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Per se Performance of Elite Accessions of Indian Pennywort (*Centella asiatica* L.) for Growth, Herbage Yield and Triterpenoid Compounds

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ABSTRACT

Background: Indian Pennywort (*Centella asiatica* L.) is an important tropical medicinal plant having pharmaceutical, neutraceutical and cosmoceutical properties but it is treated as threatened species due to indiscriminate collections from the wild. The whole herb is economically important and its biological effects have been attributed to the existence of major triterpene derivatives including madecassoside, asiaticoside, madecassic and asiatic acid. To meet the industrial requirement on sustainable basis, commercial cultivation of *Centella asiatica* L. is the only solution. The sustained supply of quality raw material to the industry is possible only through cultivation of elite genotypes with high potency as the natural resources are dwindling. Increased productivity will also facilitate the international trade with high potency raw material at a reasonable cost.

Methods: The field evaluation was carried out at Zonal Agricultural and Horticultural Research Station, Mudigere, Keladi Shivappa Nayaka University of Agricultural and Horticulture Sciences, Shivamogga during 2018-19 with nine selected accessions along with check variety Vallabh Medha in randomized complete block design with three replications. These accessions were selected based on *per se* performance with respect to triterpenoid content and its yield over three seasons among 39 accessions collected from different ecological regions of hill zone of Karnataka, India. They were evaluated for quantitative and quantitative traits.

Result: The results of the study indicated that all the parameters studied differed significantly except for chlorophyll content. Among elite accessions, Acc. 07 and Vallabh Medha performed better for most of the growth parameters, while for the yield attributes Acc. 12, Vallabh Medha, Acc. 6 and Acc.18 were found better. Regarding total triterpenoid yield per hectare, the accessions Acc. 12 (180.16 kg ha^{-1}), Acc.3 (141.01 kg ha^{-1}) and Acc.7 (137.98 kg ha^{-1}) seemed to be promising accessions.

Key words: Asiatic and total triterpenoid content, Asiaticoside, Madecassic, Madecassoside, Qualitative, Quantitative, Rosette, Stolons.

INTRODUCTION

Indian Pennywort (*Centella asiatica* L.) is an important tropical medicinal plant belongs to the family Apiaceae with a somatic chromosome number 2n=18. The plant is native to South East Asian countries including India, Sri Lanka, China and Malaysia as well as South Africa and has pantropical in distribution. It occurs throughout India in moist places from plains to hill ranges up to 2000 m (Mukherjee and Constance, 1993). The whole herb is economically important and its biological effects have been attributed to the existence of major triterpene derivatives including madecassoside, asiaticoside, madecassic and asiatic acid (Schaneberg *et al*., 2003). The use of *Centella asiatica* L. in food and beverages has increased over the years due to its health benefits such as anti-inflammatory, cardio protective, wound healing, memory enhancing and antioxidant properties. Further, it is also considered as an effective antidiabetic, antimicrobial and antiproliferative herb. The plant is also used in cosmetic masks and creams to increase the synthesis of collagen and the firming up of the skin. Owing to its innumerable phytopharmaceutical properties, the herb is used in many *Ayurvedic* formulations and products including Mentat and *Mandukaparni* (Memory booster), Geriforte (Stress care), Abana (Heart care), Weight loss tea, Nourishing skin cream, Organic baby skin care, Gotu Kola and Germanium moisturizer, Geriforte vet and Anxocare (Animal health care) *etc*.

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According to the reports of Export and Import Bank of India, *Centella asiatica* L. is one of the important medicinal plants in the international trade (Singh *et al*., 2010). Also, National Medicinal Plant Board (NMPB) reported that the total estimated annual trade of *C. asiatica* L. in the Indian market was between 500-1000 MT, with an average price of fresh herb ranging between 0.40-0.50 US dollar/kg traded annually (Prasad *et al*., 2019). However, the wild stock of this plant species has been markedly depleted, because of its large scale and unrestricted exploitation coupled with limited cultivation and insufficient attempts for its replacement. Further, it is listed as threatened plant species (Gowthami *et al*., 2021) by the International Union for Conservation of Nature (IUCN) with threat category of LC (Least concern).

The Indian Pennywort is treated as threatened species due to indiscriminate collections from the wild. To meet the industrial requirement on sustainable basis, commercial cultivation of *Centella asiatica* L. is the only solution as this is an important upcoming medicinal herb with pharmaceutical, neutraceutical and cosmoceutical properties medicinal plants. Based on a wide range of industrial applications, these medical constituents have a high demand (Nav *et al*., 2021) in the global market (US$900 billion per year).

Increased production is possible by bringing more area under cultivation and by increasing the productivity through crop improvement programmes and cultivation on sound scientific line. Genetic improvement and development of high yielding varieties in *Centella asiatica* L. largely depend on the genetic variability in the species. Identification of superior germplasm accessions for higher herbage yield and triterpenoid contents can promote its cultivation which would benefit the drug industry in terms of higher dry recovery with higher desirable chemical contents.

The sustainable and commercial cultivation of *Centella asiatica* L. largely depends on the availability of elite genotypes and developing such genotypes through planned, systematic and organized crop improvement programme. Further, sustained supply of quality raw material to the industry is possible only through cultivation of elite genotypes with high potency as the natural resources are dwindling. Increased productivity will also facilitate the international trade with high potency raw material at a reasonable cost as both producers and consumers are benefitted.

MATERIALS AND METHODS

The experimental site is situated in the Western Ghats and represents the typical hilly zone (Zone-9) of Karnataka consisting of medium sandy loam soil with acidic pH ranging from 4.8 to 5.00. It is located at an altitude of 982 m above mean sea level at 13°. 25′ North latitude and 75°. 25′ East longitude. Nine elite accessions along with the check variety Vallabh Medha used for the study and evaluated for one cropping season (main crop). These accessions were selected based on *per se* performance with respect to triterpenoid content and its yield over three seasons among 39 accessions collected from different ecological regions of hill zone of Karnataka, India (Table 1). The field evaluation

Table 1: Geographical origin of elite *Centella asiatica* L. accessions collected from hill zone of Karnataka.

Accessions	Collection site			Habitat	Geo reference		
	Village	Mandal/Taluk/Tehsil	District		Latitude (N)	Longitude (E)	Altitude (m)
Acc. 1	Halekote	Mudigere	Chikkamagaluru	Paddy field	13°.07′	75°.37′	916
Acc. 3	Heggadde	Sringeri	Chikkamagaluru	Up land arecanut	13°.27′	75°.15′	558
Acc. 4	Nagalapura	Koppa	Chikkamagaluru	Paddy field	13°.30′	75°.18′	531
Acc. 6	Ithige Seegodu	N.R. Pura	Chikkamagaluru	Paddy field	13°.20′	75°.27′	639
Acc. 7	Kymanahalli (Rakshidi Estate)	Sakaleshpura	Hassan	Coffee + Pepper plantation	13°.01′	75°.4′	859
Acc. 8	Kallarahalli	Sakaleshpura	Hassan	Low land coffee + arecanut plantation	12°.56′	75°.42′	820
Acc. 12	Madikeri	Madikeri	Kodagu	Paddy field	12°.25′	75°.43′	1024
Acc. 18	Bhramana Manchale	Sagara	Shivamogga	Forest	14°.10′	75°.51′	531
Acc. 38	Ammadi	Koppa	Chikkamagaluru	Coffee plantation	13°.33′	75°.20′	660

was carried out at Zonal Agricultural and Horticultural Research Station, Mudigere, University of Agricultural and Horticulture Sciences, Shivamogga during 2018-19 in randomized complete block design with three replications.

The experimental field was brought to fine tilth by repeated ploughing and clod crushing. The plots measuring 2.1 m × 1.8 m were made as per the layout plan leaving a space of 1.0 m between replications and 0.5 m between plots. Well grown stolons from each accession were selected and planted at a spacing of 30 cm × 30 cm in the raised bed system as per the layout plan with protective irrigation under 50% shade green colored net. The recommended agronomic practices as per the DMAPR, Anand, Gujarat and plant protection measures were followed to ensure a normal healthy crop.

Observations were recorded on five randomly selected rosettes in each plot leaving the border rosettes. The mean value of the data was taken to represent a particular accession with respect to a character as per the NBPGR descriptors. The crop was harvested five months after planting at full leaf growth stage. The aerial parts were harvested in 1 m^2 area in the center of the micro plot. The harvested herb was shade dried at room temperature by spreading in thin layers for a week with frequent up turnings till attain constant weight.

Terpenoids content of whole herb was estimated from composite samples of each accession using High Performance Liquid Chromatographic (HPLC) technique. The shade dried herbage samples were thoroughly ground using Matrix mixer and the powder sieved finely and used for the extraction of total terpenoids *viz.*, madecassoside, asiaticoside, madecassic acid and asiatic acid.

Analysis of variance was performed to assess the variability among elite accessions for various quantitative and qualitative traits as per the procedure suggested by Panse and Sukhatme (1967).

RESULTS AND DISCUSSION

The results of the study on various morphometric traits are presented in the Tables 2 to 4. and which indicated significant variations for all the traits studied except for chlorophyll content.

The nine selected accessions along with check variety Vallabh Medha were evaluated for quantitative (stolon length, internodal length, number of nodes per stolon, number of stolons per rosette, leaf length, leaf width, rosette length, rosette diameter, petiole length, chlorophyll content, fresh and dry herbage yield per square meter and per hectare) and qualitative (madecassoside, asiaticoside, madecassic, asiatic and total triterpenoid content and their yield) parameters differed significantly except for chlorophyll content.

The check variety Vallabh Medha registered maximum mean values (Table 2) for number of stolons per rosette (3.70), leaf length (3.88 cm) and width (7.33 cm), rosette length (23.53 cm) and diameter (29.67 cm) and petiole length

Table 2: *Per se* performance of elite *Centella asiatica* L. accessions for morphometric traits.

Accessions	Stolon length (cm)	Internodal length (cm)	Number of nodes per stolon	Number of stolons per rosette	Leaf length (cm)	Leaf width (cm)	Rosette length (cm)	Rosette diameter (cm)	Petiole length (cm)	Chlorophyll content (SPAD units)
Acc. 1	49.53^{ab}	8.97^{cd}	5.67^{ab}	3.27^{ab}	2.19^{d}	3.87^{ef}	16.13^{bc}	19.53^{cd}	15.47^{bcde}	35.00
Acc. 3	42.67^{bc}	8.97^{cd}	4.60^{de}	2.93^{bc}	2.19^{d}	3.59^{f}	15.01^{cd}	19.80^{cd}	16.26^{bc}	37.06
Acc. 4	39.08^{c}	9.07^{cd}	4.47^{e}	3.13^{bc}	2.21^{cd}	3.83^{ef}	13.23^{d}	17.00^{d}	14.59^{cde}	35.62
Acc. 6	51.40^{ab}	8.75^{cd}	5.40^{abcd}	2.93^{bc}	2.58^{c}	4.73^{bc}	18.33^{b}	22.20^{bc}	17.73^{b}	36.23
Acc. 7	57.10^{a}	12.62^{a}	4.67^{cde}	3.20^{b}	3.02^{b}	5.05^{b}	21.67^{a}	26.50^{ab}	22.33^{a}	36.55
Acc. 8	53.87^{a}	10.47^{bc}	5.87^{a}	3.13^{bc}	2.33^{cd}	4.50^{bcd}	15.60^{cd}	21.20^{cd}	14.31^{cde}	35.52
Acc. 12	50.20ab	10.07^{bc}	5.33^{abcd}	3.40^{ab}	2.55^{cd}	4.38^{cde}	17.53^{bc}	21.73^{c}	16.05^{bcd}	35.77
Acc.18	42.00^{bc}	7.99^{d}	5.13^{abcde}	3.00^{bc}	2.27^{cd}	3.99^{def}	13.27^{d}	17.13^{d}	12.80^{e}	34.31
Acc.38	50.27ab	9.39^{bcd}	5.47^{abc}	2.67^{c}	2.33^{cd}	4.39^{cde}	15.07^{cd}	18.87^{cd}	13.50^{de}	35.08
Vallabh Medha*	54.27^{a}	10.97^{ab}	4.87^{bcde}	3.73^{a}	3.88^{a}	7.33^{a}	23.53^{a}	29.67^{a}	22.72^{a}	33.93
S. Em ±	3.27	0.62	0.28	0.18	0.13	0.20	0.92	1.44	0.89	0.82
CD @ 5%	9.71	1.85	0.83	0.52	0.38	0.59	2.73	4.28	2.69	NS
CV (%)	11.54	11.08	9.36	9.71	8.55	7.55	9.39	11.69	9.38	

*Check variety.

(22.67 cm). The highest mean stolon length and internodal length were recorded in accession Acc. 7 (57.10 and 12.62, cm resp.), while maximum number of nodes per stolon were found in accession Acc. 8 (5.87). The manifestation of maximum plant growth attributes in these accessions could be due to inherent genetic makeup and the differential ability of accessions for synthesis of phytohormones which resulted in high growth parameters. Similar variations in growth attributes among genotypes were reported by Krishnamurthy *et al.* (2006) and Singh (2017) in *Centella asiatica* L.; Kumar (2017) in brahmi and Venkatesha *et al.* (2018) in Indian oregano.

Fresh and dry herbage yield per m^2 and per hectare (Table 3) was significantly higher in Acc. 12 (1895.81 and 371.07 g m^{-2} and 18.96 and 3.71 t ha^{-1} resp.) followed by Vallabh Medha (Check variety). The highest fresh and dry herbage yield recorded in Acc. 12 was due to inherent genetic makeup exhibited in terms of quick growth, thick ground cover, large reniform dark green leaves, long petiole and more number of leaves per unit area might have attributed to highest yield. These results are in consonance with the earlier reports of Krishnamurthy *et al.* (2006); Prasad *et al.* (2014); Lal *et al.* (2017) and Singh (2017) in *Centella asiatica* L.; Kumar (2017) in brahmi and Venkatesha *et al.* (2018) in Indian oregano.

Variation in triterpenoid contents had been recorded among the accessions (Table 4), Acc. 7 registered highest madecassoside (3.514% w/w) and asiaticoside content (2.641% w/w). The accessions 3 and 6 recorded highest madecassic acid and asiatic acid content (0.398 and 0.252% w/w), respectively.

The total triterpenoid content among the accessions differed significantly and was maximum in accession Acc.

Table 3: Mean performance of elite *Centella asiatica* L. accessions for yield.

Accessions	Fresh herbage yield		Dry herbage yield	
	Per squaremeter (g)	Per hectare (t)	Per squaremeter (g)	Per hectare (t)
Acc. 1	1576.57^{bc}	15.77^{bc}	279.07^{cd}	2.79^{cd}
Acc. 3	1327.25^{def}	13.27^{def}	241.96^{def}	2.42^{def}
Acc. 4	1161.96^{ef}	11.62^{ef}	207.00^{f}	2.07^{ef}
Acc. 6	1713.65^{ab}	17.14^{ab}	332.73^{ab}	3.33^{ab}
Acc. 7	1115.67^{f}	11.16^{f}	213.33^{ef}	2.13^{ef}
Acc. 8	1355.00^{de}	13.55^{de}	278.00^{cd}	2.78^{cd}
Acc. 12	1895.81^{a}	18.96^{a}	371.07^{a}	3.71^{a}
Acc.18	1517.00^{bcd}	15.17^{bcd}	301.67^{bc}	3.02^{bc}
Acc.38	1401.77^{cd}	14.02^{cd}	259.13^{cde}	2.59^{cde}
Vallabh Medha*	1860.55^{a}	18.61^{a}	356.96^{a}	3.57^{a}
S. Em ±	71.37	0.71	17.04	0.17
CD @ 5%	212.04	2.12	50.63	0.51
CV (%)	8.28	8.27	10.38	10.38

*Check variety.

Table 4: Mean performance of elite *Centella asiatica* L. accessions for quality traits.

Accessions	Triterpenoids content (% w/w dry weight basis)					Triterpenoids yield (kg ha^{-1})				
	Madecassoside	Asiaticoside	Madecassic acid	Asiatic acid	Total	Madecassoside	Asiaticoside	Madecassic acid	Asiatic acid	Total
Acc. 1	1.002^{g}	0.797^{i}	0.280^{f}	0.227^{b}	2.306^{i}	27.96^{f}	22.24^{e}	7.81^{cd}	6.33^{d}	64.34^{d}
Acc. 3	3.457^{a}	1.792^{c}	0.398^{a}	0.179^{d}	5.827^{b}	83.66^{b}	43.37^{cd}	9.63^{b}	4.33^{ef}	141.01^{b}
Acc. 4	3.123^{b}	1.925^{b}	0.343^{b}	0.200^{c}	5.590^{c}	64.65^{cd}	39.85^{d}	7.10^{cd}	4.14^{ef}	115.71^{c}
Acc. 6	1.896^{d}	1.469^{e}	0.291^{e}	0.252^{a}	3.909^{e}	63.07^{d}	48.87^{bc}	9.68^{b}	8.38^{ab}	130.04^{bc}
Acc. 7	3.514^{a}	2.641^{a}	0.173^{i}	0.139^{e}	6.468^{a}	74.97^{bc}	56.34^{ab}	3.69^{e}	2.97^{g}	137.98^{b}
Acc. 8	1.351^{f}	1.053^{g}	0.229^{h}	0.184^{d}	2.816^{h}	37.56^{ef}	29.27^{e}	6.37^{d}	5.12^{e}	78.28^{d}
Acc. 12	2.591^{c}	1.695^{d}	0.325^{c}	0.246^{a}	4.856^{d}	96.13^{a}	62.88^{a}	12.06^{a}	9.13^{a}	180.16^{a}
Acc.18	0.905^{h}	0.772^{j}	0.257^{g}	0.247^{a}	2.181^{j}	27.30^{f}	23.29^{e}	7.75^{cd}	7.45^{bc}	65.79^{d}
Acc.38	1.796^{e}	0.973^{h}	0.307^{d}	0.136^{e}	3.213^{g}	46.58^{e}	25.23^{e}	7.96^{c}	3.53^{fg}	83.32^{d}
Vallabh Medha*	1.875^{d}	1.171^{f}	0.289^{e}	0.185^{d}	3.521^{f}	66.94^{cd}	41.80^{cd}	10.32^{b}	6.60^{cd}	125.70^{bc}
S. Em ±	0.03	0.01	0.001	0.00	0.04	3.98	2.56	0.51	0.34	7.33
CD @ 5%	0.10	0.02	0.01	0.01	0.11	11.81	7.60	1.52	1.02	21.78
CV (%)	2.80	0.65	1.57	2.32	1.57	11.67	11.28	10.74	10.29	11.31

*Check variety.

07 (6.468 % w/w). Differences in triterpenoid contents among the accessions could be due to inherent genetic makeup and their interaction with the prevailing environment, which can be attributed to differential ability of the accessions for synthesis and accumulation of secondary metabolites. Rudriamampionona *et al.* (2007), Thomas *et al.* (2010), Puttarak and Panichayupakaranant (2012), Prasad *et al.* (2014), Srivastava *et al.* (2014), Lal *et al.* (2017) and Singh (2017) recorded wide variations among accessions in *Centella asiatica* L.

The highest total triterpenoid yield in terms of madecassoside, asiaticoside, madecassic and asiatic acid yield per hectare was registered by accessions Acc. 12 (180.16 kg ha^{-1}), Acc.3 (141.01 kg ha^{-1}) and Acc.7 (137.98 kg ha^{-1}) is mainly due to higher dry herbage yield per m^2 and total triterpenoids content. Singh (2017) observed similar differences among *Centella asitica* accessions.

CONCLUSION

Among elite accessions studied, the accession Acc. 07 and Vallabh Medha performed better for most of the growth parameters, while for the yield attributes such as fresh and dry herbage yield, the accession Acc. 12, Vallabh Medha, Acc. 6 and Acc.18 were found better. Regarding total triterpenoid yield per hectare, the accessions Acc. 12 (180.16 kg ha^{-1}), Acc.3 (141.01 kg ha^{-1}) and Acc.7 (137.98 kg ha^{-1}) seemed to be promising accessions. These accessions could be tested further for stability under multi location trials.

Conflict of interest

Authors declares that their is no conflict of interest for publishing this research paper.

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