



Evaluation of Plumbagin in Roots of *Plumbago zeylanica* L. from Different Locations of Central India for Quality Assessment

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Received: 26 April 2016;

Accepted: 15 July 2016;

Published online: 10 August 2016;

AJC-18027

Plumbagin is a major active ingredient and phytochemical marker compound for the quality assurance of the roots of *Plumbago zeylanica* L. The variations in active ingredients according to environmental and edaphic factors have been described for many plant species for their quality assessment and identification of elite chemotype. In present investigation, the roots of *P. zeylanica* collected from twelve locations belonging to nine agroclimatic regions of Madhya Pradesh state (Central India) were evaluated for plumbagin content to find out variation for their quality assessment using high performance thin layer chromatography (HPTLC) technique. The result showed that the accessions of *P. zeylanica* collected from Tropical Forest Research Institute, Jabalpur are of best quality in terms of plumbagin content and thus, the elite chemotypes.

Keywords: *Plumbago zeylanica*, Roots, Plumbagin, HPTLC, Superior chemotype.

INTRODUCTION

Medicinal plants have been used as herbal drugs since times immemorial. In the era of 21st century too, according to World Health Organization (WHO), approximately 80 % of the world's population currently uses herbal medicines directly as teas, decocts or extracts with easily accessible liquids such as water, milk or alcohol [1,2]. Global market for herbal medicines including drug products and raw materials is growing at the rate of 7-15 % annually and total world herbal drug market is estimated as US\$ 62 billion and is expected to grow to the tune of US\$ 5 trillion by 2050 [3,4]. Although, the Indian system of medicine, the Ayurveda, is the most ancient and still a practicing tradition, is facing typical western bias due to absence of scientific validation and standardization of herbal drugs [5]. The medicinal property of a plant lies in their active/therapeutic ingredients and high concentration of active ingredients provides elite germplasm. Active ingredients being the secondary metabolites are often influenced by the environmental and edaphic factors and vary from region to region [6]. Therefore, the standardization of herb to fulfill the therapeutic requirement based on quality and efficacy (active ingredient concentration) by applying suitable standards is the need of the hour to meet the public health challenges and also to capture the global market. WHO and modern herbal pharmacopoeias also lay a strong emphasis on the need for scientific validation

and quality standardization of herbs with respect to their active ingredients [7-9].

Plumbago zeylanica Linn. (family: Plumbaginaceae) is a large perennial undershrub, found throughout the tropical and subtropical countries of the world. It grows throughout India in moist places [10,11] and commands an important place among medicinal herbs. In Indian indigenous systems of medicine, the plant has been described as tumor-negating, anti-dyspeptic, appetizer, anti-saturative, antianorexic, anti-haemorrhoidal and pain-reliever. The plant has also been found significant in different clinical conditions, especially inflammation, leprosy, scabies, ringworm, dermatitis, ulcers, hemorrhoids and hook-worm [12]. Herbal medicines such as Dabur Chitrak Haritaki, Medohar Guggulu, Morslim-Z, Divya Chandraprabhavati etc. use *P. zeylanica* extracts in different amounts [13]. In Africa, Nigeria, Ethiopia, Mauritius and Rodrigues, different plant parts (leaves, roots, bark, etc.) of species are reported to be used to treat gonorrhoea, diarrhea, dyspepsia syphilis, tuberculosis, rheumatic pain, rheumatic swellings, wounds, influenza, black water fever, shortness of breath, inflammation in the mouth, throat and chest, as a counter-irritant and vesicant [11]. *P. zeylanica* is listed among the high trade medicinal plant species in India [14] and annual turnover of its roots is estimated approximately 500 Mt tonnes [15]. Due to being high trade species, over exploitation made it vulnerable in Madhya Pradesh, endangered in Chhattisgarh and in many other parts of the country [16-18].

Plumbagin (5-hydroxy-2-methyl-naphthalene-1,4-dione) is a major active ingredient mainly found in the roots of *P. zeylanica* (Fig. 1). It is credited with a number of pharmacological activities such as antimicrobial, antihepato toxic, antitumor, antifertility, antidiabetic, anticoagulant, hypolipidaemic, antiatherosclerotic, GST, antioxidant, blood coagulation, anti-*Helicobacter pylori*, synergistic etc. [19].

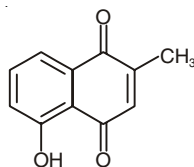


Fig. 1. Chemical structure of plumbagin

Madhya Pradesh is a large state of India and can be divided into 11 agro-climatic regions on the basis of its climatic conditions, which include temperature, rainfall, soil conditions etc. Various medicinal plants viz., *Terminalia arjuna*, *Embelia tsjeriam-cottam*, *Andrographis paniculata*, etc. have been studied to find out variations in active ingredients in the state [20-22]. As far as *P. zeylanica* is concerned, no information on the extent of variability in the concentration of plumbagin in *Plumbago* populations of Madhya Pradesh was available. Therefore, this study has been planned for evaluation of plumbagin in roots of this prestigious species for quality assessment as well as selection of elite chemotype.

EXPERIMENTAL

The root samples of *P. zeylanica* were collected in the month of December from twelve locations of nine agroclimatic regions of Madhya Pradesh following purposive sampling. GPS locations of collection sites were also recorded (Table-1). Root samples of three accessions representing the whole diversity of the species on that location were taken for the study. Taxonomic identification of the plant was confirmed by Biodiversity & Sustainable Management Division of Tropical Forest Research Institute (T.F.R.I.), Jabalpur, Madhya Pradesh, India (Identification No. 1764) and the voucher specimen was deposited in the same division of Tropical Forest

Research Institute. Plumbagin standard was purchased from Sigma Aldrich, India. All chemicals and solvents used were of AR grade.

Processing of plant material: Root samples of each location were homogenized separately and washed in running water to remove the unnecessary foreign particles, cut into small pieces and dried in shade. The shade dried roots were grinded to make powder and stored in air-tight polythene bags for chemical analysis.

Evaluation of plumbagin in root extracts using HPTLC technique: Quantitative evaluation of plumbagin in root extracts of *P. zeylanica* was carried out by standard method [23] with some modification.

Statistical analysis: Each sample on TLC plates was applied in triplicate and results were expressed as mean $\pm$ SD ($n = 3$). Analysis of variance was carried out to check the results as significant and non-significant.

RESULTS AND DISCUSSION

Plumbagin content in roots of *P. zeylanica* collected from twelve different locations is given in Table-1 which showed the highest content of plumbagin in root samples of Tropical Forest Research Institute, Jabalpur (0.25 %) followed by Jaraudhi, Singrauli (0.19 %) and Tamia, Chhindwara (0.17 %) respectively and the lowest content in root samples of Pamakhedi, Khandwa (0.009 %). Variation in plumbagin content in root samples of different locations can also be seen in Fig. 2.

The result showed significant variation in plumbagin content in the roots of *P. zeylanica* collected from different locations of Madhya Pradesh. The variations in the secondary metabolites may be due to various environmental/agroclimatic factors viz. temperature, humidity, drought, light intensity, high salinity, supply of water, minerals, freezing temperatures and CO₂ which influence the growth of a plant and secondary metabolites production [6]. It has also been reported that stress conditions trigger the accumulations of secondary metabolites in plants and help them to adapt according to the environment and in overcoming those conditions [24]. Chemical composition of *P. zeylanica* root is affected by the plant physiological stage and rainfall patterns in the locality [25] which is

TABLE-1
COLLECTION PLACES, GPS LOCATIONS AND PLUMBAGIN CONTENT IN ROOTS OF *Plumbago zeylanica*

Agroclimatic regions	Districts	Places of collection	GPS location	Plumbagin (% dry wt.)
Northern Hill zones of Chhattisgarh	Anuppur	Amarkantak	N 22°40'59.1"; E 81°45'07.7"	0.150 $\pm$ 0.01
Bundelkhand zone	Chhatarpur	Bijawar	N 24°37'27.7"; E 79°29'44.6"	0.037 $\pm$ 0.00
Nimar Valley	Khandwa	Pamakhedi	N 21°53'28.0"; E 77°05'02.0"	0.009 $\pm$ 0.00
Grid zone	Gwalior	Audhpur	N 26°10'17.3"; E 78°10'33.3"	0.160 $\pm$ 0.01
	Seopur kalan	Khori	N 25°30'10.5"; E 77°07'00.6"	0.100 $\pm$ 0.03
Satpura Plateau	Chhindwara	Tamia	N 22°20'40.5"; E 78°39'57.4"	0.170 $\pm$ 0.01
Vindhyan Plateau	Sehore	Pandado	N 22°46'33.4"; E 77°37'23.8"	0.041 $\pm$ 0.00
Central Narmada Valley	Hoshangabad	Pachmarhi	N 22°26'28.0"; E 78°25'05.3"	0.130 $\pm$ 0.01
Malwa Plateau	Mandsaur	Navali	N 24°39'04.0"; E 75°45'61.6"	0.043 $\pm$ 0.00
Kymore plateau & Satpuda Hills	Jabalpur	TFRI	N 23°05'55.3"; E 79°59'21.7"	0.250 $\pm$ 0.01
	Panna	Shyamagiri	N 24°10'35.2"; E 80°29'03.6"	0.110 $\pm$ 0.00
	Singrauli	Jaraudhi	N24°02'16.9"; E82°31'00.5"	0.190 $\pm$ 0.03
CD _{0.05}				0.0325
CD _{0.01}				0.0441
SE $\pm$				0.0156

TFRI = Tropical Forest Research Institute

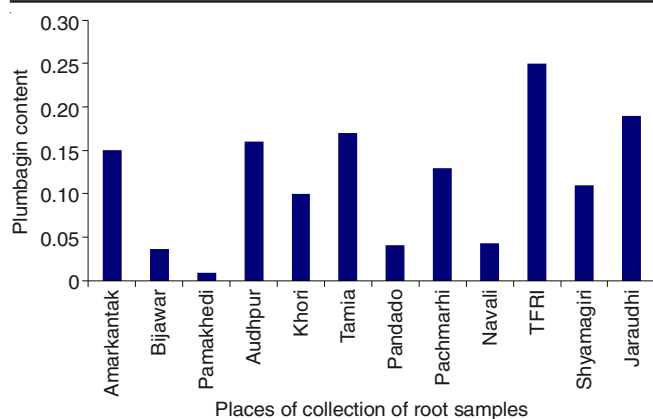


Fig. 2. Plumbagin content in roots of *Plumbago zeylanica* of different locations

supported by a study that water-logged conditions during rainy season caused damage of apical shoots and consequently stunted vegetative growth of field-grown seedlings of *P. zeylanica* [26]. Moreover, a recent study confirmed that water stressed conditions have positive response to root length, dry herbage, plumbagin, potassium and proline contents probably due to increase in many hormones levels in the roots, including the growth hormone which may alter and affect the growth of the plant in the altered environment [27,28]. Similarly, denitrification and accumulation of copper in the water logged conditions stunted vegetative growth and lowers the plumbagin content due to less availability of nitrogen to the plant and great affinity of plumbagin to form copper chealates respectively and thus, only free plumbagin is detected by the instrument [29,30].

Madhya Pradesh state is much diversified in environmental, edaphic and rainfall patterns and here also, the rainfall pattern may be the reason for causing the variation in plumbagin content. The five locations viz., Tropical Forest Research Institute (Jabalpur), Jaraudhi (Singrauli), Tamia (Chhindwara), Audhpur (Gwalior) and Amarkantak (Anuppur) were found to contain more content of plumbagin than reported earlier (0.145 %) [23].

Conclusion

India is among the richest biodiversity countries in the world and the global resurgence in traditional and alternative health care systems provide an opportunity for emerging the Indian trade and commerce in the field of pharmaceuticals, phytochemicals, nutraceuticals, cosmetics and other herbal products. The study investigated that plumbagin content among *Plumbago* populations varied with geographical locations of Madhya Pradesh significantly. Accessions from Tropical Forest Research Institute, Jabalpur was found to contain maximum plumbagin content and thus, of best quality. These accessions may be considered as elite chemotypes and may be *in situ* conserved for further exploitation. Tropical Forest Research

Institute, Jabalpur has also been found the best location out of twelve locations in terms of quality of roots.

ACKNOWLEDGEMENTS

The authors thank Director of Tropical Forest Research Institute, Head of Chemistry Department, Govt. Model Science College, Jabalpur, India and Head of NWFP Division, Tropical Forest Research Institute for providing necessary facilities for carrying out the work.

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