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Full Length Research Paper

Phyllanthin biosynthesis in *Phyllanthus amarus*: Schum and Thonn growing at different altitudes

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Phyllanthus amarus **Schum and Thonn is a member of family *Euphorbiaceae* and highly used in Indian system of medicine to cure numerous human ailments. The lignans, Phyllanthin and hypophyllanthin present in plant are reported as therapeutically active constituents and serve as hepatoprotective agents. We studied on 23 natural populations of *P. amarus*, collected from different geographical regions of India, which were differed in altitude (114 - 5295 ft). The phyllanthin content was extracted and quantified in different parts of *P. amarus* using high performance thin layer chromatography (HPTLC) method at 282 nm wavelength. Out of 23 populations, 16 populations showed increased phyllanthin content with elevated altitude. The highest amount of phyllanthin was found in the leaves followed by fruits and stem, whilst the roots have the least amount of phyllanthin. The highest and lowest phyllanthin content on dry weight basis was 3.163 mg/g in population P_{21} at 2214 ft and 1.431 mg/g in population P_2 at 114 ft and a positive correlation was observed in 16 populations for phyllanthin content with the increased altitude. These results showed the importance of habitat characteristics in the biosynthesis of phyllanthin compound and possible mechanism may involve their antioxidant activity.**

Key words: High performance thin layer chromatography, phyllanthin, quantification, secondary metabolite.

INTRODUCTION

Phyllanthus amarus Schum and Thonn has long history in traditional system of medicine in every tropical country, where it grows and well known for their biologically active compounds, it possesses. It is a common Arabic weed of disturbed ground in Southern Florida, Bahmas, West Indies, Tropical America, and is naturalized in the old world tropics. Medicinal plant produce a wide variety of secondary metabolites, which are indispensable for the survival of plants, since many of these secondary metabolites have important ecological functions such as resistance against diseases and herbivores (Hartmann, 1996). The biosynthesis of secondary metabolites varies among plants, even in different organs of plants and their biosynthesis depends on the environmental factors in which they grow. Intra-specific variation in phytoconstituents has been documented extensively among the plants (Johnson and Scriber, 1994; Chew and Rodman, 1979).

Differences in biosynthesis can result from both genetic and phenotypic variations. Phenotypic variation is especially pronounced in the physiological responses of a plant under growth conditions. The phytoconstituents present in *P. amarus* are alkaloids, flavanoids, hydrolysable tannins, polyphenols and major lignans respectively. The lignans found in a wide range of plant species and display numerous pharmacological activities (Pool-Zobel et al., 2000; Arroo et al., 2002). In view of the important pharmacological properties, numerous studies have been carried out in an attempt to get a better knowledge of the biological events linked to the biosynthesis and the accumulation of lignans (Seidel et al., 2002;

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Sicilia et al., 2003).

Limited information is available regarding lignan biosynthesis accumulation and regulation. The lignan; phyllanthin and hypophyllanthin is the main therapeutically active constituents of *P.* amarus, and widely used as hepatoprotective agent (Shyamsundar et al., 1985; Padma and Setty, 1999). Besides, these phytoconstituents a variety of natural products have been found in *P. amarus* to inhibit the activity of unique enzymes and proteins, which are crucial to the life cycle of HIV including efficient intervention with the reverse transcription process, but also virus binding, the integrase or protease (Vlietinck et al., 1998; De Clercq, 2000; Jung et al., 2000; Cos et al., 2004). The aerial parts of *P. amarus* are used in traditional medicine for its healing properties in different countries (Foo and Wong, 1992). The different plant parts of it, are ethnobotanically used in various diseases and disorders e.g. leaves as expectorant and diaphoretic, fruits as carminative, laxative, astringent, diuretic, diaphoretic and tonic to the liver (Kirtikar and Basu, 2001).

P. amarus is distributed all over India and is considered as the most widely occurring species of *Phyllanthus* (Chaudhary and Rao, 2002). A factor rarely assessed is the altitude of the growing site. Many environmental factors like precipitation, mean temperature, soil, wind speed, low and high temperature extremes, duration of snow-cover, length of the vegetation period and the intensity of radiation under clear sky conditions have been reported to differ between low and high altitude sites (Korner, 1999).

Moreover, study on phytochemicals of wild populations of plant at different altitudes were performed and it is not conclusive whether the observed variations are a response of individual plants to environmental factors related to altitude or a genetic adaptation of the populations growing at different altitudes to their specific environment (McDougal and Parks, 1984; Polle et al., 1992; Veit et al., 1996; Ruhland and Day, 2000; Zidorn and Stuppner, 2001a; Zidorn et al., 2005b).

However, *P. amarus* grows in different geographical regions of India and environmental factors fluctuate at various altitudes. In view of the importance of this species, its large scale multiplication and cultivation of quality planting material (based on the content of active ingredients) is urgently required. The present study was, therefore, carried out to determine phyllanthin content in different populations and their parts of *P. amarus*, spread across an altitudinal gradient, in the different geographical regions of India.

MATERIALS AND METHODS

The present study was taken on *P. amarus* to study phyllanthin content variation in different populations using HPTLC technique. Research work was carried out at Center for Transgenic Plant Development, Jamia Hamdard, Hamdard Nagar, New Delhi, India.

Three populations were selected from the top, middle and lower stands for each altitudinal range and ten quadrats (1 x 1 m) were laid at random, within each stand. Twelve individuals/stand were sampled randomly for recording morphological characters (plant height, leaflet per compound leaf, compound leaf per plant, length of compound leaf and number of fruits per compound leaf).

The populations of *P. amarus* were collected at mature fruiting stage in the month of August (2007) from 23 different geographical locations of India (Table 1). Three mature individuals from each stand were removed for the estimation of phyllanthin. The samples of each location (leaf, stem root and fruit) were oven dried at 40°C until constant weight; net dry weight was used for extraction of phyllanthin.

Phyllanthin extraction

The dried plant material was ground in a pestle mortar to obtain a homogenous drug powder. The crude phyllanthin was extracted according to the method described by (Tripathi et al., 2006). Three subsamples (1.0 g) of each population were extracted with 25 ml of 96% CH_3OH at (25 ± 5°C) on water bath for 10 h and extracted phyllanthin was estimated by HPTLC technique. All chemicals used in phyllanthin extraction and estimation were HPLC grade and purchased from Merck, Germany.

HPTLC analysis

The extracted phyllanthin from different populations were spotted in the form of bands (width 4 mm) with a Camag microlitre syringe on precoated silica gel aluminium plate 60F - 254 (20 cm x 10 cm with 0.2 mm thickness, E. Merck, Germany) using a Camag Linomat V (Switzerland). A constant application rate 150 nl/s was employed and space between two bands was 7.2 mm. The slit dimension was kept at 5.00 x 0.1 mm Micro, and 20 mm/s scanning speed was employed. The mobile phase consisted of toluene: ethyl acetate (2: 1, v/v). Linear ascending development was carried out in twin trough glass chamber, saturated with mobile phase. The optimized chamber saturation time for mobile phase was 15 min at room temperature. The length of chromatogram run was 80 mm. Subsequent to the development; TLC plates were dried with air current provided by an air-dryer. Densitometric scanning was performed on Camag TLC scanner IV in absorbance mode at 282 nm.

Deuterium and tungsten lamps were used as radiation sources. The solvent system produced sharp and compact peak of phyllanthin (R_f 0.30 ±/0.05, (Figure 1). Densitometric analysis of phyllanthin was carried out at 282 nm in absorbance modes. The regression analysis data showed good linear relationship with r = 0.9955 with respect to concentration vs peak area in the concentration range of 10 - 500 ng per spot. The regression equation obtained was Y = 24.302X + 788.98. Specificity of the method was checked by comparing superimposed UV spectrum (Figure 2) of populations with standard. Intra and inter-day variation of phyllanthin at two different concentration levels showed low percent Relative Standard Deviation (0.88 - 1.31%). The content of phyllanthin in different populations was analyzed from the regression equations using values of area obtained from wincats software.

Different volumes of standard solution of phyllanthin 0.1, 0.2, 0.4, 0.5, 1.0, 2.0, 4.0 and 5.0 µl of 100 µg/ml were spotted in triplicate on TLC plate to obtain 10, 20, 40, 50, 100, 200, 400 and 500 ng per spot of phyllanthin respectively. The data of peak area vs. phyllanthin concentration were treated by linear least-square regression and the regression equation thus obtained from standard curve was used to estimate phyllanthin in different

Table 1. Morphometric traits of *P. amarus* plant populations.

Populations	Locations	Altitude (ft)	Plant height (cm)	Leaflet per compound leaf	Compound leaf per plant	Length of compound leaf	No of seeds per compound leaf
P_1	Gowahti (Assam)	170	29.651 ± 5.52	15.33 ± 1.24	19.33 ± 2.62	4.93 ± 0.12	25.66 ± 1.24
P_2	Goalpara (Assam)	114	27.371 ± 3.72	13.33 ± 2	16.33 ± 1.69	4.55 ± 0.42	24.33 ± 1.24
P_3	Pantnagar (Uttaranchal)	793	30.377 ± 1.68	14.0 ± 1.63	19.66 ± 1.69	5.05 ± 0.12	25.33 ± 1.24
P_4	Dehradun (Uttaranchal)	2155	32.518 ± 1.61	14.33 ± 2.35	20.33 ± 0.47	5.22 ± 1.68	27.66 ± 0.09
P_5	Kathua (J&K)	1007	29.963 ± 3.81	17.33 ± 3.29	20.00 ± 0.81	5.14 ± 0.25	27.00 ± 2.16
P_6	Bhadarwah (J&K)	5295	31.926 ± 2.56	17.66 ± 0.35	21.00 ± 0.81	4.86 ± 0.26	27.66 ± 1.69
P_7	Jammu (J&K)	1072	32.89 ± 2.00	17.33 ± 1.24	21.66 ± 1.24	5.49 ± 0.4	27.00 ± 0.81
P_6	Udhampur (J&K)	2480	34.25 ± 3.27	17.33 ± 0.47	17.00 ± 2.16	5.53 ± 0.39	25.66 ± 1.24
P_9	Hyderadad (A.P)	1607	46.904 ± 2.58	23.66 ± 1.69	30.00 ± 1.63	6.52 ± 1.71	38.33 ± 1.24
P_{10}	Karimnagar (A.P)	869	40.636 ± 3.71	20.00 ± 0.81	23.33 ± 2.05	6.43 ± 0.8	34.66 ± 5.24
P_{11}	Agra (U.P)	561	37.733 ± 3.72	19.00 ± 0.81	20.66 ± 0.94	6.22 ± 1.68	32.33 ± 4.02
P_{12}	Gorakhpur(U.P)	229	35.56 ± 4.45	19.66 ± 2.05	16.33 ± 1.69	10.3 ± 2.89	28.66 ± 2.35
P_{13}	Basti (U.P)	255	35.584 ± 6.08	18.33 ± 1.24	16.33 ± 1.24	6.47 ± 1.43	29.66 ± 3.07
P_{14}	Aligarh (U.P)	295	35.516 ± 3.08	19.33 ± 0.62	20.66 ± 1.69	5.93 ± 2.16	31.00 ± 4.96
P_{15}	Kota (Rajsthan)	889	42.545 ± 6.18	20.00 ± 0.00	23.33 ± 1.69	6.78 ± 0.48	36.33 ± 4.02
P_{16}	Jaipur (Rajsthan)	1417	43.711 ± 5.78	22.33 ± 1.24	23.66 ± 1.24	6.94 ± 0.37	39.00 ± 4.32
P_{17}	Indore (M.P)	1791	51.933 ± 6.4	27.00 ± 2.16	31.00 ± 1.41	10.33 ± 1.64	41.33 ± 3.29
P_{18}	Sagar (M.P)	1689	48.188 ± 6.63	24.66 ± 0.94	29.43 ± 1.41	11.47 ± 1.3	40.66 ± 4.49
P_{19}	Betul (M.P)	2181	57.004 ± 6.47	27.66 ± 1.69	31.66 ± 2.05	10.79 ± 0.45	44.00 ± 4.32
P_{20}	Guna (M.P)	1555	39.581 ± 4.06	23.33 ± 1.24	24.66 ± 1.88	8.57 ± 0.42	37.66 ± 2.35
P_{21}	Chhindwana (M.P)	2214	58.71 ± 4.49	26.66 ± 1.88	33.66 ± 1.69	11.78 ± 0.37	42.66 ± 1.24
P_{22}	Jamia Hamdard (Delhi)	780	39.044 ± 3.70	19.66 ± 1.69	22.00 ± 1.63	6.14 ± 0.13	34.66 ± 3.85
P_{23}	Gurgaon(Haryana)	721	38.708 ± 4.44	20.00 ± 2.16	23.33 ± 2.05	6.82 ± 1.14	35.66 ± 6.64

Values are mean ± SD for three replicate in each group.

populations. Each extract was analyzed in duplicate and the mean value was used for the calculation of phyllanthin concentrations in plant material.

RESULTS AND DISCUSSION

Characterization of the phytochemical variation is an essential first step towards executing any organized plant conservation or improvement programs. Environmental factors have important role in physiology of plants as well as in shaping the vegetation. In dynamic environments, plants can respond to the changing conditions through altered production of chemical compounds phyllanthin. In this study, we examined phyllanthin content variation in the different populations collected from natural dynamic environment with morphometric traits and content of phyllanthin were varied with increased altitudes (Tables 1 and 2). The morphometric traits of collected populations viz., plant height, leaflet per compound leaf, compound leaf per plant, length of compound leaf and number of fruits had negative correlation with increased altitudes.

The morphometric traits including plant height (27.371 ± 3.72 cm), leaflet per compound leaf (13.33 ± 0.47), length of compound leaf (4.55 ± 0.42 cm) and number of fruits per compound leaf (24.33 ± 1.24) were more reduced in population (P_2) at low altitude 114 ft as compared to high altitude. Some populations P_2, P_{12} and P_{13} had similar compound leaf per plant at various altitudes. In contrast to P_2 population, the population (P_{21}) showed augmented variation in morphometric traits including plant height (58.71 ± 4.49 cm), compound leaf per plant (33.66 ± 1.69) and length of compound leaf (11.78 ± 0.37 cm) respectively. The number of leaflet and fruits per compound leaf were highest in population P_{19} at altitude 2181 ft, and these were 27.66 ± 1.69 and 44.00 ± 4.32 as compared to populations collection at low altitude.

At elevated altitude, some populations P_4 (2155 ft) and P_6 (5295 ft) showed slight variation in plant height to each other, and it was 32.518 ± 1.61 (cm) and 31.926 ± 2.56 (cm), even as these population had wide difference in altitude. The reason behind wide variation in morphometric traits may be other various environmental factors presented at various locations along with altitudes. Similarly, the most of the morphological data such as length of petiole in sago palm was highly variable

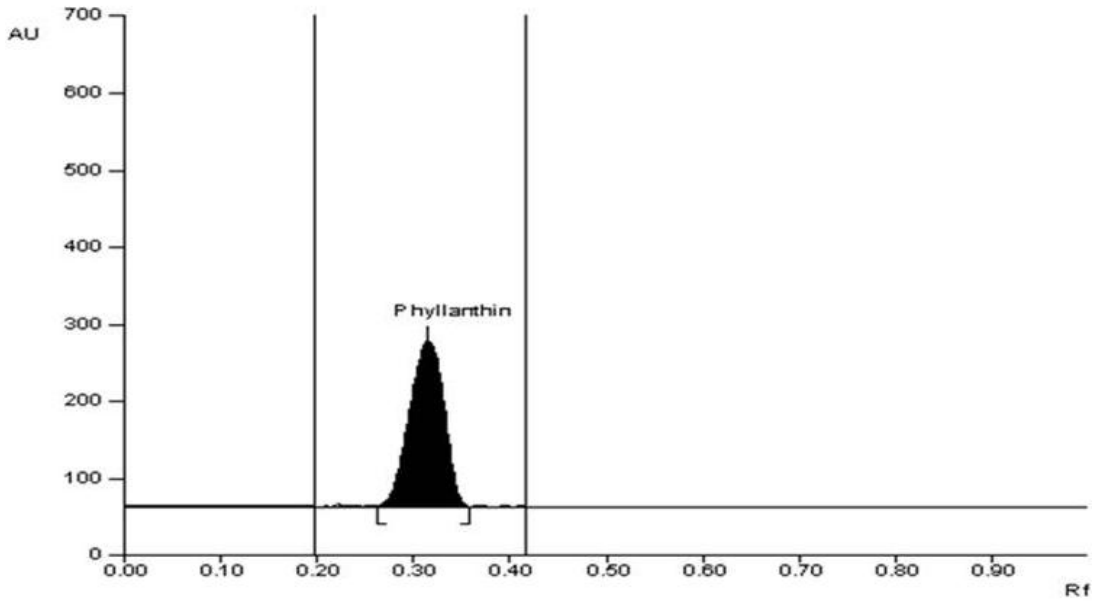

Figure 1. Chromatogram of phyllanthin compound.

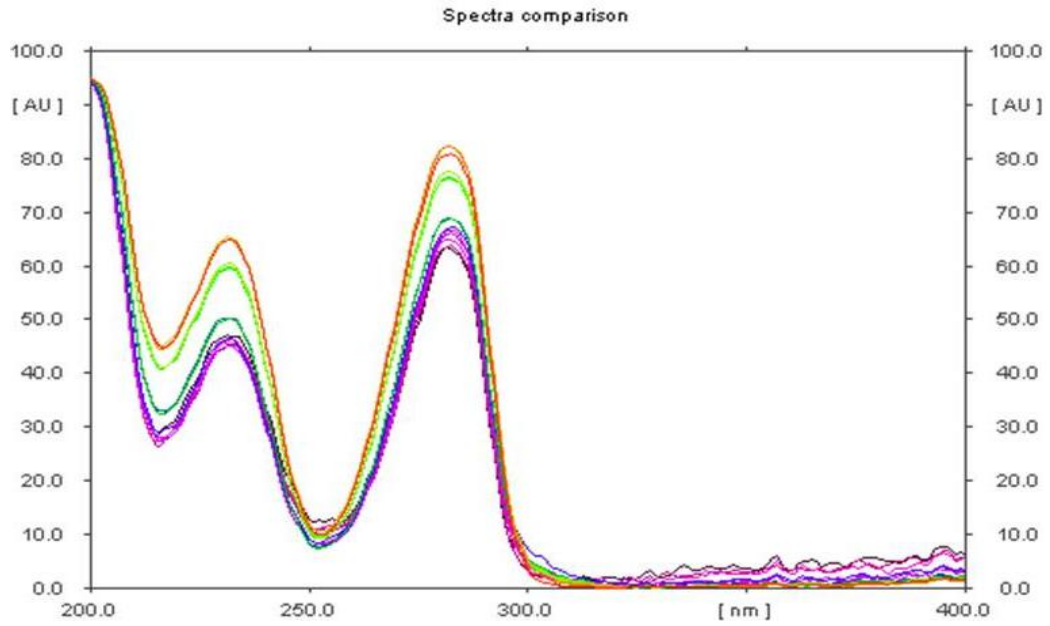

Figure 2. UV spectra of phyllanthin compound.

according to environmental conditions (Kjaer et al., 2004).

The morphometric traits and phyllanthin content were varied under different environmental conditions at elevated altitude and both had strong correlation to thephyllanthin biosynthesis. The phyllanthin content was also varied in plant parts of same site of collected (Table 2). As the morphometric traits varied from one geographical region to another geographical region, in the same way phyllanthin content also varied and high variation was found in the population (P_{21}) as compared to other populations. Out of 23 populations, 16 populations showed increased biosynthesis with the increased altitude. The 7 populations including P_3, P_{10}, P_5, P_7, P_4, P_8 and P_6 at altitude 793 ft, 869 ft, 1007 ft, 1072 ft, 2155 ft, 2480 ft and 5295 ft had total phyllanthin content 2.147 mg/g, 2.707 mg/g, 2.02 mg/g, 2.345 mg/g, 2.313 mg/g, 2.368 mg/g and 2.24 mg/g respectively. They

Table 2. *Phyllanthin* content in root, stem, leaf and seed of different populations of *P. amarus* estimated at wavelength 282 nm.

Populations	Locations	Altitude (ft)	Root Phyllanthin (mg/g dry wt)	Stem Phyllanthin (mg/g dry wt)	Leaf Phyllanthin (mg/g dry wt)	Seed Phyllanthin (mg/g dry wt)	Total Phyllanthin (mg/g dry wt)
P_1	Gowahti (Assam)	170	0.014 ± 0.004	0.024 ± 0.004	6.61 ± 0.283	0.183 ± 0.004	1.708
P_2	Goalpara (Assam)	114	0.014 ± 0.004	0.013 ± 0.004	5.58 ± 0.360	0.113 ± 0.004	1.431
P_3	Pantnagar (Uttaranchal)	793	0.026 ± 0.004	0.047± 0.004	8.38 ± 0.139	0.23 ± 0.004	2.147
P_4	Dehradun (Uttaranchal)	2155	0.033 ± 0.004	0.05 ± 0.008	8.92 ± 0.182	0.25 ± 0.004	2.313
P_5	Kathua (J&K)	1007	0.033 ± 0.004	0.043 ± 0.004	8.90 ± 0.307	0.233 ± 0.004	2.302
P_6	Bhadarwah (J&K)	5295	0.033 ± 0.004	0.056 ± 0.004	8.62 ± 0.457	0.263 ± 0.004	2.244
P_7	Jammu (J&K)	1072	0.036 ± 0.004	0.066 ± 0.004	9.00 ± 0.090	0.276 ± 0.004	2.345
P_6	Udhampur (J&K)	2480	0.033 ± 0.004	0.07 ± 0.008	9.07 ± 0.054	0.293 ± 0.004	2.368
P_9	Hyderadad (A.P)	1607	0.08 ± 0.008	0.186 ± 0.012	11.59 ± 0.216	0.543 ± 0.004	3.1
P_{10}	Karimnagar (A.P)	869	0.063 ± 0.012	0.160 ± 0.008	10.14 ± 0.049	0.463 ± 0.004	2.707
P_{11}	Agra (U.P)	561	0.043 ± 0.04	0.113 ± 0.009	9.59 ± 0.137	0.39 ± 0.008	2.535
P_{12}	Gorakhpur(U.P)	229	0.036 ± 0.004	0.073 ± 0.004	9.21 ± 0.117	0.30 ± 0.008	2.405
P_{13}	Basti (U.P)	255	0.04 ± 0.008	0.09 ± 0.008	9.19 ± 0.148	0.333 ± 0.004	2.413
P_{14}	Aligarh (U.P)	295	0.043 ± 0.004	0.106 ± 0.012	9.27 ± 0.228	0.371 ± 0.004	2.448
P_{15}	Kota (Rajsthan)	889	0.053 ± 0.004	0.16 ± 0.008	11.04 ± 0.054	0.473 ± 0.004	2.932
P_{16}	Jaipur (Rajsthan)	1417	0.063 ± 0.004	0.173 ± 0.004	11.07 ± 0.086	0.483 ± 0.004	2.947
P_{17}	Indore (M.P)	1791	0.116 ± 0.012	0.24 ± 0.008	11.56 ± 0.084	0.620 ± 0.008	3.134
P_{18}	Sagar (M.P)	1689	0.10 ± 0.008	0.213 ± 0.012	11.41 ± 0.163	0.596 ± 0.009	3.018
P_{19}	Betul (M.P)	2181	0.126 ± 0.004	0.22 ± 0.016	11.58 ± 0.265	0.633 ± 0.004	3.14
P_{20}	Guna (M.P)	1555	0.07 ± 0.008	0.183 ± 0.004	11.18 ± 0.135	0.506 ±0.004	2.986
P_{21}	Chhindwana (M.P)	2214	0.133 ± 0.004	0.255 ± 0.004	11.61 ± 0.108	0.65 ± 0.00	3.163
P_{22}	Jamia Hamdard (Delhi)	780	0.06 ± 0.008	0.137 ± 0.004	10.31 ± 0.227	0.44 ± 0.008	2.736
P_{23}	Gurgaon (Haryana)	721	0.05 ± 0.008	0.130 ± 0.008	9.49 ± 0.408	0.423 ±0.004	2.524

Values are mean ± SD for three replicate in each group.

were negatively of phyllanthin correlated for phyllanthin biosynthesis with increased altitudes.

The concentration of phyllanthin was higher in leaf (Figure 4) than fruit, stem and root and it was found 11.61 ± 0.108 mg/g and 0.65 ± 0.00 mg/g, 0.255 ± 0.004 mg/g and 0.133 ± 0.004 mg/g in population (P_{21}) and 5.58 ± 0.360 mg/g, 0.113 ± 0.004 mg/g, 0.014 ± 0.004 mg/g and 0.013 ± 0.004 mg/g in population (P_2) respectively. Some populations of various altitudes had similar content of phyllanthin in root, stem and seed. The populations P_4, P_5, P_6 and P_8 had similar content of phyllanthin in root approximately 0.033 mg/g at altitude 2155 ft, 1007 ft, 2295 ft and 2480 ft; and population P_{10} and P_{16} had 0.063 mg/g in root at altitudes 869 ft and 1417 ft; and population P_{11} and P_{14} had 0.043 mg/g in root at altitude 561 ft and 1417 ft respectively. Two populations P_{10} and P_{15} had similar content of phyllanthin 0.160 mg/g in stem at altitudes 869 ft and 889 ft.

Similarly, populations P_3 and P_5 had similar content of phyllanthin in seed at altitudes 793 ft and 1007 ft respectively. The reason behind this similarity of phyllanthin content may be environmental or genetic factors. However, leaf had varied content of phyllanthin in all collected populations from different altitudes and it may be due to the different rate of photosynthesis at various altitudes. Our results was supported by the result of

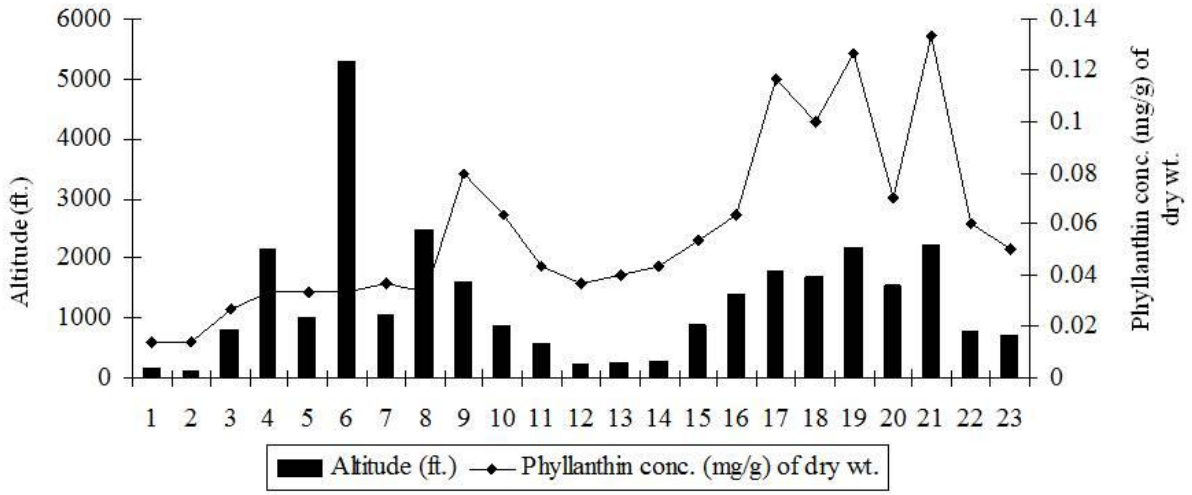

Figure 3. Phyllanthin content in *P. amarus* root collected from different populations growing at various altitudes.

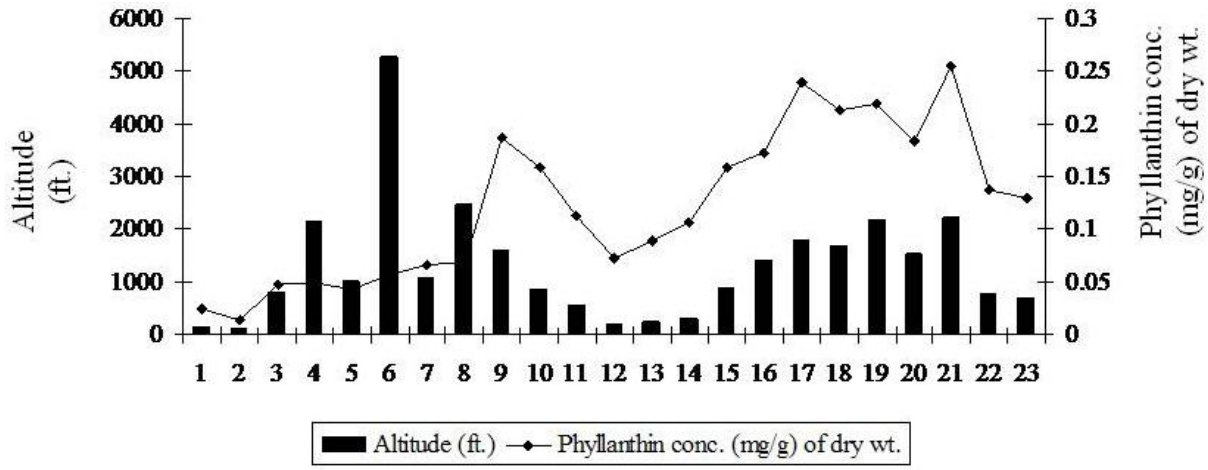

Figure 4. Phyllanthin content in *P. amarus* stem collected from different populations growing at various altitudes.

Murugaiyah and Chan (2007) who found lignans higher in leaf than followed by fruit, branch, stem and least lignans was found in root of *Phyllanthus niruri*. The results were in contrast to the study by Chang et al. (2003) who reported that phyllanthin was the highest in both the aerial part and the roots of *P. urinaria*. Recently, Khatoon et al. (2006) reported that phyllanthin and hypophyllanthin were present in the methanolic extract of *P. amarus* but not in *P. fraternus* and *P. maderaspatensis* using an HPTLC method. The variation in biosynthesis of secondary metabolites in different parts of plant may be due to expression or activity of genes/enzymes (Jiang et al., 2006).

As the altitude increased of collected populations, phyllanthin content also increased in the same way in all parts of plant (Figures 3, 4, 5 and 6) except some populations which had similar content of phyllanthin in root as altitude increased and it was 0.033 ± 0.004 mg/g at (2155 ft) in P_4, 0.033 ± 0.004 mg/g at (5295 ft) in P_6 and 0.033 ± 0.004 mg/g (2480 ft) in P_8 respectively. Similarly, the phyllanthin content in stem and leaf of populations P_5 and P_6 had negative correlation to the increased altitude and these populations had slight variation in phyllanthin biosynthesis and it was found 0.043 ± 0.004 mg/g (1007 ft) and 8.62 ± 0.457 mg/g at (5295 ft) respectively. Beside altitudes, the observed high degree of variability in the phyllanthin content (total and individual) from the different geographical locations could be due to growing conditions or the age of the plants during sample collection.

Our work was in line with the work of Nadeem et al. (2007), who estimated the lignan; podophyllotoxin, which found in *Podophyllum hexandrum* and its content in rhizomes ranged between 0.36 - 1.08% (on dry wt. basis) in different populations and a positive correlation was observed between podophyllotoxin content and an

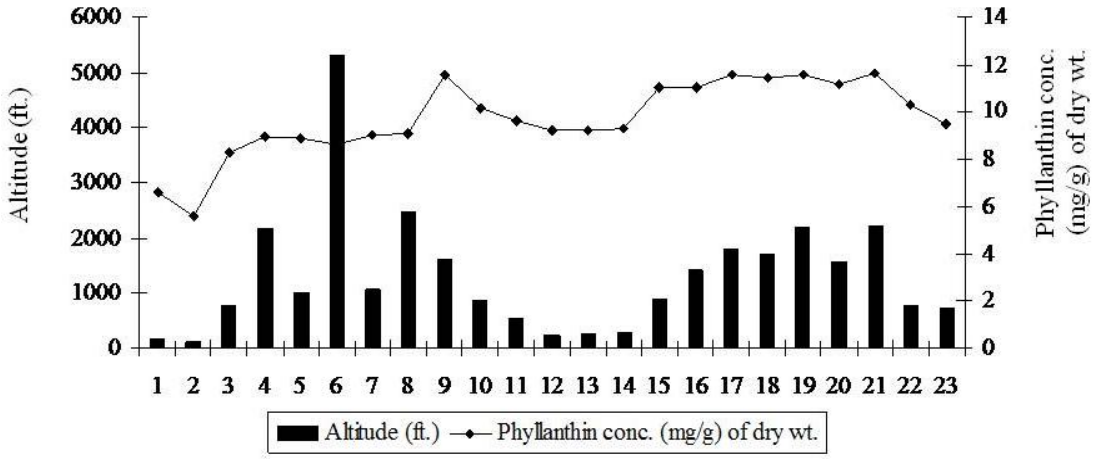

Figure 5. Phyllanthin content in *P. amarus* leaf collected from different populations growing at various altitudes.

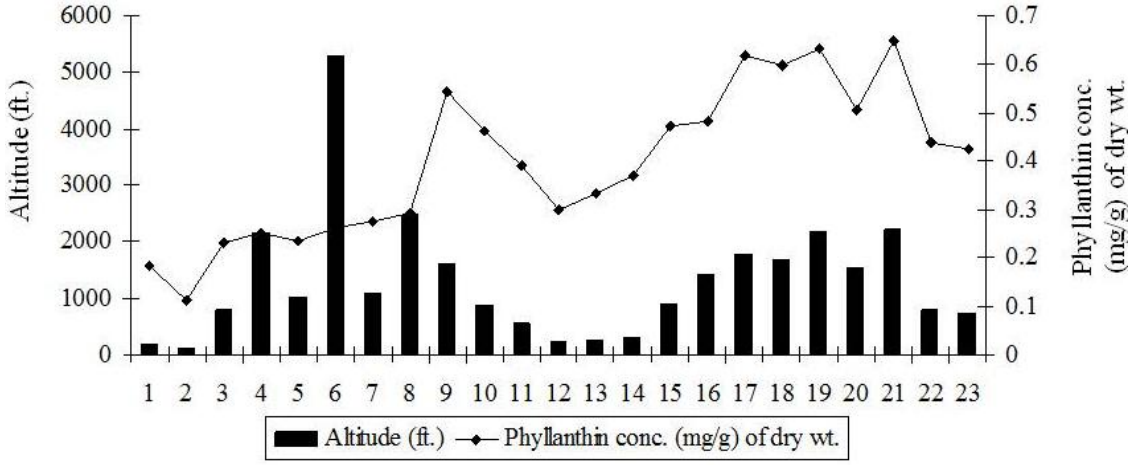

Figure 6. Phyllanthin content in *P. amarus* seed collected from different populations growing at various altitudes.

increase in an altitude. Similarly, the lignan, 3-O-beta-D-Galactopyranoside of quercetin increased at elevated altitude in *P. hexandrum*, which showed radioprotective, metal chelating and DNA protective properties (Chawla et al., 2005). Similarly, phenolics level in *P. arachenoideum* and *Salix mirsinifolia* was high at elevated altitude as compared to low altitude (Miguel et al., 2007; Tegelberg and Julkunen-Tiitto, 2001). Total amount of caffeic acid derivatives, flavanoids and phenolics significantly increased with increase of altitude in *Arnica montana* cv. ARBO (Ganzera et al., 2008; Italer et al., 2008).

In summary, the results from this study suggest that the *P. amarus* examined in this study, the variation in phyllanthin biosynthesis was highly influenced by environmental factors prevailing in different geographical locations. Thus, varied environmental factors have profound effects on phyllanthin biosynthesis and further, phyllanthin compound of elevated altitude of different geographical regions could be best source for pharmacological study.

Conclusion

Phyllanthin biosynthesis in natural condition was highly affected at various altitudes. The biosynthesis was positively correlated at higher altitudes except few populations. The content of phyllanthin was higher in those populations which were collected from higher altitudes as compared to lower altitude. Besides altitude, the variation in phyllanthin content in various populations may be other environmental factors, because plant was growing under open environment.

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