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Variation of Andrographolide Content in *Andrographis paniculata* from Different Sites of Balaghat Region of Jabalpur (M.P.)

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ABSTRACT

Aim: The main objective of this investigation was to show the variation of Andrographolide content in *Andrographis paniculata* from thirty seven different sites of Balaghat (Jabalpur MP) region.

Methodology: In order to study the variation in andrographolide content, plant material was collected from different sites and were analyzed through quick and accurate HPLC chromatographic method using C-18 column (4.6 x 250 mm, 5µ particle size).

Result and Discussion: The average andrographolide content varied from 0.01 to 1.2%. Highest percent of andrographolide in *Andrographis paniculata* were estimated from site Kera of south Balaghat 1.2%. Lowest content of andrographolide in *Andrographis paniculata* were estimated from the site of Salhen north Balaghat region that is 0.02%.

Conclusion: The content of this active ingredient in this plant varies with in the plant parts and with the geographical distribution.

Key Words: *Andrographis paniculata*, Andrographolide, Balaghat region, HPLC instrumentation

INTRODUCTION

Andrographis paniculata is an important medicinal plant of family acanthaceae. It is also called king of bitters. It is an annual herb widely used in different parts of Asia. Used mainly for treating fever, liver disease, common cold, chickengunia fever, snake bite etc. It is to be used extensively for medicinal purpose in India, Thailand, China and Mauritius (Niranjan, *et. al*., 2010). The leaves and aerial parts of the plant are used in Indian traditional medicine for the treatment of fever, malaria, some throat infection (Shanker, *et. al*., 2012), hepatoprotective, antioxidant (Lin, *et. al*., 2009). Whole plant has variety of therapeutic uses. Phytochemical data revealed that this plant can be used for the treatment of common cold pharyngotonsillitis and diarrhea (Mishra *et al* 1992 and Najib *et al* 1999). Antioxidant properties of *Andrographis paniculata* were recorded by (Trivedi and Raval, 2001). This plant is used for blood purifier, liver diseases, dermatological diseases (Prathanturarug *et al*., 2007). Aqueous extract of this plant are having antimicrobial activity due to presence of arabinogalactan proteins and andrographolide (Singh *et al.,* 2003). Leaf part was found to content highest amount of andrographolide and the seeds were estimated to content lowest amount of andrographolide (Sharma *et al.,* 1991). Andrographolide is bitter crystalline compound, colorless having antithrombotic, hyphoglycemic and antipyretic properties. A recent study conducted by USA University that this plant is having anti HIV activity (Calabrese, 2000). This plant ranks seventeenth position among their thirty two prioritized medicinal plants and annual growth of this plant is 3.1 percent (Anonymous, 2007).

MATERIALS AND METHODS

Plant material: *Andrographis paniculata* was collected from different sites of Balaghat region. Areal plant parts were collected for comparative study of andrographolide

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content. Leaves/ plant material at different sites were collected and dried in shade for 15 days followed by grinding to form powder of it. The fine powdered 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^{0}C). Class VP software was used for integration and calibration. Evaluation was via peak areas with linear regression.

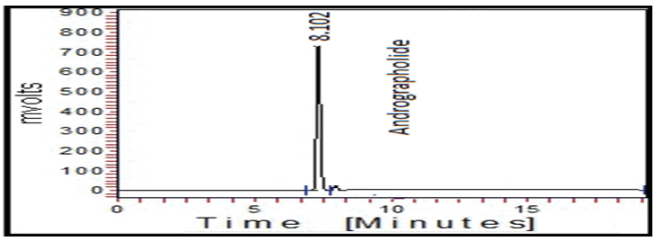
Preparation of herbal extract: Fresh aerial part of the plant sample collected from 37 different sites of Balaghat region of Jabalpur (M.P). Reflux 1 gm dried powder along with 50 ml of methanol was kept in soxhlet for one hour. After one hour the refluxing load was subjected to Rota-vapor at 60 RPM and heated at 60^{0}C. Filter & subject the marc for another two cycles of refluxes (1 hrs. 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 sample.

RESULTS

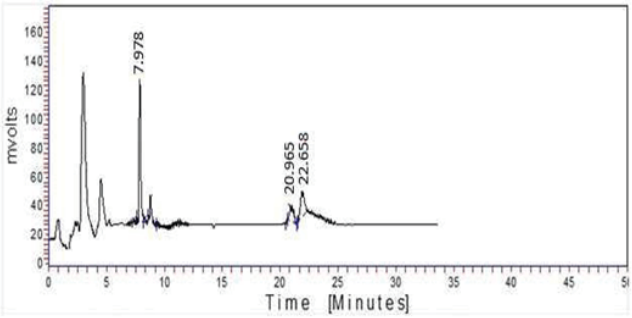
Table 1: Variations of Andrographolide content from *Andrographis paniculata*

Forest division	Forest Range	Name of forest beats	Sample code Number	Percent of andrographolide
South Balaghat	South Balaghat	Gangulpara	SAP-58	0.87
		Kera	SAP-59	1.20
		Manjhara	SAP-60	0.19
			SAP-61	0.73
		Payli	SAP-62	0.11
		Dhansua	SAP-63	0.11
		Sonewani	SAP-64	0.18
		Peepertola	SAP-65	0.17
		Bori	SAP-66	1.07
	East Lanjhi	Bodhadalkha	SAP-67	0.11
		Mataghat	SAP-68	0.12
		Sulsuli	SAP-69	0.19
			SAP-70	0.72
	West Lanjhi	Kandri	SAP-71	0.13
		Gatapar	SAP-72	0.16
		Kandri	SAP-73	0.11
		Gatapar	SAP-74	0.18
		Timkitola	SAP-75	0.18
	Kirnapur	Salhen	SAP-76	0.15
		Nandora	SAP-77	0.16
		Belgaon	SAP-78	0.12
		Salhen	SAP-79	0.04
		Gopalpur	SAP-80	1.01
	Hatta	Paraswada	SAP-81	0.61
			SAP-82	1.00
		Garda	SAP-83	1.00
	South Balaghat	Bori	SAP-84	0.91
North Balaghat	South Ukwa	Bithli	SAP-53	0.08
		Lathri	SAP-54	0.09
		Bithli	SAP-55	0.14
	North Ukwa	Dongaria	SAP-56	0.12
		Lood	SAP-57	1.06
	West Baihar	Tikariya	SAP-85	0.91
	East Baihar	Umardoni	SAP-86	0.71
			SAP-87	0.94
North Balaghat	North Lamta	Mehkapatha-II	SAP-29	0.16
			SAP-30	0.20
			SAP-31	0.07
		Mehkapatha-I	SAP-32	0.18
		Basegaon	SAP-33	0.10
	South Lamta	Dhooti	SAP-34	0.07

	North Lamta	Kuman-gaon	SAP-35	0.11
		Arandia	SAP-36	0.06
		Kuman-gaon	SAP-37	0.11
		Arandia	SAP-38	0.07
		Kuman-gaon	SAP-39	0.13
		Salhen	SAP-40	0.08
		Badgaon-II	SAP-41	0.26
		Badgaon-I	SAP-42	0.20
		Badgaon-I	SAP-43	0.07
		Salhen	SAP-44	0.02
			SAP-45	0.47
		Badgaon-I	SAP-46	0.65
			SAP-47	0.06
			SAP-48	0.19
			SAP-49	0.09
		Lamta	SAP-50	0.20
		Janamkhar	SAP-51	0.25
			SAP-52	0.12

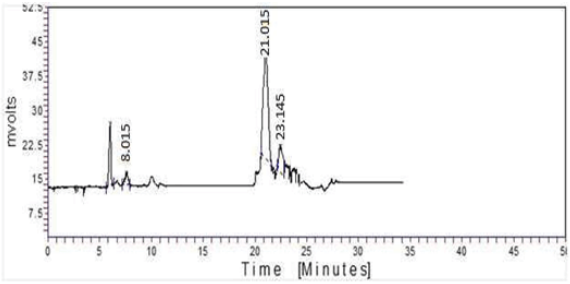


Graph 1: Standard of andrographolide prepared by HPLC method.



SAP-59			
Peak Name	Retn. Time	Area	Percent of andrographolide
Andrographolide	7.978	6025.328	1.2

Graph 2: Maximum % andrographolide of SAP-59.



SAP-44			
Peak Name	Retn. Time	Area	Percent of andrographolide
Andrographolide	8.015	99.821	0.02

Graph 3: Minimum% andrographolide of SAP-44.

DISCUSSION

The data pertaining to the percent concentration of andrographolide in *Andrographis paniculata* from different sites of Balaghat region is presented in Table 1.The estimated data gives the content of andrographolide in the shoot part of the plant. It has been estimated that highest concentration of andrographolide was found in Kera site of south Balaghat region (1.2%), followed by Bori site of south Balaghat (1.06%), Lood- north Ukwa north Balaghat (1.06%), Gopalpur- Kirnapur- south Balaghat (1.01%), Paraswada-Hatta-south Balaghat (1.00%).

Lowest percent of andrographolide was found in Salhen-north Lamta-north Balaghat (0.02%), Salhen-Kirnapur-south Balaghat (0.04%), Arandia-north-Lamta-north Balaghat (0.06%) , Badgon-1-north Lamta (0.06%) and Mehkapatha-north Lamta- north Balaghat (0.07%). Climatic conditions also showed great variation in the content of andrographolide. However the information regarding the content of andrographolide in *Andrographis paniculata* of Balaghat region was not available before my research findings. Hence a deep study was taken to show the variation of andrographolide content from different sites of Balaghat region of Jabalpur Madhya Pradesh. Result showed high variation of andrographolide from one site to another site of Balaghat region.

CONCLUSION

In my study, all the collected samples from different sites of Balaghat region were analyzed by quick and accurate HPLC method. Maximum amount of andrographolide was found in Kera site of south Balaghat region (1.2%), while lowest concentration of andrographolide was estimated from the site of Salhen-north Lamta-north Balaghat (0.02%). Andrographolide content varied from one site to another site

of Balaghat region. However the information regarding the content of andrographolide in *Andrographis paniculata* was not available before my research findings. Hence a deep study was taken to show the variation of andrographolide content from different sites of Balaghat region of Jabalpur Madhya Pradesh.

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