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Variation in Morphological Characteristics and Andrographolide Content in *Andrographis paniculata* (Burm.f.) Nees of Central India

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Abstract: *Andrographis paniculata* (king of bitters), commonly known as Kalmegh is used both in Ayurvedic and Unani system of medicines for a number of ailments related to digestion, hepatoprotection, hypoglycemic and as anti-bacterial, analgesic, anti-inflammatory, vermicidal and antipyretic. *A. paniculata* collected from five locations of Madhya Pradesh and Chhattisgarh were studies for their morphological characteristics and diterpene contents to find out superior genotype. The average amount of dry weight per individual was between 16.58 to 18.92 g. The average andrographolide content varied from 1.07 to 2.24 percent in dried leaves. The differences in growth characteristics among the plants collected from different locations were not significant. However andrographolide content among the plants from different locations were statistically significant. The results indicated that those populations are potentially important sources for breeding, improvement of cultivars and best sources for obtaining higher drug yield.

Key words: Kalmegh · Variations andrographolide · Madhya pradesh · Chhattisgarh

INTRODUCTION

Andrographis paniculata (Burm. f.) Wall. ex Nees, commonly known as "king of bitters" belongs to family Acanthaceae, is a annual herb widely used in tropical Asia. It is a hardy and erect plant which grows mainly as undershrub in tropical, moist deciduous forest. It is distributed southwards through Thailand and Peninsular Malayasia to Indonesia and in India it is found in the states of Madhya Pradesh, Chhatisgarh, Orissa, Maharastra, Assam, Bihar, West Bengal, Uttar Pradesh., Tamil Nadu, Karnataka and Kerala. *A. paniculata* (Kalmegh) has been used for centuries in Asia for the treatment of various ailments. It has an important place in the Indian Pharmacopoeia and is being prominently used in at least 26 Ayurvedic formulas [1]. *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 [2]. Clinical data indicate the effectiveness of the plant for the treatment and prevention of the common cold, pharyngotonsillitis and diarrhea. The plant is also reported effective against malaria [3,4]. Hepatoprotective and antioxidant properties of *A. paniculata* were reported by Trivedi and Rawal [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. Tincture of the root is a tonic and stimulant. In Thailand, the plant has been recommended for the use in primary health care in cases of sore throat and diarrhea [7].

The aqueous extract of Kalmegh showed antimicrobial activity, which may be due to combined effect of the isolated arabinogalactan proteins and andrographolides [8]. The plant has antityphoidal properties against *Salmonella typhae* and antifungal against *Helminthosporium sativum*. Shoot extract in saline and ether showed potential antibiotic properties against *Micrococeus pyogenes* var. aureus. Kalmegh also reported to possesses antifertility effect against male albino rats [9].

The plant contains a number of diterpenoids. However, the major bitter constituent is andrographolide, which is a diterpene lactone. The second most important

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and non bitter constituent is neoandrographolide. Twelve other minor andrographolide related compounds have been isolated from the plant. There are some other minor constituents as well, which include seven flavonoid compounds, two long chain hydrocarbons, four dimers of diterpenoids-bis-andrographoloides A,B,C,D and sitosterol, tannins, traces of essential oil and two acidic polysaccharides. Four xanthones were isolated from the roots of *A. paniculata* and reported to possess anti-malarial activity [10]. Two flavonoids, identified as 5,7,2',3'-tetramethoxyflavonone and 5-hydroxy-7,2',3'-trimethoxyflavone, as well as several other flavonoids were obtained from the whole plant [11].

Andrographolide, chief constituent extracted from the leaves of the plant, is exhibited protective effects in carbon tetra chloride induced hepatopathy [12]. The bitter principle was isolated in pure form by Gorter [13]. Andrographolide is also attributed with some other activities like liver protection under various experimental conditions of treatment with galactosamine and paracetomol induced hepatotoxicity [14,15]. Antihepatotoxic effects of major diterpenoids of Kalmegh were reported by Kapil, *et al.* [16]. The hepatoprotective action of andrographolide is related to activity of certain metabolic enzymes [17]. Andrographolide showed anticancer activity on diverse cancer cells representing different types of human cancers [18]. Unlike cytotoxic anticancer drug eisplatin andrographolide is rapidly metabolized when taken orally. Andrographolide has also shown antidiabetic activity [19]. Calabrese, *et al.* [20] has conducted a trial on HIV patients and found effective. Considering extensive reports of clinical efficacy in preventing restenosis in angioplasty patients and its role in the various malignancies andrographolides may become a welcome addition to the physician's armoury of non-toxic therapeutic agents.

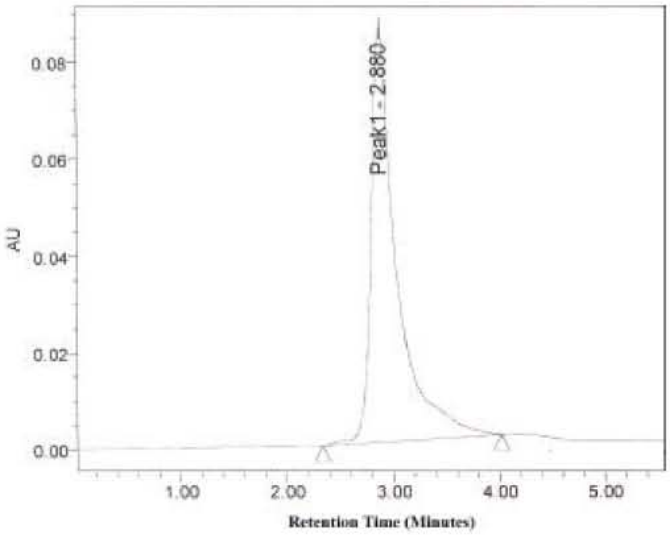
The plant is usually collected from wild sources for domestic consumption and its cultivation is confined only to the gardens especially maintained by the traditional users of medicinal plants in traditional system of medicine. It is placed at 17th position among the 32 prioritized medicinal plants of India with a demand of 2,197.3 tonnes (2005-06) and annual growth of 3.1%. Mishra, *et al.* [21] has studied patterns of genetic variability for different traits in a Kalmegh. However, no studies were conducted from Madhya Pradesh. The aim of the present study was to find out phenotypic and biochemical variations in the plants grown in different agro-climatic regions of Madhya Pradesh and Chhattisgarh. The present study was performed to analyse the variation in yield parameters and diterpene lactone content of wild *A. paniculata* from various agro-climatic regions of central India (Madhya Pradesh and Chhattisgarh). Furthermore, we also quantified andrographolide content in different plant parts of Kalmegh to find out the plant part rich in major active ingredient (andrographolide).

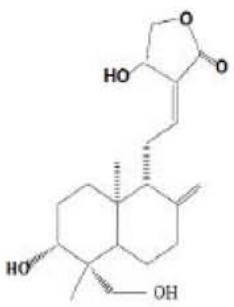
MATERIALS AND METHODS

Plant Material: Kalmegh was collected from the forest areas of Chhindwara, Balaghat and Amarkantak, Madhya Pradesh; Dhamtari and Jagdalpur, Chhattisgarh. Collected seeds were sown in nursery and seedlings were planted in field to evaluate their performance in tropical climate of Jabalpur. Kalmegh seedlings were planted at 30x30 cm spacing in first week of July. The crop was grown as per cultivation practices developed by Pandey and Mandal [22]. Leaves/plant material at different stages of maturity were collected and dried in shade for 7 days followed by grinding. The fine leaf powder samples were then used for extracting the andrographolide. Different plant parts were also collected and analysed for their andrographolide content.

Determination of Fresh and Dry Weight: Morphological parameters including characteristics e.g., plant height, collar diameter, number of branches, leaf length, leaf width, fresh and dry weight of whole plant were recorded for exploring the phenotypic diversity. Kalmegh plants were collected from the nursery and washed under tap water to remove soil. Then they were placed under a fan for drying and weighed with an electronic balance. After fresh weight determination the plants were dried in shade and dry biomass weight was also recorded.

Andrographolide Estimation: Fully grown Kalmegh plants of each genotype were screened for the active principle (andrographolide) contents. The methanolic extract of the shade-dried leaves was analysed by HPLC to estimate its andrographolide concentration. Andrographolide content was estimated as per methods of Jain, *et al.* [23] and Rajani, *et al.* [24]. 5 g dried, powdered and defatted plant material was extracted with methanol in a soxhlet for 5 h on a water bath. Solvent was removed under reduced pressure. The residue thus obtained, was dissolved in HPLC grade methanol and subjected to HPLC for qualitative and quantitative analysis by Waters system, equipped with dual 515 pump binary system, 2996 PDA detector, Waters C-18 column (x bridge) and data was integrated with Empower software. The temperature was maintained at 25°C, with injection volume of 20 μL and





Structure of Andrographolide

Fig. 1: Chromatogram of standard andrographolide

flow rate of 1 ml/min. The total run time was 10 min at 223 nm. A good resolution of the compound was achieved in acetonitrile-water (80:20, v/v). The andrographolide content was quantified by comparison with authentic sample.

The standard andrographolide (Sigma, USA) (22.3 mg) was dissolved in 100 ml methanol in which 20 µl of standard solution was injected into the HPLC column for making calibration curve. Standard andrographolide took 2.880 min as retention time (Figure 1). The retention time and peak area were recorded for calculating the total andrographolide content in each genotype to compute an average content.

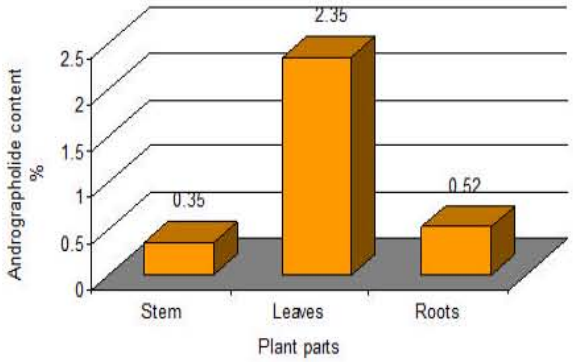


Fig. 2: Andrographolide content in different plant parts of *Andrographis paniculata*

RESULTS AND DISCUSSION

Data on growth parameters like plant height, collar diameter, leaf size, leaf stem ratio, number of branches and fresh/dry biomass were recorded and presented in Table 1. The andrographolide content was also estimated from different plant parts of Kalmegh and presented in Figure 2. Andrographolide content varied from 0.35 to 2.35% in various plant parts of Kalmegh. The maximum andrographolide content (2.35 %) was found in the leaves harvested after 110 days of planting. However, lowest andrographolide content was found in stem (0.35%). The andrographolide content was also estimated at various stages of maturity of the crop. It varied from 0.65 to 1.97 % in various aerial parts of Kalmegh, collected from various stages of maturity. The maximum andrographolide content was found in the in the crop harvested after 110 days of plantation i.e. just before flowering, making it ideal harvesting time. The andrographolide content also depended upon growing region and season.

Evaluation of Kalmegh Germplasm: Maximum plant height (60.20 cm), leaf length (6.22 cm) and width (1.98 cm) was recorded from Amarkantak source. Minimum height (52.30 cm), collar diameter (4.96 mm), number of branches (16.80), leaf length (5.84 cm), width (1.83 cm) and fresh weight/plant (58.30 gm) was recorded from Dhamtari source. Andrographolide content varied from 1.07 to 2.24 % in Kalmegh collected from various places. The maximum andrographolide (2.24 %) was estimated from Dhamtari source. However, minimum andrographolide content (1.07%) was determined from Balaghat. There was no significant difference found in the growth

Table 1: Growth characteristics and andrographolide content of Kalmegh collected from various locations

Locations	Height (cm)	Collar diameter (mm)	No. of Branches	Leaf size Length (cm)	Breadth (cm)	Leaf/stem ratio	Fresh wt. (g)	Dry wt. (g)	Andrographolide content (%)
ChhindwaraAP-1	56.56	5.20	19.40	5.93	1.85	0.648	64.80	18.74	1.87
Balaghat AP-2	59.70	5.36	17.20	6.19	1.92	0.67	61.00	18.10	1.07
AmarkantakAP-3	60.20	5.32	18.80	6.22	1.98	0.71	63.10	18.92	1.95
Dhamtari AP-4	52.30	4.96	16.80	5.84	1.83	0.72	58.30	17.58	2.24
Jagdalpur AP-5	56.28	5.21	18.26	6.28	1.89	0.71	59.25	16.58	2.05
SE±0.5	2.04	0.75	1.16	0.303	1.32	0.064	2.73	0.97	0.12
CD at 5%	NS	NS	NS	NS	NS	NS	NS	NS	0.28

characteristics and androgrpholide content among the germplasm collected from various places.

The HPLC estimation showed the considerable phytochemical (andrographolide) variation in the studied *A. paniculata* genotypes. The phytochemical diversity measured as quantitative difference in the accumulated andrographolide ranged from 1.07 to 2.24% of dry weight with a mean value of 1.83%. The highest andrographolide content was detected in the Dhamtari population AP-4(2.24%) followed by AP-5 (2.04%), AP-3 (1.95%), while the least amount was found in genotype AP-2 (1.07%) (Table 1). Phytochemical marker compound (andrographolide) showed quantitative variations among the plants of different locations. Earlier, Sabu *et al.* [25] carried out a study and reported andrographolide content 0.73% to 1.47% in leaves of 12 accessions collected from southern regions of India and 3 accessions collected from other tropical Asian countries. Sharma, *et al.* [26] have also studied variability at morphplogical, molecular and biochemical level of *A. paniculata*. Raina, *et al.* [27] conducted a study and also reported variation of andrographolide content in dry leaves from 1.14% to 2.60% amongst their collections. A similar study conducted in Thailand, reports average andrographolide content varied from 12.44 to 33.52 mg/g dried leaves and 14-deoxy-11,12-didehydroandrographolide content varied from 0.23 to 2.08 mg/g dried leaves [7]. In our studies we have identified promising accessions having andrographolide content above 2% i.e. AP-4 (2.24%) and AP-5 (2.04%). Hence, these can be cultivated to obtain drug of uniform potency and to increase Andrographis supply for meeting requirements of drug industry.

The better understanding of variation help in identifying superior genotype(s) for crop improvement as well as to evolve strategies for the effective *in situ* and *ex situ* conservation programmes. The results have shown non-significant level of variation among the collected genotypes at phenotypic level. However, there is significant variation at phytochemical level i.e. andrographolide content. The results obtained in the study are important in the exploration of *A. paniculata* genotypes for commercial production of andrographolide due to its high andrographolide content in the Dhamtari genotype (AP-4).

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