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CHEMOPROFILING OF ANDROGRAPHIS PANICULATA (KALMEGH) FOR ITS ANDROGRAPHOLIDE CONTENT IN MADHYA PRADESH, INDIA

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ABSTRACT

Andrographis paniculata (Burm.f.) Nees (Acanthaceae) is a medicinal plant traditionally used for the treatment of cold, fever, laryngitis and several infectious diseases ranging from malaria to dysentery and diarrhoea. The plant is widely used in Ayurvedic and Homeopathic systems of medicines. 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. In order to study the variation in andrographolide content, plant material was collected from 15 districts of Madhya Pradesh and evaluated through a simple, quick and accurate HPLC method using C-18 ODS-2 column. The chemoprofiling study showed significant variations in the concentration of active ingredients in the leaves as well as in whole plant. The study also revealed that andrographolide content was maximum in leaves of the accession collected from Seoni(1.82%) followed by Chhindwara (1.48%).

KEYWORDS:

Andrographis paniculata, Kalmegh, Madhya Pradesh, andrographolide, HPLC.

INTRODUCTION:

In recent years focus on use of non-traditional approaches to treat diseases has been revived world wide. Kalmegh (*Andrographis paniculata* Nees), commonly known as "king of bitter" belonging to family Acanthaceae, is an important annual medicinal herb widely distributed in Madhya Pradesh, India. It is hardy and erect herb which grows mainly as undershrub in tropical, moist deciduous forest. It is one of the most widely used plant in Ayurvedic formulations [1] and whole plant part known as "*Panchang*" (stem, leaf, flower, seed and root) is being used in various formulation of Indian system of medicine. It was recommended in *Charaka Samhita* (175 BC) for treatment of jaundice along with other plants in multi plant preparations [2]. It has also been used traditionally for sluggish liver, as antidote in case of colic dysentery and dyspepsia[3]. Long known in traditional Asian medicine as an immune system booster, *Andrographis* has demonstrated significant activity in fighting common cold, flu and upper respiratory infections[4,5]. It is used as bitter tonic, antispasmodic, antiperistaltic, stomachic and also an antihelmintic. It has been employed with benefit in case of general debility in convalescence after fevers,

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disorders of liver and advanced stages of dysentery[6]. The juice of fresh leaves is a domestic remedy in the treatment of colic pain, loss of appetite, irregular stools and diarrhea[7]. The pharmacological studies suggest anti-inflammatory[8], antipyretic, anti-viral, immunostimulatory, potential cancer therapeutic agent, anti-hyperglycemic and anti-oxidant [9] properties.

The major bioactive constituents of *Andrographis paniculata* are a group of diterpene lactones[10]. Leaves of the plant contain several derivatives of diterpene lactones out of which andrographolide (bitter constituent) and neoandrographolide (non bitter constituent) are important one[11]. 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. From the results, it is evident that there is wide variation in the andrographolide present in leaves and whole plant.

To find out which part of the plant have maximum concentration of andrographolide a systematic study has been done from leaves and whole plant parts saparetaly. The present study should come in conclusion that leaves of the plant contains highest amount of andrographolide while roots contains lower. To assess the andrographolide content in *Andrographis paniculata*, a simple, quick and accurate HPLC method using C-18 ODS-2 column was followed.

MATERIALS AND METHODS

Plant material

A systematic survey was conducted at different regions of Madhya Pradesh to find out Kalmegh growing areas. Kalmegh plants were collected from the forest divisions of Anooppur, Betul, Chhatarpur, Chhindwara, Dindori, Gwalior, Hosangabad, Jabalpur, Katni, Mandla, Sahdole, Sagar and Seoni districts belonging to different agroclimatic regions of Madhya Pradesh in the month of October and November. Leaves were collected from different size of plants such that the sample contained both tender and mature leaves. However, whole plants were also collected for comparative study of andrographolide content. While harvesting, care was taken not to destroy the plants in wild. After harvesting the leaves and whole plant collected from each districts were shade dried separately for 7 days and finely powdered.

Chemicals and reagents

Solvents used for chromatographic analysis were Methanol and HPLC grade water (E Merck, India). Mobile phase (Methanol) and the samples were filtered through 0.45µm membrane filter using Millipore filtration unit (Instrument Company, Bangalore) and for degassing of mobile phase ultra sonicator (Flexit, Pune) was used. The standard solution was prepared by dissolving 5mg of standard andrographolide (99%, pure, Sigma) in 5ml methanol 100% (v/v).

Sample extraction

2 gm of dried material with 20 ml of methanol was refluxed for 10 hours. It was then concentrated on in rotary vacuum evaporator at 61-65^{0}C. The concentrated material contains andrographolide with some impurities. The collected concentrated material was purified with the help of several chemicals, after

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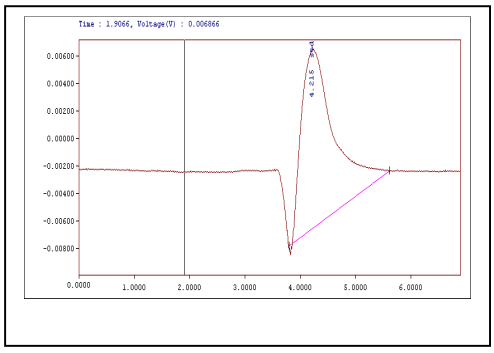
purification 20 ml methanol was added and filtered with Millipore. The extracted sample was ready for further analysis[12].

Chemoprofiling

Analysis was performed using high performance liquid chromatography with a C-18 ODS-2 column, manual injector valve (Rheodyne) and CIC software to control the equipment and for analysis of data. During the analysis, methanol:water (90:10) was used as mobile phase wavelength of the UV detector was 232nm and column temperature was ambient (35^0C). Linear calibrations of standards at accuracy of more than 99% were carried out for the quantification of the *A. paniculata* extracts. Single injection of solvent (blank) was also made to determine the retention time of the solvent. The chromatograms of andrographolide from plant samples were obtained and compared with standard chromatogram of andrographolide on the basis of their retention time and peak area (Fig-1).

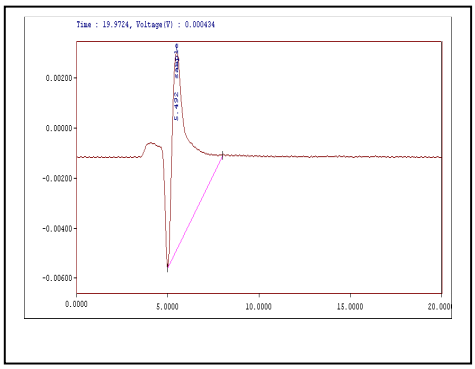
Fig. 1

Standard chromatogram of Andrographis paniculata



RT(min) Peak name Area(mV*sec)
std 533.826

Chromatogram of Andrographis paniculata sample



RT(min) Peak name Area(mV*sec)
4.49 sample 517.082

RESULT AND DISCUSSION

The data pertaining to the percent concentration of andrographolide in plant samples of different locations is presented in Table-1. The data showed significant variations in andrographolide concentration in the leaves as well as in whole plants. Mature leaves of the plant have higher amount of andrographolide (1.82%) as compared to whole plant (0.831%). It has been observed that andrographolide content in leaves was maximum in Seoni (1.82%) followed by Betul (1.53%) and minimum in Chhatarpur (0.67%). However, the content in whole plant was maximum in Chhindwara (0.83%) followed by Seoni (0.78%) and minimum in Rewa (0.33%). The results revealed that mature leaves have higher amount of andrographolide (1.82%) as compared to whole plant (0.831%). Sharma *et al.* (1992) have also reported that leaves of the plant contains highest amount of andrographolide[13].

Agroclimatic conditions also evident great variations in the percent concentration of andrographolide. Considerable variations exist among

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the morphological traits in *Andrographis* accessions from different agroclimatic zones of Madhya Pradesh. However, information on the extent of variability in the concentration of andrographolide in the *Andrographis* populations of Madhya Pradesh is not available. Hence, a study was undertaken to characterize the biochemical variations among the *Andrographis* germplasm accessions from diverse agroclimatic regions of Madhya Pradesh. Documenting the biochemical variations will provide an efficient tool for identifying useful genotypes that could be used as cultivars for extraction of standard drugs. Andrographolide concentration in the leaves was estimated by HPLC were used as the biochemical marker. The results indicated high variations in biochemical characters of *Andrographis* collected from various regions of Madhya Pradesh.

Table .1

Variations in Andrographolide content in Andrographis paniculata

No	Accessions source	Andrographolide (%) in leaves	Andrographolide (%) in whole plant
1	Seoni	1.820	0.780
2	Chhindwara	1.480	0.831
3	Betul	1.530	0.480
4	Hosangabad	0.880	0.530
5	Jabalpur	0.750	0.480
6	Mandla	0.720	0.550
7	Dindori	0.810	0.420
8	Anooppur	0.906	0.510
9	Sahdole	0.731	0.506
10	Rewa	0.729	0.331
11	Satna	0.679	0.429
12	Katni	0.732	0.479
13	Chhatarpur	0.670	0.432
14	Gwalior	0.970	0.470
15	Sagar	1.030	0.770

CONCLUSION

In the present study, all the collected accessions were analyzed under controlled conditions and following variations were observed-

(i) Maximum andrographolide content was found in leaves (1.82%) of Seoni accession as compared to whole plants (0.83%) of Chhindwara accession.

(ii) Andrographolide content among the population varied with geographical locations and it was maximum in accession collected from Seoni district of Madhya Pradesh.

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(iii) Documenting the biochemical variation will provide an efficient tool for identifying better accession that could be exploited as cultivars for extraction of standard drugs.

REFERENCES

1. Hooker, J. D. 1885. "*Flora of British India*", vol: IV, L. Reeve & CO. LTD, Ashford, Kent.
2. Sharma, P. V. 1983. "*Charka Samhita*", Ed. Chankhambhia Orientalia, Vol.II, Varanasi.
3. Handa, S. S., and Sharma, A. 1990. Hepatoprotective activity of andrographolide from *Andrographis paniculata* against carbon tetrachloride. *Indian Journal of Medical Research*, B 92: 276-283.
4. Coon, J.T. and Ernst, E. 2004. *Andrographis paniculata* in the treatment of upper respiratory tract infections: A systematic review of safety and efficacy. *Planta medica*, 70:293-298.
5. Melchior, J., Spasov, A. A., and Ostrovskij, O. V. 2000. Double blind placebo controlled pilot and phase III study of activity of standardized *Andrographis paniculta* Herba Nees extract fixed combination (Kan jang) in the treatment of uncomplicated upper respiratory tract infection. *Phytomedicine*, 7:341-350.
6. Dastur, J. F. 1959. "*Medicinal plants of India and Pakistan*", Meyer Books.
7. Saxena, S., Jain, D. C., Bhakuni, R. S. and Sharma, R. P. 1998. Chemistry and pharmacology of Andrographis species. *Indian Drugs*, 35: 458-467.
8. Shen, Y. C., Chen, C. F., and Chiou, W. F. 2002. Andrographolide prevents oxygen radical production by human neutrophils: possible mechanism(s) involved in its anti - inflammatory effect. *British Journal of Pharmacology*, 135: 399-406.
9. Zhang, X. F. and Tan, B. K. 2000. Antihyperglycaemic and anti-oxidant properties of *Andrographis paniculata* in normal and diabetic rats. *Clinical Experimental Pharmacology and Physiology*, 27: 358-363.
10. Wongkittipong, R., Prat, L., Damronglerd, S., and Gourdon, C. 2000. Solid-Liquid Extraction of Andrographolide from Plants-Experimental Study, Kinetic Reaction and Model. *Sepn. Purifn. Technol.*, 40:47-154.
11. Akowuah,G.A., Zhari,I., Norhayati,I., Mariam,A. 2006. HPLC and HPTLC densitometric determination of andrographolides and antioxidant potential of *Andrographis paniculata J. of Food Composition and Analysis*, 19**:**118-126.
12. Sharma, A., Krishan, L. and Handa S.S. 1992. Standardization of the Indian crude drug Kalmegh by high pressure liquid chromatographic determination of andrographolide. *Phytochem. Anal.*, 3: 129-31.
13. Rajani, M., Shrivastava, N. and Ravishankara, M. N. 2000. A Rapid Method for Isolation of Andrographolide from *Andrographis Paniculata* Nees (Kalmegh). *Pharmaceut. Biol.*, 38: 204 - 209.