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Identification of elite germplasm of medicinally important *Andrographis paniculata* (Burm. f.) Nees with high content of four active diterpenoids in aerial parts from wild populations of eastern India

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Short Communication

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Abstract

Andrographis paniculata is an Indian medicinal plant with tremendous therapeutic values due to the presence of active diterpenoids in its aerial parts. However, high domestic and export demand has led to overexploitation of wild populations of this species. With a view to bringing *A. paniculata* into cultivation and to reduce the pressure on wild populations, the present study was undertaken to identify elite germplasm from different locations of eastern India by analysing intraspecific variation in the content of four major active diterpenoids. A total of 166 wild accessions of *A. paniculata* analysed through high-performance liquid chromatography (HPLC) revealed remarkable variation in the sum of four active diterpenoids in the aerial parts, ranging from 0.41 to 8.55% on a dry weight basis. Three elite accessions (AP-6, AP-8, AP-46) having respectively 8.02, 8.36 & 8.55% of the sum of four major active diterpenoids were identified. These germplasm could be used for commercial cultivation and genetic improvement of *A. paniculata*.

Introduction

Andrographis paniculata (Burm. f.) Nees (family Acanthaceae), popularly known as 'Kalmegh', is an important medicinal plant harvested from the wild in India. Although the species is widely distributed in tropical Asian countries, in eastern India the wild populations are rapidly declining due to large-scale harvesting of the whole plant from the wild for local use and transportation to adjoining states of India. The species has been assigned the 'vulnerable' threat category in states like Chhattisgarh and Madhya Pradesh (Gowthami *et al.*, 2021). The aerial parts of the plant are mostly used in Indian, Chinese and Thai systems of medicine (Kumar *et al.*, 2004; Prathanturarug *et al.*, 2007; Chao and Lin, 2010) for their wide spectrum pharmacological activities such as antidiabetic, anti-HIV, anticancer, anti-inflammatory, hepatoprotective, cardiovascular protection, and antioxidant properties (Dai *et al.*, 2019). The high medicinal value of this species is attributed to the presence of active diterpenoids such as andrographolide, neoandrographolide, 14-deoxyandrographolide and 14-deoxy-11,12-didehydroandrographolide, which are present in the aerial parts (Bhan *et al.*, 2006).

In spite of the ever-increasing demand for the species, the quality of the herbal products is not satisfactory. Inconsistency in drug quality caused by variability in the bioactive principles poses a major problem for industrial application of *A. paniculata* (Bhan *et al.*, 2006). Moreover, extensive use of *A. paniculata* for its varying pharmaceutical applications, traditional use and export value leads to over-exploitation of wild populations, thereby causing scarcity of elite germplasm with high content of bioactive compounds. Although, *A. paniculata* has been cultivated in countries such as China, Thailand, East & West Indies, Mauritius and some regions of India (Kataky and Handique, 2010), this important medicinal plant has not so far been brought into cultivation in eastern India.

Although aerial parts are used in herbal medicine, no work has been carried out for the selection of elite germplasm on the basis of a high content of major bioactive compounds. Quality evaluation of *A. paniculata* is so far limited to andrographolide content only (Raina *et al.*, 2016; Pandey *et al.*, 2019). Andrographolide is known to show anticancer activity with immunomodulatory activities (Rajagopal *et al.*, 2003). Neoandrographolide possesses antiradical activity (Kamdem *et al.*, 2002) and 14-deoxyandrographolide is effective in treating hepatocyte apoptosis in liver dysfunctions (Roy *et al.*, 2010).

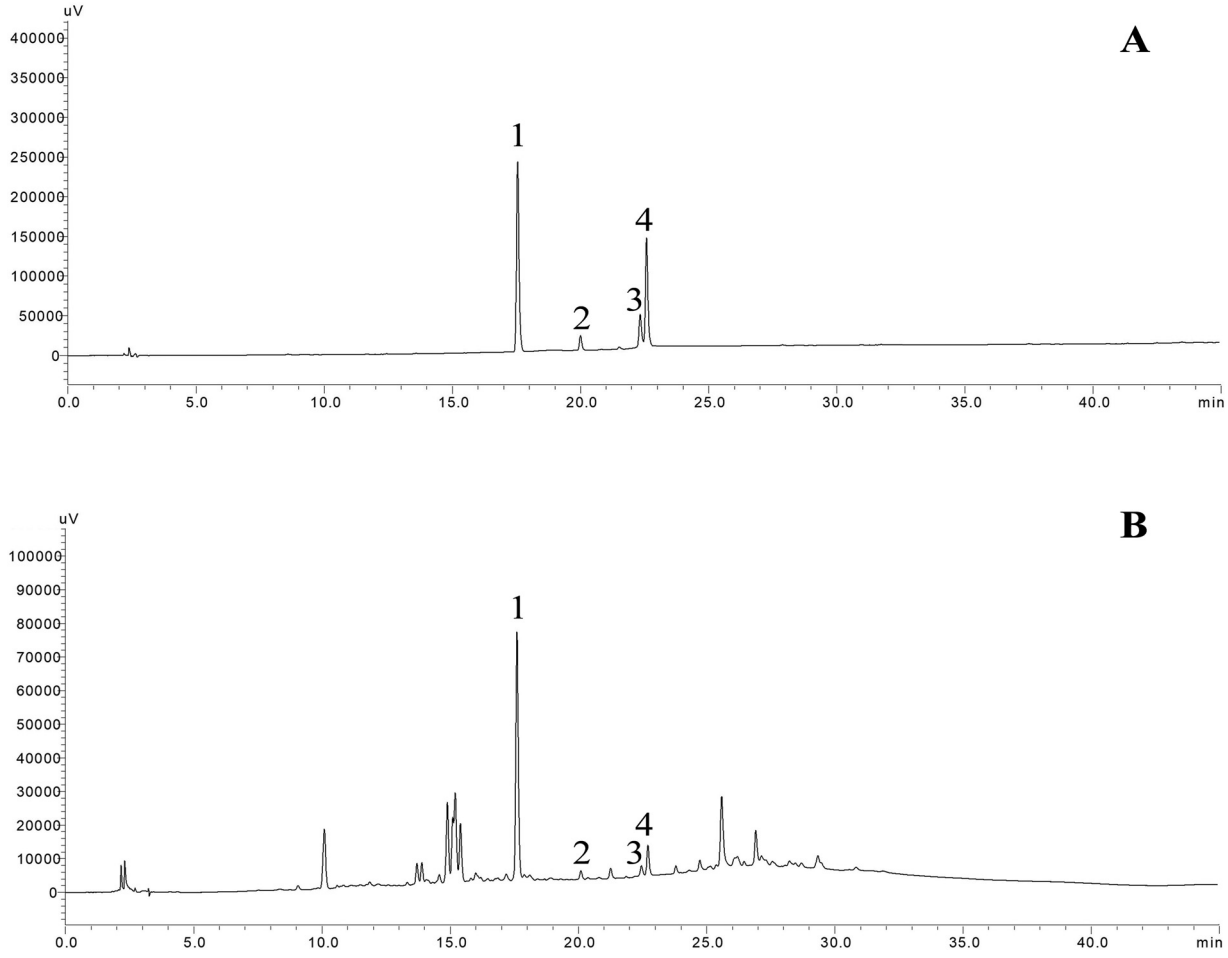

Fig. 1. HPLC Chromatogram; A-HPLC Chromatogram of four standard compounds, B-HPLC Chromatogram of a *Andrographis paniculata* accession. (1) Andrographolide, (2) Neoandrographolide, (3) 14-deoxyandrographolide, (4) 14-deoxy-11,12-didehydroandrographolide.

14-deoxy-11,12-didehydroandrographolide has vasorelaxation & hypotensive activity (Zhang *et al.*, 1998).

The present study was undertaken to identify elite germplasm by analysing intraspecific variations of four major active diterpenoids in 166 wild accessions of *A. paniculata* collected from different geographical locations of eastern India.

Experimental

High-performance liquid chromatography (HPLC) analysis was carried out on 166 wild accessions of *A. paniculata* collected from different geographical locations of Odisha and West Bengal. The fresh aerial parts of the healthy mature plants with similar growth habit were collected during October–November 2019 in order to avoid the developmental and seasonal variations of bioactive compounds. The shade dried powdered samples were accurately weighed to 25 mg and extracted with 50 ml methanol in an ultrasonic bath for 20 min at 60°C. Stock solutions of four reference standards were prepared in methanol. A Shimadzu HPLC system (Kyoto, Japan) with LC-20 AD pump, an SPD-20A diode array detector and Shimadzu Shimpak C18 column was used. The chromatograms were developed at 223 nm (Fig. 1). The samples were analysed in triplicates and the contents (%) were calculated using the calibration curve. The detailed procedure of HPLC analysis and method validation is given as supplementary material. The method validation was carried out according to the regulatory guidelines (International Conference on Harmonization, 2005).

Discussion

The therapeutic activity of *A. paniculata* is attributed to the presence of major active diterpenoids i.e., andrographolide, neoandrographolide, 14-deoxyandrographolide and 14-deoxy-11, 12-didehydroandrographolide (Rafi *et al.*, 2020). HPLC analysis of dried aerial parts of 166 wild accessions showed a great variation in these four bioactive compounds (Table 1). The quantitative HPLC method validation criteria showed satisfactory results which are given in the supplementary material. Among the germplasm analysed, the highest content of diterpenoids (8.55%) was identified in accession AP-46. The variation in the active diterpenoids was reported previously in leaves only and the highest content was reported as 6.96% (Bhan *et al.*, 2006). Usually, in *A. paniculata*, the aerial part has been used for the preparation of traditional medicines (USP, 2012). For the very first time, four active diterpenoids from aerial parts of the species were identified in the current research work. On the basis of a higher percentage (>8%) of sum of four major active diterpenoids, three elite germplasm accessions (AP-6, AP-8, AP-46) have been identified in *A. paniculata* from Gajapati district, Odisha. Variation of andrographolide in leaves and other aerial parts

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Table 1. Quantitative estimation of diterpenoids in 166 different accessions of *A. paniculata*

Acc. No.	Sum of four D (%)	Acc. No.	Sum of four D (%)	Acc. No.	Sum of four D (%)	Acc. No.	Sum of four D (%)
AP-1	3.95 ± 0.07	AP-43	6.16 ± 0.23	AP-85	4.85 ± 0.12	AP-127	1.61 ± 0.05
AP-2	3.83 ± 0.07	AP-44	5.27 ± 0.12	AP-86	5.81 ± 0.05	AP-128	0.79 ± 0.00
AP-3	2.36 ± 0.05	AP-45	7.13 ± 0.25	AP-87	4.17 ± 0.06	AP-129	1.30 ± 0.04
AP-4	2.91 ± 0.05	AP-46	8.56 ± 0.30	AP-88	6.19 ± 0.14	AP-130	1.10 ± 0.03
AP-5	2.70 ± 0.08	AP-47	7.39 ± 0.27	AP-89	4.78 ± 0.08	AP-131	1.55 ± 0.04
AP-6	8.02 ± 0.22	AP-48	6.27 ± 0.17	AP-90	1.91 ± 0.04	AP-132	1.82 ± 0.04
AP-7	5.13 ± 0.16	AP-49	6.21 ± 0.14	AP-91	3.50 ± 0.13	AP-133	4.42 ± 0.13
AP-8	8.36 ± 0.29	AP-50	4.78 ± 0.11	AP-92	3.50 ± 0.11	AP-134	4.52 ± 0.16
AP-9	7.02 ± 0.23	AP-51	5.64 ± 0.17	AP-93	3.74 ± 0.12	AP-135	4.04 ± 0.09
AP-10	5.49 ± 0.07	AP-52	4.98 ± 0.10	AP-94	5.01± 0.07	AP-136	6.18 ± 0.14
AP-11	6.30 ± 0.16	AP-53	3.10 ± 0.09	AP-95	5.08 ± 0.03	AP-137	3.83 ± 0.16
AP-12	1.79 ± 0.08	AP-54	4.50 ± 0.14	AP-96	2.52 ± 0.05	AP-138	2.14 ± 0.06
AP-13	1.92 ± 0.08	AP-55	2.61 ± 0.05	AP-97	3.79 ± 0.10	AP-139	2.28 ± 0.05
AP-14	4.32 ± 0.12	AP-56	2.38 ± 0.04	AP-98	1.62 ± 0.03	AP-140	2.09 ± 0.07
AP-15	5.19 ± 0.13	AP-57	2.21 ± 0.04	AP-99	4.50 ± 0.10	AP-141	6.90 ± 0.26
AP-16	2.68 ± 0.06	AP-58	5.86 ± 0.20	AP-100	1.42 ± 0.01	AP-142	1.78 ± 0.06
AP-17	4.18 ± 0.15	AP-59	4.97 ± 0.15	AP-101	6.39 ± 0.20	AP-143	0.75 ± 0.00
AP-18	3.96 ± 0.14	AP-60	5.64 ± 0.16	AP-102	2.08 ± 0.04	AP-144	3.63 ± 0.16
AP-19	4.95 ± 0.19	AP-61	0.42 ± 0.00	AP-103	2.19 ± 0.07	AP-145	3.07 ± 0.09
AP-20	5.64 ± 0.22	AP-62	5.11 ± 0.14	AP-104	0.97 ± 0.02	AP-146	1.06 ± 0.02
AP-21	5.51 ± 0.13	AP-63	4.96 ± 0.09	AP-105	2.22 ± 0.07	AP-147	4.65 ± 0.18
AP-22	1.75 ± 0.04	AP-64	3.05 ± 0.12	AP-106	1.49 ± 0.03	AP-148	0.83 ± 0.02
AP-23	7.93 ± 0.34	AP-65	3.18 ± 0.08	AP-107	1.04 ± 0.03	AP-149	0.82 ± 0.01
AP-24	3.22 ± 0.09	AP-66	2.38 ± 0.04	AP-108	5.91 ± 0.20	AP-150	2.79 ± 0.11
AP-25	3.26 ± 0.13	AP-67	4.43 ± 0.13	AP-109	1.94 ± 0.06	AP-151	3.88 ± 0.15
AP-26	2.40 ± 0.07	AP-68	4.45 ± 0.10	AP-110	0.89 ± 0.01	AP-152	4.68 ± 0.16
AP-27	1.40 ± 0.02	AP-69	5.20 ± 0.17	AP-111	0.98 ± 0.00	AP-153	2.02 ± 0.07
AP-28	2.87 ± 0.09	AP-70	5.13 ± 0.14	AP-112	5.20 ± 0.12	AP-154	1.97 ± 0.04
AP-29	4.11 ± 0.13	AP-71	4.95 ± 0.16	AP-113	0.68 ± 0.01	AP-155	4.03 ± 0.15
AP-30	4.46 ± 0.14	AP-72	4.37 ± 0.14	AP-114	0.90 ± 0.02	AP-156	3.65 ± 0.14
AP-31	4.10 ± 0.13	AP-73	3.05 ± 0.06	AP-115	4.69 ± 0.14	AP-157	2.78 ± 0.08
AP-32	4.85 ± 0.18	AP-74	3.19 ± 0.10	AP-116	3.70 ± 0.12	AP-158	2.96 ± 0.08
AP-33	3.71 ± 0.11	AP-75	4.30 ± 0.08	AP-117	5.33 ± 0.15	AP-159	4.50 ± 0.14
AP-34	4.30 ± 0.13	AP-76	3.15 ± 0.12	AP-118	5.31 ± 0.11	AP-160	1.69 ± 0.06
AP-35	4.74 ± 0.16	AP-77	5.19 ± 0.08	AP-119	3.72 ± 0.09	AP-161	2.75 ± 0.07
AP-36	2.10 ± 0.06	AP-78	3.74 ± 0.02	AP-120	4.64 ± 0.14	AP-162	1.31 ± 0.04
AP-37	1.85 ± 0.05	AP-79	5.35 ± 0.16	AP-121	2.27 ± 0.06	AP-163	3.33 ± 0.08
AP-38	1.63 ± 0.05	AP-80	4.19 ± 0.16	AP-122	3.97 ± 0.09	AP-164	4.52 ± 0.18
AP-39	1.05 ± 0.02	AP-81	3.65 ± 0.05	AP-123	3.75 ± 0.06	AP-165	2.02 ± 0.07
AP-40	4.89 ± 0.15	AP-82	3.01 ± 0.08	AP-124	3.59 ± 0.09	AP-166	1.47 ± 0.05
AP-41	6.36 ± 0.20	AP-83	3.01 ± 0.05	AP-125	3.96 ± 0.10		
AP-42	5.47 ± 0.20	AP-84	2.47 ± 0.11	AP-126	1.26 ± 0.02		

Data are presented as Mean ± SD (n = 3), D = Diterpenoids. Acc. No = Accession Numbers.
Sum of four D% = (Andrographolide + Neoandrographolide + 14-deoxyandrographolide + 14-deoxy-11,12-didehydroandrographolide).
From accession number AP-1 to AP-104, the samples were collected from different districts of Odisha.
From accession number AP-105 to AP-166, the samples were collected from different districts of West Bengal.

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was reported earlier (Mishra *et al.*, 2010; Raina *et al.*, 2016; Pandey *et al.*, 2019). Reported HPLC studies are based on single or few accessions of *A. paniculata*, but a simultaneous estimation of four active diterpenoids is still lacking.

The production and accumulation of plant secondary metabolites are strongly influenced by genetic, morphogenetic, ontogenic and environmental factors (Yang *et al.*, 2018). *A. paniculata* plants collected in the mature stage have a higher content of andrographolide as compared to other diterpenoids, and the present finding is in close agreement with previous report (Pholphana *et al.*, 2013). Therefore, the variations of four active diterpenoids among the samples might not be due to the growth stage. Abiotic factors such as soil, temperature, rainfall and humidity might account for the variation in the content as the samples were collected from different phytogeographical regions (Basak *et al.*, 2012; Akbar *et al.*, 2018). The current study revealed that altitude has insignificant relation ($R^2 = 0.048$) with the sum of four major active diterpenoids content (Fig. S1). Therefore, the variation in the level of four diterpenoids reported in the present study might be due to environmental × genetic factors, which needs to be explored further.

The three elite accessions (AP-6, AP-8, AP-46) of *A. paniculata* identified in the present study with significantly higher amounts of sum of four major active diterpenoids (>8%) could be used as potential sources of quality germplasm for commercial cultivation, future breeding programs, and genetic improvement to improve the quality of the herbal products and to reduce the pressure on the threatened wild populations.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S1479262121000575

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