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Research Paper

Herbage and phytochemicals yield of *mandukaparni* (*Centella asiatica*) as influenced by time of harvest

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ABSTRACT

Indian pennywort or *mandukaparni* [*Centella asiatica* (L.) Urban] containing four triterpenes; asiaticoside, madecassoside, asiatic acid and madecassic acid as principal bioactive phytochemicals, is an important medicinal crop and valued as brain tonic worldwide. An experiment was conducted during 2012–14 at Anand, Gujarat, to investigate the effects of harvest time of main crop of *mandukaparni* as well as ratoon on herbage yield, and triterpenes content and yield. Significantly higher leaf area was found at the first harvesting of main crop at 6 months (21.9 cm^2/leaf) and ratoon harvesting at 4 months (22.1 cm^2/leaf), whereas leaves/node were found markedly higher at 4 months of first harvest (MFH) (11.3) and 2 months of ratoon harvest (MRH) (11). The first harvesting of the main crop at 5 months and ratoon harvesting at 4 months recorded markedly higher dry herbage yield (1,404 kg/ha), being higher by 54.8% over 4 MFH and 2 MRH. The highest triterpenes content were recorded at the first as well as ratoon harvesting at 4 months (2%) and increased by 42.8% over 4 MFH and 2 MRH. The triterpenes yield was markedly higher at the first harvesting of main crop at 5 months and ratoon harvesting at 4 months (26.7 kg/ha), which was 107% higher over 4 MFH and 2 MRH. Thus, *mandukaparni* should be harvested first at 5 months after planting and ratoon at 4 months interval for the production of maximum herbage and phytochemicals on unit area basis under commercial cultivation.

Key words **:** Harvest time, Herbage yield, *Mandukaparni*, Phytochemicals, Triterpenes yield

Indian pennywort or *mandukaparni* [*Centella asiatica* (L.) Urban], is among the top 10 drugs in the category of anti-ageing and central nervous system (CNS) used worldwide (Bhavna and Jyoti, 2011). It is a rich source of pentacyclic triterpenes like asiaticoside, centelloside, brahmoside, brahminoside, thankuniside, sceffoleoside, centellose and madecassoside, and asiatic, brahmic, centellic and madecassic acids (James and Dubery, 2009). It is a popular rasayana drug and is used as medhya rasayana in the CNS disorders like epilepsy, schizophrenia and cognitive dysfunction. It is also used as wound-healing agent and as constituent in brain tonics for developmentally disabled people (Shrestha and Dhillion, 2003; Zainol *et al*., 2003; Mamedov, 2005).

It is necessary to understand the biosynthesis and metabolism of secondary metabolites in medicinal and aromatic plants to increase their production for medicinal, aromatic and culinary uses (Marchese *et al*., 2009). The biosynthesis of secondary metabolites although controlled genetically, is affected strongly by environmental influences (Yazdani *et al*., 2002; Malik *et al*., 2011) and biotic and abiotic stresses (Smetanska, 2008). Agricultural practices have critical effect on quantitative and qualitative characteristics of the medicinal and aromatic plants, which finally result in plant growth, yield and quality determination. Content of bioactive compounds in *C. asiatica* varies with location, climate and agronomic practices (Jamil *et al*., 2007; Randriamampionona *et al*., 2007). Harvesting of medicinal plants at optimum phenological stage for required medicinal quality and efficacy, is very crucial which vary from one species to other. The concentration of biologically and medicinally active secondary metabolites varies with the time of plant growth and development stage of the plant which determine the choice of the optimal harvesting time. Harvesting at optimum stage ensure the best possible quality of raw material and products (Kumar and Kumar, 2013; Mansoori, 2014). Kumar (2000) observed higher alkaloid content in the roots of *ashwagandha* at fruiting stage followed by flowering

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stage. Plant growth and development is also affected strongly by environmental conditions. The production of secondary metabolite trigger under stress due to adverse environment (Thompson *et al.,* 2003; Cristina *et al.,* 2008). Thus, stage of harvesting in any medicinal crop is most important for harvesting of maximum herbage yield as well as phytochemicals production which ultimately leads to good quality raw material for industries. The scientific information on right stage of harvest of *mandukaparni* was not available so far. Hence, present investigation was undertaken to study the effect of harvesting time on herbage yield and principal bioactive compounds (asiaticoside, madecassoside, asiatic acid and madecassic acid, etc.), and to find out the optimum harvest time to produce better quality raw material on unit area basis of *mandukaparni.*

MATERIALS AND METHODS

The experimental site was located at the ICAR-Directorate of Medicinal and Aromatic Plants Research, Anand, Gujarat, India (22°35' N, 72°55 E' and 45.1 m above mean sea-level). It has a semi-arid, sub-tropical climate with hot dry summers and mild winters. The mean annual rainfall received was 946 mm during 2012–13 and 1,142 mm during 2013–14, out of which 95% rainfall was received from June–September. The minimum temperature was recorded in January (10°C in 2013 and 12°C in 2014), whereas, maximum temperatures was in May (40°C in both the years). Evaporation was also recorded higher in May. The minimum relative humidity was in March (25–28%), whereas maximum humidity was highest in September (up to 95%). Soil was sandy clay loam (clay 25%, silt 22% and sand 53%), moderately calcareous, hyperthermic and Vertic Ustochrepts.

Mandukaparni is a perennial crop and can be harvested 150–200 days after planting. Field experiments were conducted during 2012–14 to find out the right time of first harvesting of main crop after transplanting and subsequent harvests at time of ratoon crop for maximum herbage yield, and triterpenes content and yield. The treatment consisted of 3 times; 4, 5 and 6 months of first harvesting (MFH) after planting and 3 times; 2, 3 and 4 months of ratoon harvest (MRH) after first harvesting. The experiment was laid out in split-plot design with MFH in main-plots and MRH in sub-plots, and replicated thrice. The crop was planted during July on the onset of monsoon at 30 cm × 15 cm planting pattern of row-to-row and plant-to-plant in the plots of 5.0 m × 3.6 m size. Since, *mandukaparni* is a partial shade-loving crop, field was covered with 50% green shade net 2 m above the surface. The first crop as well as ratoons were harvested as per respective treatments. After completion of the first year cycle in June, the crop was uniformly harvested and maintained for the second year cycle. The crop was harvested from the net plot area, washed, sun dried and weighed, and calculated herbage yield/ha. For leaf area and number of leaves per node, 5 plants were selected randomly from the net plot area. Leaf area was measured with LI-COR leaf area meter (LI-2100C area meter) and average leaf area was calculated. The number of leaves was counted and calculated the average number of leaves/node or plant.

The leaf samples of *mandukaparni* were collected, dried, powdered and extracted with 90% methanol in water (3 × 75 ml, 1.0 hr.) under reflux in a thermostatically controlled water bath maintained at 90°C temperature. The extracts were combined, filtered, and then evaporated under reduced pressure in rotary evaporator. The residues were dissolved in HPLC-grade methanol and diluted to fit into reference standard calibration curve.

A modular HPLC (High Performance Liquid Chromatography) system; LC system consisted of 2 LC-20AD pumps, SPD-20A UV/VIS detector, DGU-20A3 degasser, SIL-20AC HT auto sampler, CTO-10ASvp column oven, CBM-20 communications bus module and Grace make Altima column (100 mm × 4.6 mm, 3 µm). The column temperature was kept at 40°C for better resolution. The mobile phase composed of acetonitrile (A) and 0.1% formic acid (B) in gradient condition as per procedure described by Rafamantanana *et al.* (2009). The sample injection volume was kept at 20 µL and the UV/VIS detector was set at 205 nm. The samples were injected and measured the concentration (mg/litre) of all 4 triterpenes (asiaticoside, madecassoside, asiatic acid and madecassic acid) in *mandukaparni*. These values were converted and presented in the percentage on dry weight basis of the sample.

The data obtained were subjected to statistical analysis using Microsoft Excel. Analysis of variance was done as per the procedure outlined by Gomez and Gomez (1984).

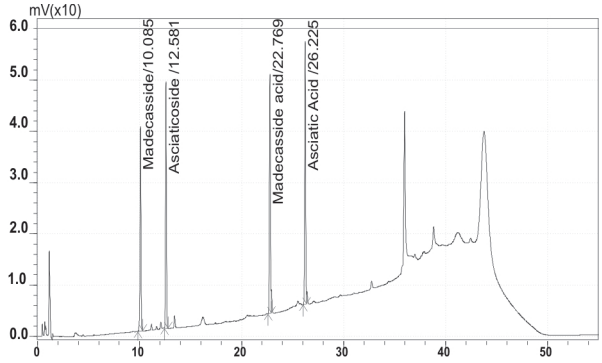

Fig. 1. HPLC chromatogram of reference compounds of *Centella asiatica*

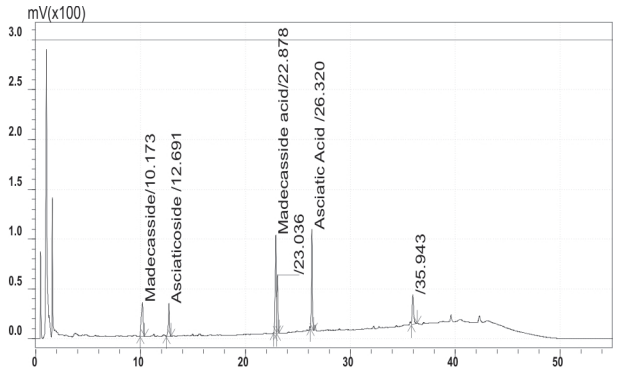

Fig. 2. HPLC chromatogram of leaf extract of *Centella asiatica*

The signicant differences between treatments were compared using standard error of mean (SEm±) and critical difference (CD) at 5% level of probability (*P*=0.05).

RESULTS AND DISCUSSION

Growth and herbage yield

Harvesting of main crop as well as ratoon at different times intervals markedly influenced plant growth and yield (Table 1). The first harvesting of main crop at 6 months showed significantly higher leaf area, which was 15.8 and 12.8% higher over 4 and 5 months, respectively. The ratoon harvesting at 4 months recorded significantly higher leaf are a being 20.7 and 12.2% higher over 2 and 3 months respectively. The number of leaves/node or plant was markedly higher at first harvesting of main crop at 4 months and decreased at 5 and 6 months. First harvesting at 6 months decreased number of leaves by 14% over 4 months. Harvesting of ratoon at 2 months recorded higher number of leaves and decreased at delayed harvesting at 3 and 4 months. Ratoon harvesting at 4 months decreased number of leaves by 10% over 2 months.

The significantly higher dry herbage yield recorded at first harvesting of main crop at 5 months. First harvesting at 5 months increased the herbage yield of main crop by 11.4% over 4 months and decreased by 11.8% at 6 months. Ratoon harvesting at 4 months interval revealed markedly higher dry herbage yield which was increased by 31.3 and 18.7% over 2 and 3 months respectively.

The interaction between time of first harvesting and ratoon harvesting showed significant response to the dry herbage yield (Table 2). The first harvesting of main crop at 5 months and subsequent harvesting of ratoon at 4 months interval recorded highest dry herbage yield, being higher by 54.8% over 4 MFH and 2 MRH. The next best time of harvesting was at 6 MFH and 4 MRH (1,090 kg/ha) followed by 4 MFH and 4 MRH (1,063 kg/ha).

Table 1. Effect of harvesting time on leaf area, leaf number and herbage yield (mean data of 2 years)

Treatment	Leaf area (cm^2/leaf)	Leaves/ node	Dry herbage yield (kg/ha)
Time of first harvesting			
4 MFH	18.9	11.3	992
5 MFH	19.4	10.5	1106
6 MFH	21.9	9.9	989
SEm±	0.10	0.20	15
CD (P=0.05)	0.40	0.60	58
Time of ratoon harvesting			
2 MRH	18.3	11.0	903
3 MRH	19.7	10.7	999
4 MRH	22.1	10.0	1186
SEm±	0.40	0.20	20
CD (P=0.05)	1.40	0.60	61

MFH, Months of first harvest; MRH, months of ratoon harvest

Table 2. Interaction effects of harvest time of main crop and ratoon on herbage yield (kg/ha) (mean of 2 years)

Time of ratoon harvest	Time of first harvesting		
	4 MFH	5 MFH	6 MFH
2 MRH	907	908	895
3 MRH	1,008	1,005	983
4 MRH	1,063	1,404	1,090
SEm±		34	
CD (P=0.05)		106	

MFH, Months of first harvest; MRH, months of ratoon harvest

The year-wise variation in dry herbage yield was also recorded (Fig. 3). The dry herbage yield was comparatively higher in the second year than in the first year across all the harvesting stages. In both the years, the first harvesting of main crop at 5 months and ratoon harvesting at 4 months resulted in higher herbage yield. The first harvesting either early at 4 months or delayed to 6 months and

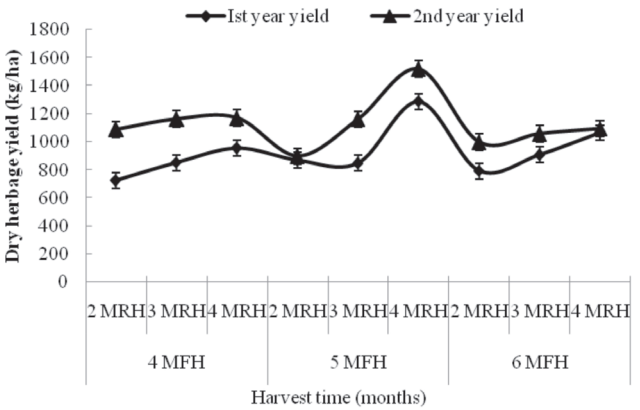

Fig. 3. Year-wise variation in herbage yield (mean of number of harvests at each stage). Error bars represent standard error of mean

ratoon harvesting at early stages at 2 and 3 months recorded less yield in both the years.

The time of harvesting in *mandukaparni* markedly influenced leaf area, leaf number and herbage yield. Leaf area was the highest when main crop was harvested at 6 months and ratoon at 4 months. Leaf area increased with crop duration which might be owing to increased cell-division and cell-expansion that contributed to the final organ size. Besides this, it might be owing to favourable environmental conditional that temporally coordinate cell expansion and size. The first harvesting of main crop at 6 months and ratoon at 4 months coincide with the months of December and April, respectively, when ambient temperature was quite favourable for crop growth and development (maximum temperature 30–35°C and minimum temperature 15°C) and resulted in to increased leaf area. The maximum leaves were found at first harvesting of main crop at 4 months and ratoon at 2 months and thereafter decreased. The decrease in number of leaves at late harvesting stages might be due to leaf senescence. Senescence might be caused due to either age factor or the ethylene-induced abscission of petioles. *Mandukaparni* is a frequently irrigated crop with partially submerged conditions which favoured the ethylene production and abscission of leaf petioles thus, decreased the leaves/plant at late harvests. *Mandukaparni* showed the higher herbage yield when main crop was first harvested at 5 months and subsequently ratoon harvesting at 4 months and decreased at late harvest stages. It might be owing to the optimum levels of number of leaves, and leaf area and favourable environmental conditions for crop growth and development. This period of harvest coincides with November and March, respectively, where plant experienced favourable temperature and humidity. The plant growth is initially slow then exponential growth phase followed by a plateau and subsequently reduction due to leaf senescence. At the first harvesting of main crop at 5 months in November, the crop might have reached to exponential growth stage with optimum leaf number and leaf area after the wet season. After first harvesting, the crop experienced a winter break and grow slowly due to low temperature during January followed by favourable spring season and simultaneously increased availability of reserves food stored in the winter season and thus, increased herbage yield of ratoon at 4

Table 3. Effect of harvest time on total triterpenes content and yield (mean of 2 years)

	Asaticoside (%)	Made cassoside (%)	Asiatic acid (%)	Madecassic acid (%)	Total triterpenes content (%)	Total triterpenes yield (kg/ha)
Time of first harvesting						
4 MFH	0.47	0.99	0.13	0.09	1.7	16.8
5 MFH	0.45	0.90	0.12	0.08	1.5	17.6
6 MFH	0.39	0.87	0.12	0.08	1.5	14.5
SEm±	0.01	0.02	0.01	0.003	0.02	0.08
CD (P=0.05)	0.03	0.07	NS	NS	0.07	0.33
Time of ratoon harvesting						
2 MRH	0.39	0.83	0.09	0.06	1.4	12.3
3 MRH	0.40	0.86	0.14	0.09	1.5	14.9
4 MRH	0.52	1.06	0.14	0.10	1.8	21.7
SEm±	0.01	0.03	0.01	0.004	0.03	0.32
CD (P=0.05)	0.03	0.08	0.02	0.01	0.10	0.98

MFH, Months of first harvest; MRH, months of ratoon harvest

Table 4. Effect of time of first harvest of ratoon on triterpenes content and yield (mean of 2 years)

Time of ratoon harvest	Time of first harvesting					
	Triterpenes content (%)			Triterpenes yield (kg/ha)		
	4 MFH	5 MFH	6 MFH	4 MFH	5 MFH	6 MFH
2 MRH	1.4	1.4	1.3	12.9	11.5	12.5
3 MRH	1.6	1.5	1.4	16.0	14.6	14.1
4 MRH	2.0	1.9	1.5	21.6	26.7	16.7
SEm±		0.06	0.55			
CD (P=0.05)		0.18	1.69			

MFH, Months of first harvest; MRH, months of ratoon harvest

months in March. The results showed the better performance of the crop in the second year than in the first year in terms of herbage yield. This might be owing to more rainfall and relative humidity experienced in the second year which favoured the growth and yield. Another reason could be the development of better root system in the second year which might have increased the nutrient and water absorption and supply for crop growth and development. In some of the medicinal plants like, *Withenia somnifera* and *Dracocephalum moldavica*, the best harvesting time was reported at 100% flowering stage (Wankhade *et al*., 2010; Prshang and Reza, 2014), whereas in garden thyme 50% blooming stage was found best harvest time for highest economical yield (Golparvar, 2011). Similarly, harvesting of kalmegh at 135 days after transplanting resulted in the maximum dry herbage yield and it decreased until seed maturity (Kumar and Kumar, 2013). The reduction in herbage yield of kalmegh was mainly due to lesser dry-matter yield/plant and senescence of leaves at later stages of crop growth (Maheshwari *et al.*, 2002; Pandey *et al.*, 2003; Singh *et al.*, 2011).

Triterpenes content and yield

The harvesting stage of *Mandukaparni* at different biological stages markedly influenced the content and yield of all 4 triterpenes (asiaticoside, madecassoside, asiatic acid and madecassic acid) (Table 3). The first harvesting of main crop at 4 months recorded the highest asiaticoside, madecassoside and total triterpenes content which further decreased significantly at 5 and 6 months. However, asiatic acid and madecassic acid were not affected due to time of first harvesting. Ratoon harvesting at 4 months interval recorded significantly higher asiaticoside, madecassoside, asiatic acid, madecassic acid and total triterpenes content. The total triterpenes yield was significantly higher at first harvesting of main crop at 5 months, which was increased compared to 4.8% over 4 months and decreased by 17.6% at 6 months harvest. Ratoon harvesting at 4 months recorded significantly higher triterpenes yield which was increased by 76.4 and 45.6% over 2 and 3 months respectively.

The interaction effects of time of first harvesting as well as ratoon harvesting showed significant variation in triterpenes content and yield (Table 4). The first harvesting of main crop at 4 months and subsequent harvesting of ratoon at 4 months recorded significantly higher triterpenes content, which was increased by 42.8% over the first harvesting of main crop at same stage of 4 months and ratoon harvesting at 2 months. However, triterpenes yield per unit area was recorded markedly higher at the first harvesting of main crop at 5 months and ratoon harvesting at 4 months. The first harvesting at 5 months and ratoon harvesting at 4 months increased triterpenes yield by 107% over early harvesting of main crop at 4 months and ratoon harvesting at 2 months.

The concentration of biologically and medicinally active ingredients varied with the time of growth and development of *mandukaparni*. Composition of secondary metabolites is strongly dependent on developmental stage of the plant (ontogeny), and therefore harvesting time is one of the most important factor. The present study in *mandukaparni* showed the highest triterpenes content at first harvesting of main crop at 4 months and ratoon harvesting at 4 months. It might be owing to better activation of enzymes and biosynthesis of triterpenes at these stages. The results are corroborated with the studies in many medicinal plants where certain biological stages proved better in terms of secondary metabolites content: *ashwagandha* showed the total alkaloids content significantly highest at 50% flowering (Wankhade *et al*., 2010), *Prunella vulgaris* recorded highest concentrations of bioactive compounds at full-flowering stage (Chen *et al*., 2012), *Boehraavia diffusa* at flowering stage (Verma and Kasera, 2007), and *Origanum majorana* revealed the highest levels of volatile terpenes at the budding and full-flowering stage (Sellami *et al*., 2009). The present results showed the maximum triterpenes content at 4 months growth cycle and decreased with further delayed harvest might be due to breakdown of these compounds and decreased content at late harvests. Similar results were also reported in kalmegh for andrographolide content (Pandey *et al*., 2003; Bhan *et al*., 2006). For more practical point of view, the total triterpenes yield was calculated on unit area basis and interpreted thoroughly. The results showed markedly higher triterpenes yield at first harvesting of main crop at 5 months and ratoon harvesting at 4 month which might be owing to optimum herbage yield and triterpenes content at these stages.

Significant variation in leaf area, leaf number, triterpenes content and herbage and triterpenes yield were recorded at different harvests of *mandukaparni*. The maximum dry herbage yield recorded when main crop was harvested at 5 months after planting and ratoon crop harvested at 4 months interval. However, the triterpenes content were found maximum when main crop harvested at 4 months after planting and ratoon at 4 months. The harvesting of *mandukaparni* first at 5 months after planting and ratoon crop at 4 months interval found optimum time to get maximum triterpenes yield on unit area basis. This study provided basic information with respect to harvest time of *mandukaparni* to produce better-quality raw drugs. However, significant amount of information about other aspects of agronomy of *mandukaparni* is still remained to be studied.

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