesh, Karnataka, Gujarat, Haryana, Odisha and Uttarakhand. and also from research institutions like CIMAP Lucknow; NBRI Lucknow; SFRI Jabalpur; Anand Agricultural University, Anand; IIHR Bangalore; FRLHT Bangalore; Natural Remedies Bangalore; Dabur India Ltd. and Patanjali Ayurvet Haridwar. Plant cuttings/poly potted plants of five to fifty (randomly sampled individual plants) from each location were brought to Forest Research Institute, Dehradun and planted together in polybags/beds filled with soil and farmyard manure. These plant cuttings/germplasms were maintained in the nursery of the institute as mother plants. The plants were watered regularly and allowed to grow and proliferate. The plants were harvested, washed, shade dried and finally ground to powder for further analysis.

Chemicals and analytical equipment

All chemical used for extraction and analysis were of analytical grade and referred to Emerck. For quantitative assessment of kalmegh accessions by HPTLC, solvents including methanol, toluene, chloroform, acetone, vanillin, sulphuric acid and water used were of HPLC grade procured from Merck Life Science Private Limited (Mumbai, India). TLC Aluminium pre-coated plates with Silica gel 60 F_{254} (20X20 cm^2) used were obtained from E. Merck Ltd. (Mumbai, India). Andrographolide standard 99.8% was procured from Natural Remedies, Bangalore and was used as standard biomarker to confirm the results and to find correct percentage of andrographolide. A CAMAG (Switzerland) HPTLC system equipped with a sample Linomat V, Twin trough Glass Chamber (20 x 10) with SS lid, TLC Scanner III and Wincats an integrated Software 4.02(Switzerland).

Extraction and Sample preparation for HPTLC Analysis

Powdered plant material (1 gm) of each accession were extracted with methanol (15ml x 3) through refluxing on water bath for 20 min and filtered. The extracts obtained were subjected to estimation of andrographolide content using HPTLC. Extracts obtained from all accessions were makeup upto 50ml with methanol in volumetric flask.

Estimation of Andrographolide

For HPTLC analysis conditions were as follows- application volume- 10 µL, as 5-10 mm bands; developing solvent system- chloroform, acetone and toluene (2:2:1) and spray reagent– a mixture containing 1% vanillin in alcohol and 10% sulfuric acid in alcohol (1:1) and wavelength 223nm.

Results and Discussion

The germplasm of *A. paniculata* were collected from different locations of India and was phytochemically evaluated for the key therapeutically important compound, andrographolide. In HPTLC, andrographolide band of plant extract was detected at Rf value 0.31± 0.03 in Chloroform: Acetone: Toluene (2:2:1) mobile phase. The spectra of andrographolide showed maximum absorption at 223nm (Fig.1). The chromatogram of andrographolide from plant samples were obtained and compared with chromatogram of andrographolide standard on the basis of retention factor and peak area (Fig. 2 and Fig. 3). Twenty eight accessions of *A. paniculata* were analysed for active ingredient andrographolide by HPTLC.

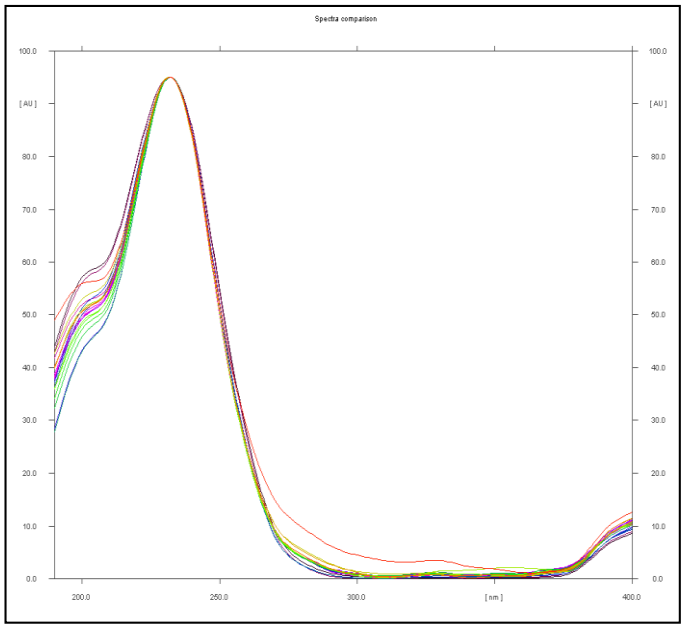

Fig 1: Andrographolide HPTLC Spectrum

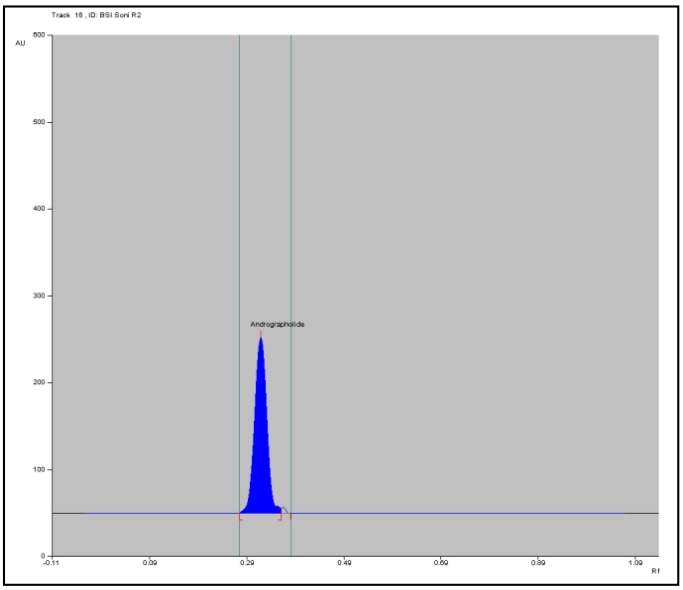

Fig 2: Andrographolide Standard Peak

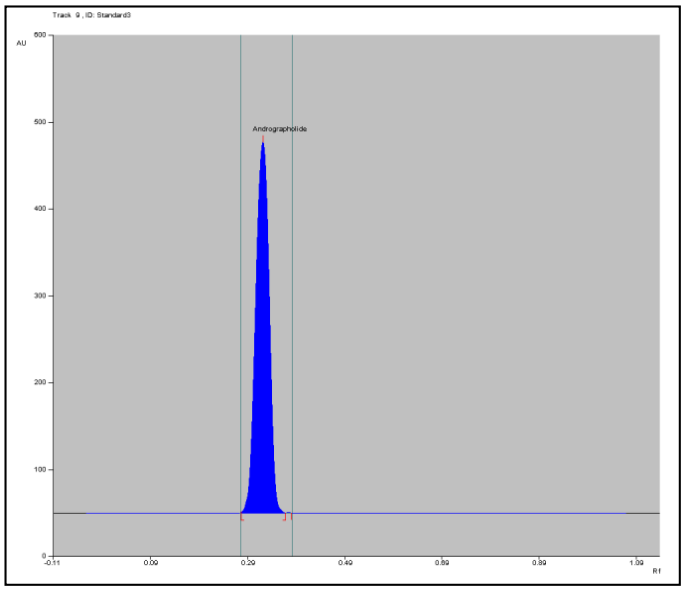

Fig 3: *Andrographis paniculata* Sample peak

Table 1: Percentage of Andrographolide among different accessions of *Andrographis paniculata*

Accession	Location	Andrographolide content % on DWB
1.	IIHR Bangalore	3.12
2.	Ranchi	1.94
3.	Patanjali Haridwar	2.17
4.	CIMAP, Lucknow	2.59
5.	Rajeshwari Nursery, Haridwar	1.84
6.	F.R.I, Dehradun	2.58
7.	Gujarat Agriculture University-1	2.92
8.	Gujarat Agriculture University -2	2.00
9.	Anand Gujarat 1	2.50
10.	Anand Gujarat 2	1.84
11.	Anand Gujarat 3	2.49
12.	Anand Gujarat 4	1.92
13.	Anand Gujarat 5	2.50
14.	FRLHT, Bangalore	2.31
15.	Natural Remedies, Bangalore	1.75
16.	Ayurvet	2.11
17.	Dabur	2.05
18.	Bhopal	2.35
19.	R.K.Mission, Kolkata	2.25
20.	BSI, Kolkata	2.17
21.	NBPGR, Delhi	1.56
22.	RPRC, Bhuvneshwar	1.91
23.	SMPB, Bhuvneshwar	2.16
24.	Shantikunj, Haridwar	1.78
25.	Dr. Sushila Devi Herbal Garden, Rishikesh	2.04
➢	CD value at 5 % level	0.226
➢	CD value at 1 % level	0.313

*DWB- Dry Weight Bases

The studies revealed the variation in andrographolide content ranged from 1.38 to 3.12 % on dry weight basis (Table 1). The highest andrographolide content was found in IIHR Bangalore accession (3.12%), followed by 2.92% in GAU 1 and 2.59% in CIMAP (Fig. 4)

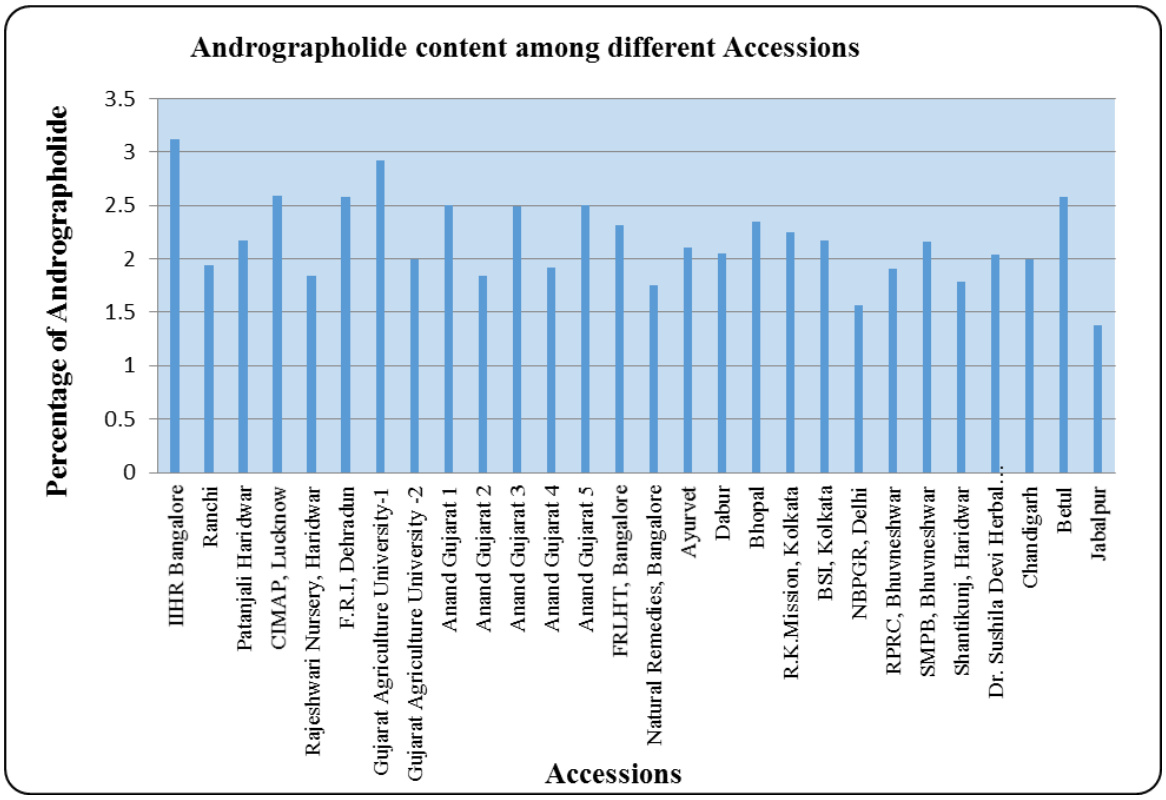

Fig 4: Graphical representation of Variation in Andrographolide content.

Variability in andrographolide content has previously been assessed by various workers. Mishra *et al*. 2001 has reported 0.67% to 1.82% of andrographolide content in leaves while 0.33% to 0.83% in complete plant of *A. paniculata* collected from Madhya Pradesh [10]. Another study carried out by Sabu *et al*. 2001 reported 0.73% to 1.47% andrographolide in leaves [14]. While Pandey and Mandal 2010 reported a variability of 1.07 to 2.24 % in andrographolide content in dried leaves collected from five locations of Madhya Pradesh and Chattisgarh [11]. Further Raina *et al*. 2007 has reported a variation of andrographolide content in dry leaves from 1.14% to 2.60% [15], Sharma *et al.* (2013) conducted a study on different harvesting time reporting the content vary from 0.81% to 1.86% before and after flowering [16]. Bhan *et al.* (2006) has concluded that the total andrographolide concentration in leaves increased from 6.23% to 6.96% from September to November [17].

Conclusion

Study was conducted with the objective to find out superior material of Kalmegh in terms of active ingredient (andrographolide) to obtain quality drug. The average andrographolide content varied from 1.38 to 3.12 % on dry weight basis. The differences in andrographolide content among Kalmegh collected from different locations were statistically significant. The highest andrographolide content was found in IIHR Bangalore accession (3.12%), followed by 2.92% in GAU 1 and 2.59% in CIMAP. The study revealed that the andrographolide content being secondary metabolite may be influenced by the environmental, seasonal factors and soil characteristics. Genetic makeup of the germplasm also contributed in the quality of raw material. The results indicated that populations/germplams having highest andrographolide content may be potential source for good quality raw material vis-à-vis production of more efficacious drugs.

Acknowledgement

Authors are grateful to the Director, Forest Research Institute, Dehradun, India for providing necessary facilities to carry out the study. The authors are also thankful to the National Medicinal Plants Board, New Delhi for financial assistance.

Conflict of Interest

Conflict of interest declared none.

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