Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; SP1: 568-573

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Screening of traits for higher biochemical constituents production in Kalmegh (*Andrographis paniculata*) plants harvested at pre-flowering stage

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Abstract
In case of various medicinal plants, understanding the association of biochemical constituents with its contributing component is of paramount importance for utilizing the best use of their relationship because it is a complex character and depending upon several components, polygenic in inheritance and prone to influenced by the environment. In this regard, an experiment was conducted to know the traits relation and impact of various traits on andrographolide, neo-andrographolide and diterpenoid yield from the plants of Kalmegh harvested at pre-flowering stage. Highly significantly positive correlation of andrographolide % and neo-andrographolide % was observed with andrographolide yield (0.634 and 0.599 respectively) and diterpenoid lactones yield (0.523 and 0.766 respectively) in Kalmegh plants harvested at pre-flowering stage. Multiple regression analysis of biochemical parameters showed that andrographolide yield and neo-andrographolide yield contributed significantly towards higher diterpenoid lactones yield in Kalmegh plants at pre-flowering stage. Path value analysis of biochemical parameters showed maximum direct positive effect by neo-andrographolide yield (0.572) on diterpenoid lactones yield in plants of Kalmegh at pre-flowering stage. Principal component analysis of biochemical characters in Kalmegh plants at before flowering stage showed that neo-andrographolide, diterpenoid lactones and andrographolide yield had maximum genetic advance (111.34%, 90.66% and 79.81% respectively), which indicates further scope of improvement in Kalmegh for higher biochemical production and indicating its significance to be selected as important biochemical parameters for its crop improvement programme.

Keywords: Kalmegh, *Andrographis paniculata*, andrographolide, neo-andrographolide, diterpenoid lactones yield

Introduction
Probing of structure of biochemical constituents involves assessment of mutual relationship among various characters contributing to it. The estimate of correlation coefficient mostly indicate interrelationship of different characters but it does not furnish information on cause and effect. Under such situation, path analysis helps the breeder to identify the index of selection, which needs due importance while practicing selection with aimed to improve biochemical yield of Kalmegh. Further, path coefficient analysis helps in partitioning of correlation coefficients into direct and indirect effects and in the assessment of relative contribution of each component character to biochemical constituents and extensively been used in breeding experiments (Nagvanshi *et al.,* 2015).

In this regard, genotypic and phenotypic correlation reveals the degree of association between different characters and thus aid in selection to improve the biochemical constituents and its attributing characters simultaneously. Both additive and non-additive component of gene action were reported to be important for biochemical constituents and its components, a clear understanding and knowledge of genotypic association and contribution of various biochemical constituents is essential for any selection programme aimed at its improvement. Phenotypic and genotypic characterization provides vast scope of identifying useful and unique germplasm resources for utilization in crop improvement (Upadhyaya *et al.,* 2005). Keeping in view the importance of trait analysis and path value analysis to screen out suitable parameters affecting biochemical contents and to determine the heritability and genetic advance for its biochemical constituents of Kalmegh, a systematic research trial was undertaken to assess variability for biochemical contents of Kalmegh germplasm and further subjected to various analysis to draw meaningful conclusion.

Kalmegh (*Andrographis paniculata* Wall. ex Nees.) is an important indigenous medicinal plant, native to India, commonly known as 'King of bitters' (Saraswathy *et al.,* 2004; Chauhan

et al., 2009; Gomathinayagam *et al.,* 2009) found throughout tropical and sub-tropical Asia. It belongs to the tribe andrographideae of the subfamily Acanthoideae. It is an erect, branched annual herb, extremely bitter in taste, grows to a height of 30-110 cm in moist shady places with glabrous leaves and white flowers with rose-purple spots on the petals (Varaprasad *et al*., 2006). Concerns about conservation of Kalmegh as an endangered species have been initiated during the past decade (Pushpangadan *et al.,* 2001; Natarajan *et al.,* 2004). It is one of the 32 prioritized medicinal plants of India with a demand of 2197 tones and annual growth of 3.10% (Anonymous, 2007). Several studies highlighted the fact that the most relevant factor on the quality of *Andrographis paniculata* depends on collecting location and the harvesting time of the plant (Sabu, 2006; Bhan *et al*., 2006; Lattoo *et al*., 2006; Valdiani *et al*., 2012a).

Andrographolide is the major bioactive phytoconstituent found in various parts of Kalmegh particularly in the leaves. Extensive datamining of the phytochemistry and pharmacology of *Andrographis paniculata* revealed more than 50 diterpene lactones, 30 flavonoids, 8 quinic acid derivatives, and 4 xanthones (Subramanian *et al.,* 2012). The extract of Kalmegh is used as anti-pyretic, anti-periodic, antibacterial, anti-malarial, anti-inflammatory, anti-thrombogenic, blood purifier, hepatoprotective, antihepatotoxic, antibiotic, antimalarial, antihepatitic, antiinflammatory, antisnakevenom, immunostimulant besides the treatment of jaundice, dermatological diseases, dyspepsia, febrifuge and anthelmintic disorders (Saraswathy *et al.,* 2004; Chauhan *et al.,* 2009; Gomathinayagam *et al.,* 2009; Kapadi *et al.,* 2010; Niranjan *et al.,* 2010). The plant extracts exhibits antityphoid and antifungal activities (Anonymous, 1985). Kalmegh exhibited a wide scope of pharmaceutical properties such as anti-HIV (Basak *et al.,* 2006), anti-H_1N_1 (Ko *et al.,* 2006), anti-hepatitis (Dumrongsak *et al.,* 2009), anticancer (Chun *et al.,* 2010) and anti-HIV (Calabrese *et al.,* 2000).

Materials and Methods

The trail was conducted at Research Farm of BAU, Ranchi during 2013-14 and 2014-15 under the climatic and edaphic conditions of Jharkhand. Geographically, it is located at $23^0$26'30" N latitude and $85^0$18'20" E longitude in Chotanagpur plateau, situated in north eastern part of India and at an altitude of 646 m above the mean sea level. The soil of the site is lateritic, developed from granite-gneiss, sandy loam in texture, sedentary in nature and well drained with low water holding capacity and poor consistency. The experimental materials comprised of twenty five genotypes of Kalmegh, for which seeds were collected from its natural habitat across six states of India and NBPGR, New Delhi including wild and cultivated varieties. Out of 25 Kalmegh accessions, 4 each were collected from Jharkhand, Chhattisgarh, Madhya Pradesh, Karnataka, 6 from Odisha, 2 from NBPGR and one from Gujarat. Experiment was laid out in Randomized Block Design with twenty five treatments and three replications at 30 cm × 30 cm spacing. Ten plants selected randomly from all the treatments were harvested at pre-flowering stage for the estimation of major active principles, *viz.,* andrographolide and neo-andrographolide using standard HPLC protocol given by Vijaykumar *et al.* (2007). Diterpenoid lactones yield (kg/ha) in plant at before flowering stage was calculated by adding the value of andrographolide and neo-andrographolide yield. Collected data on bio-chemical parameters was subjected to statistical analysis like correlation analysis, multiple regression analysis, path value analysis and principal component analysis for validation of results.

Genotypic variance was derived by the difference of mean square for treatment and mean square due to error and dividing it by number of replications as per Burton and de Vane (1953).

$$б^2g = \frac{MST - MSE}{r}$$

Where, $б^2g$ = genotypic variance, MST = mean square due to treatment

MSE = mean square due to error, r = number of replications

Phenotypic variance – It was calculated as $б^2p = б^2g + б^2e$

Where, $б^2p$ = phenotypic variance, $б^2g$ = genotypic variance $б^2e$ = environmental variance

Genotypic coefficient of variation (GCV) – It was calculated by dividing the square root of genotypic variance by the population mean and multiplying by 100 Burton and de Vane (1953).

$$G.C.V. = \frac{\sqrt{б^2g}}{x} \times 100$$

Where, $б^2g$ = genotypic variance, x = population mean of a particular character.

Phenotypic coefficient of variation (PCV) - It was calculated by dividing the square root of phenotypic variance by the population mean and multiplying by 100 Burton and de Vane (1953).

$$P.C.V. = \frac{\sqrt{б^2p}}{x} \times 100$$

Where, $б^2p$ = phenotypic variance, x = population mean of the particular character

Heritability – It was calculated by the ratio of genotypic variance to the phenotypic variance and calculated for a character according to the formula suggested by Johnson *et al.,* (1955)

$$h^2 = \frac{б^2g}{б^2p} \times 100$$

Where, $б^2g$ = genotypic variance, $б^2p$ = phenotypic variance

Genetic advance is the difference between the mean genotypic value of the selected lines and the mean genotypic value of the parental population and was estimated by the formula suggested by Johnson *et al.,* (1955).

Genetic advance = $K \times бp \times h^2$

Where, h = heritability in broad sense, бp = Phenotypic standard deviation

K = Standardized selection differential expressed in terms of standard deviation units and its value varies with the intensity of selection. In the present study K = 2.06, which is the value at 5% selection intensity (Miller *et al.,* 1958).

Following statistical procedure was done for drawing meaningful conclusions from the data generated of qualitative characters

Correlation coefficient was calculated by using the formula suggested by Miller *et al.,* (1958). As biochemical constituents are one of the most important characters among all other economic characters, correlation of it with other attributes would facilitate effective selection schemes. Multiple regression analysis determines the nature and strength of the relationship between characters and calculated through software programme Indo Stat. Path value analysis is a standardized partial regression coefficient and measures the

direct influence of one variable upon another and permits the separation of correlation coefficient into components of direct and indirect effects. It was calculated by the formula suggested by Dewey and Lu (1959).

Results and Discussion

Table 1 represents correlation matrix between diterpenoid lactones yield and its different biochemical contributing factors in Kalmegh plants collected before flowering stage. Andrographolide % in plants showed highly significantly positive correlation with neo-andrographolide % (0.539), andrographolide yield (0.634) and diterpenoid lactones yield (0.523), but showed highly significantly negative relation with methonolic yield extract (–0.679) and alkaloid yield (–0.669). Neo-andrographolide % showed highly significantly positive correlation with andrographolide yield (0.599), neo-andrographolide yield (0.845) and diterpenoid lactones yield (0.766). Methonolic yield extract showed highly positively significant correlation with alkaloid yield (0.833), but significantly negatively correlated with andrographolide yield (–0.408). Alkaloid yield showed significant negative correlation with andrographolide yield (–0.433). Andrographolide yield showed highly significantly positive correlation with neo-andrographolide yield (0.836) and diterpenoid lactones yield (0.949). Neo-andrographolide yield also showed highly significant positive correlation with diterpenoid lactones yield (0.966). The positive correlation between desirable characters is favorable for simultaneous improvements of both the characters.

Valdiani *et al.,* (2012a) observed negative correlation between stem dry weight per plant and andrographolide percentage per plant in different Kalmegh accessions of Malaysia. They also observed positive correlations between andrographolide percentage with leaf/stem ratio and mentioned that andrographolide yield per hectare and andrographolide content per plant tended to increase negligibly with increasing genetic distance. Gupta and Pareek (1981) opined that the total leaf area and plant height are significantly correlated with active ingredients. Nemade *et al.,* (2003) recorded that the date of harvesting had a significant influence on yield attributes and andrographolide content.

Table 1: Correlation matrix between diterpenoid lactones yield (kg/ha) in *Andrographis paniculata* plants (pre-flowering) and its contributing factors

Variables	V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8
V_1	1.000							
V_2	0.620**	1.000						
V_3	0.981**	0.631**	1.000					
V_4	0.497*	0.981**	0.539**	1.000				
V_5	-0.799**	-0.507**	-0.679**	-0.335^{NS}	1.000			
V_6	-0.758**	-0.475*	-0.669**	-0.328^{NS}	0.833**	1.000		
V_7	0.616**	0.637**	0.634**	0.599**	-0.408*	-0.433*	1.000	
V_8	0.359^{NS}	0.820**	0.393^{NS}	0.845**	-0.204^{NS}	-0.254^{NS}	0.836**	1.000
V_9	0.496*	0.769**	0.523**	0.766**	-0.309^{NS}	-0.349^{NS}	0.949**	0.966**

*- significant at 5% level, **- significant at 1% level,

Where V_1 - Concentration of andrographolide in plant, V_2 - Concentration of neo-andrographolide in plant, V_3 - Andrographolide (%) in plant, V_4 - Neo-andrographolide (%) in plant, V_5 - Yield of methonolic extract in 10g plant herbage, V_6 - Yield of alkaloid in 10g herbage, V_7 - Andrographolide yield in plant, V_8 – Neo-andrographolide yield in plant, V_9 – Diterpenoid lactones yield in plant.

Table 2 represents regression coefficients and significance of biochemical parameters affecting diterpenoid lactones yield of Kalmegh plant harvested before flowering period.

Table 2: Regression coefficients and significance of contributing parameters affecting diterpenoid lactones yield (kg/ha) in *Andrographis paniculata* plants (before flowering)

Variables	Coefficients	Standard Error	t-value
V_1	-0.000	0.004	-0.001
V_2	0.001	0.005	0.113
V_3	-0.004	0.220	-0.016
V_4	-0.030	0.323	-0.093
V_5	0.034	0.142	0.237
V_6	-0.011	0.107	-0.099
V_7	1.001**	0.004	273.587
V_8	0.999**	0.004	239.772
Constant: -0.051			

**- significant at 1% level, $t_{(7)}$ at 5% = 2.365, $t_{(7)}$ at 1% = 3.499

Multiple regression analysis indicated that two parameters like andrographolide yield (1.001) and neo-andrographolide yield (0.999) contributed significantly towards higher diterpenoid lactones yield in Kalmegh germplasm collected before flowering stage. Bhan *et al.,* (2006) noticed significant variations in chemical characters in different Kalmegh accessions because of their different geographical locations with different genetic makeup. They mentioned that deviation in andrographolide and neo-andrographolide concentration was probably arose due to non-uniformity in flowering time and extended phenological phases in different accessions. Pholphana *et al.,* (2004) found appreciable variation in the active components of Kalmegh, which was attributed to difference in genotypes, growing environment, time of harvesting, age of leaves at harvest etc. Lattoo *et al.,* (2008) documented that geographical origin can affect both quality and quantity of the active constituents of Kalmegh.

Table 3 represents path value analysis of different biochemical parameters influencing diterpenoid lactones yield in Kalmegh germplasm collected before flowering stage. Maximum direct positive effect was shown by neo-andrographolide yield (0.572) followed by andrographolide yield (0.471). Maximum indirect positive impact of andrographolide concentration was observed through andrographolide yield (0.291). Maximum indirect positive impact of neo-andrographolide yield was observed through neo-andrographolide yield (0.469) and indirect negative impact through neo-andrographolide % (–0.001). Andrographolide % contributed maximum indirect positive impact through andrographolide yield (0.299).

Table 3: Path analysis of biochemical characters influencing diterpenoid lactones yield in plants of *Andrographis paniculata* (before flowering)

Variables	V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8	Correlation coefficient
V_1	**0.000**	0.000	0.000	0.000	0.000	0.000	0.291	0.205	0.496
V_2	0.000	**0.001**	0.000	-0.001	0.000	0.000	0.300	0.469	0.769
V_3	0.000	0.001	**0.000**	0.000	0.000	0.000	0.299	0.225	0.523
V_4	0.000	0.001	0.000	**-0.001**	0.000	0.000	0.282	0.483	0.766
V_5	0.000	0.000	0.000	0.000	**0.000**	0.000	-0.192	-0.117	-0.309
V_6	0.000	0.000	0.000	0.000	0.000	**0.000**	-0.204	-0.145	-0.349
V_7	0.000	0.001	0.000	0.000	0.000	0.000	**0.471**	0.478	0.949
V_8	0.000	0.001	0.000	0.000	0.000	0.000	0.394	**0.572**	0.966

Residual - 0.0000

Neo-andrographolide % showed maximum indirect positive impact through andrographolide yield (0.483). Methonolic extract yield showed maximum indirect negative impact through andrographolide yield (–0.192). Alkaloid yield showed same trend (–0.204). Andrographolide yield showed maximum indirect positive impact through neo-andrographolide yield (0.478) and maximum indirect positive impact of neo-andrographolide yield was observed through andrographolide yield (0.394). Jaleel *et al.,* (2006) found crucial role of growth regulatory compounds in the regulation and coordination of plant growth, morphogenesis, metabolism and role in biosynthesis of secondary metabolites.

Table 4 represents principal component analysis of biochemical characters in Kalmegh plants collected before flowering stage. Phenotypic variance for all the parameters was more or equal to its corresponding genotypic variance. Little impact of environmental effects was noticed on all the biochemical parameters. The PCV were marginally higher than the corresponding GCV. This showed that the character is governed by additive genes and selection will be rewarded for the improvement of such traits. The concentration of andrographolide was found 16.50% more than neo-andrographolide concentration in plants. Andrographolide % was found 15.78% more than neo-andrographolide %. However, andrographolide yield was 13.42% more than neo-andrographolide yield. Diterpenoid lactones yield in Kalmegh plants collected before flowering stage was 31.21 kg/ha, while methonolic extract yield in 10 g herbage was 1.81 g. Maximum value of phenotypic as well as genotypic coefficient of variation was observed for neo-andrographolide yield (54.76 and 54.40, respectively), followed by diterpenoid lactones yield (44.85 and 44.43 respectively) and andrographolide yield (39.76 and 39.25 respectively). It was observed that andrographolide yield from Kalmegh germplasm was more affected by environmental factors than of neo-andrographolide yield.

All the biochemical parameters showed high heritability %, but neo-andrographolide concentration (99.95%), neo-andrographolide % (99.75%), andrographolide concentration (99.73%) and andrographolide % (99.47%) showed maximum heritability %. Maximum genetic advance was observed for neo-andrographolide yield (142.69%), followed by diterpenoid lactones yield (116.18%) and andrographolide yield (102.28%) indicating their significance to be adopted in crop improvement programme in Kalmegh for higher biochemical production. Herms and Mattson (1992) mentioned that environmental conditions which suppress the growth, will increase the synthesis of secondary metabolites as a defensive reaction.

Burton and de Vane (1953) suggested that genetic coefficient of variation together with the estimates of heritability may give the best idea for the amount of genetic advance to be expected from selection. This could be ascertained from heritability estimates which in broad sense include both additive and non-additive gene effects and in narrow sense include the proportion of heritable variation which is due to additive component (Lush, 1949). Valdiani *et al.,* (2012a) revealed through PCA analysis that the agronomic traits such as leaf dry weight per plant, herbage yield, andrographolide yield per hectare and andrographolide content per plant are the most important characteristics to screen out best cultivar of Kalmegh for commercial cultivation. Schlichting (1989) mentioned that changes in phenotypic correlation between characters will result when the change in environment produces different types of plastic responses by characters.

Valdiani *et al*., (2012a) mentioned about both negative and positive heterosis for different traits in Kalmegh and recorded both additive and non-additive gene effects contributed in controlling phytochemical traits. As per them, non-additive effects play a major role in the genetic basis of heterosis. Andrographolide yield per hectare and andrographolide content per plant tended to increase negligibly with increasing genetic distance. Nagvanshi *et al.,* (2015) found high heritability coupled with high genetic advance for characters *viz*., fresh herbage yield and dry herbage yield in 22 accessions of Kalmegh at Raipur, Chhatisgarh.

Table 4: Components of genetic variability of biochemical characters of Kalmegh plants (before flowering)

Parameters	Grand Mean	Range	Variance			PCV	GCV	ECV	Heritability (%)	GA% of means (at 5%)	GA% of means (at 1%)
			Phenotypic	Genotypic	Environment						
Concentration of andrographolide in plants	87.32	47.10-147.97	838.41	836.11	2.30	33.16	33.11	3.01	99.73	68.12	87.30
Concentration of neo-andrographolide in plants	75.02	46.61-176.04	792.24	791.85	0.38	37.51	37.50	1.43	99.95	77.25	99.00
Andrographolide % in plants	1.54	0.93-2.39	0.16	0.16	0.0009	25.94	25.87	3.28	99.47	53.16	68.13
Neo-andrographolide	1.33	0.83-2.93	0.19	0.19	0.0005	32.60	32.56	2.80	99.75	67.00	85.86

% in plants											
Andrographolide yield (kg/ha) in plants	16.59	9.22-35.33	43.50	42.38	1.12	39.76	39.25	11.03	97.43	79.81	102.28
Neo-andrographolide yield (kg/ha) in plants	14.63	5.71-46.77	64.16	63.32	0.83	54.76	54.40	10.84	98.69	111.34	142.69
Diterpenoid lactones yield (kg/ha) in plants	31.21	16.95-82.11	196.03	192.33	3.69	44.85	44.43	10.66	98.12	90.66	116.18
Yield (g) of plant extract in 10 g herbage	1.81	1.54-2.10	0.03	0.02	0.0005	9.57	9.49	2.17	98.28	19.38	24.84

Conclusion

Correlation matrix between diterpenoid lactones yield and its contributing factors in Kalmegh harvested at pre-flowering stage showed highly significantly positive correlation of andrographolide % with neo-andrographolide %, andrographolide yield and diterpenoid lactones yield. Neo-andrographolide % showed highly significantly positive correlation with andrographolide, neo-andrographolide and diterpenoid lactones yield. Multiple regression analysis of biochemical parameters showed that andrographolide yield and neo-andrographolide yield contributed significantly towards higher diterpenoid lactones yield in Kalmegh plants at pre-flowering stage. Path value analysis of biochemical parameters showed maximum direct positive effect by neo-andrographolide yield on diterpenoid lactones yield in plants of Kalmegh germplasm at before flowering stage. Principal component analysis of biochemical characters in Kalmegh plants at before flowering stage showed that neo-andrographolide, diterpenoid lactones and andrographolide had maximum genetic advance, which indicates further scope of improvement in Kalmegh for higher biochemical production and indicating its significance to be selected as important biochemical parameters for its crop improvement programme.

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