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Original Research Article

Seasonal Impact on The Synthesis of Foliar Bioactive Components in *Centella asiatica* L. Urban

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Abstract: Estimation of foliar bioactive components (*viz.* photosynthetic pigments, nutrients, alkaloids, polyphenol and antioxidant activities) of *Centella asiatica* L. Urban were studied in four different seasons (EM: early monsoon- March, April and May; M: Monsoon-June, July and August; LM: Late Monsoon- September, October and November and W: Winter- December, January and February) within a year. A pot culture based experiment was conducted at the Botanical Garden, University of Chittagong. Maximum foliar photosynthetic pigments, nutrients were observed in monsoon and minimum in winter respectively. Whilst the highest alkaloid content was observed in early monsoon and the lowest in winter. Moreover the maximum polyphenol content and antioxidant activity were observed in the leaf extract of late monsoon and the minimum in winter respectively. The present study concludes that monsoon is suitable for efficient photosynthesis as well as nutrient accumulation in *Centella*, early monsoon is favorable for alkaloid synthesis and late monsoon is the right time for harvesting *Centella* to obtain maximum antioxidant activity for medicinal use.

Keywords: Seasonal impact, alkaloids, pigments, minerals, polyphenols, antioxidant, *Centella*.

INTRODUCTION

Centella asiatica (L.) Urban is a small edible, prostrate, perennial, herbaceous medicinal plant belongs to the family of Apiaceae commonly known as Centella, Gotu kola, Indian pennywort and Thankuni in Bengali [1-3]. The plant is native in Asia and used as vegetable and therapeutic herb. It is generally found in the marshy areas of Bangladesh, India, South Africa, Sri Lanka, Indonesia and Malaysia. It is also indigenous in China the western South Sea Island, Southern United State, Australia, Madagascar and tropical America [1, 4]. The plant was documented as "miracle elixirs of life" in China [5]. It is used as Chinese herbal about two thousand years ago and Indian Ayurvedic medicine about three thousand year ago. It has an aromatic scent. The leaves of the plant are alternate and palmately compound. The petiole of the leaf is often bulge and stipules are off. The Indian, Chinese and Malays use this herbal plant for multi disorders ranging from treatment of mental disorder, brain tonic, Immune system deficiency, skin problem, Circulatory problems, tetanus, liver aliments epilepsy, hair loss and asthma [1]. *Centella* contains diverse pentacyclic triterpenoids aswell as centellose, centelloside, and madecassoside. It is also plentiful in vitamin A, vitamin B1, vitamin B2, vitamin C, carotene and niacin. The major chemical constituents behind for its pharmacological action are triterpenes: mainly asiatic acid, asiaticoside, madecassic acid and madecassoside [1, 6]. It has various effects on different types of diseases *viz.* effects on neurological diseases[7-9], effects on endocrine diseases [10-12], effects on skin diseases [13-15], effects on cardiovascular diseases [16-18], effects on digestive diseases [19-21], effects on respiratory diseases [22-24], effects on gynecological diseases [25-27], effects on rheumatoid arthritis [28], leukemia [29], sepsis [30], glaucoma [31], periodontitis [32] and osteolytic bone disease [33]. Recently various clinical studies revealed that *Centrella* successfully enhanced the cognitive function of stroke orientate patients [6, 34, 35]. The ethanolic leaf and root extract of this plant shows antifungal and antibacterial activity [36].

Literature review reveals that no specific study has yet been done on the screening of foliar pigments, nutrients, alkaloids polyphenol and antioxidant activity in *Centella* plant with seasons and habitats in Bangladesh. The present study was under taken to

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assess the estimation of foliar bioactive components (*viz*. pigments, nutrients, alkaloids, polyphenol and antioxidant activity) of *Centella asiatica* (L.) Urban in four different seasons.

MATERIALS AND METHODS

Centella asiatica (L.) Urban was used to culture in garden soil (sandy loam) contained in plastic pots (2.5L). Growing plantlets were watered with tap water at 2-3days interval depending on season and age of plantlets. Weeding and mulching were done 2-3 times during the whole period of growth. The experimental plant samples (leaf) were collected on second week of each month of the four seasons (EM: early monsoon- March, April and May; M: Monsoon- June, July and August; LM: Late Monsoon- September, October and November and W: Winter- December, January and February). Leaves were dried in the laboratory under current air at room temperature (27^0C) and weighed for fresh weight and then after twenty four hours the materials were put in to the oven maintained at 60^0 C for 48 hours. These were then weighed separately in an electric balance and ground to pass through 0.2 mm sieve and preserved in airtight plastic vial for analysis of nutrients, alkaloid, polyphenol and antioxidant content.

Foliar photosynthetic pigments were determined by Wettstein method [37]. For this purpose fresh leaves were collected from the plants. Foliar nutrients (*viz*. N, P and K) were extracted with sulfuric-peroxide (H_2SO_4+H_2O_2) digestion mixture and determined by standard method [38]. The foliar iron content was determined by spectrophotometric method [39]. The foliar protein content was measured sensitive spectrophotometric method [40]. Alkaloids was measured using an UV-visible spectrophotometric (Shimadzu UV-160A PC, Shimadzu Corporation, Kyoto, Japan) method [41]. The polyphenol content was measured based on Roberts [42] with modification from the recent work of some researchers [43, 44].The radical scavenging activity of the leaf extracts was determined by the 2, 2-diphenyl-2-picrylhydrazyl (DPPH) radical using a modified method [45]. The assay is based on the measurement of the scavenging ability of antioxidants towards the stable DPPH radical [46]. There were three replications for each set of experiment. Experiments were designed on CRD method. Statistical analyses were done according to MS excel.

RESULT AND DISCUSSION

The results (Fig 1) disclose that the photosynthetic pigments of the leaves varied with the change of seasons. Chlorophyll-a, Chlorophyll-b, Carotenoids and total pigments changed from 0.945 mgg^{-1} FW (Monsoon) to 0.652 mgg^{-1} FW (Winter), 2.015 mgg^{-1} FW (Monsoon) to 1.531 mgg^{-1} FW (Winter), 1.237 mgg^{-1} FW (Monsoon) to 0.695 mgg^{-1} FW (Winter), 4.196 mgg^{-1} FW (Monsoon) to 2.879 mgg^{-1} FW(Winter) respectively and showed the following trend as M>EM>LM>W. ANOVA of leaves total photosynthetic pigments showed significant value ($P<0.01$) with seasons (Table-2). Maximum chlorophyll-a content in monsoon was observed in *Acacia nilotica* and *Jatropa curcas* [47]. It was also observed that maximum total chlorophyll content in monsoon and varied with seasons in the leaf of tea, kalomegh, *Gynura*, *Abrus* and *Vitex* [48-52]. These observation are corroborate with the present study.

Table 1: Change of foliar nutrient contents in the leaves of *Centella asiatica* in four different seasons

Seasons	Nutrients, %				
	Nitrogen	Phosphorus	Potassium	Iron	Protein
Early Monsoon	1.926 ±0.11	0.343 ±0.02	2.731 ±0.05	0.069 ±0.006	12.352 ±0.714
Monsoon	2.216 ±0.08	0.376 ±0.01	3.349 ±0.07	0.089 ±0.003	13.852 ±0.497
Late Monsoon	2.017 ±0.02	0.359 ±0.01	3.077 ±0.13	0.075 ±0.004	12.612 ±0.225
Winter	1.624 ±0.16	0.273 ±0.01	2.474 ±0.08	0.065 ±0.002	10.152 ±0.339
F-value	40.84*	34.5*	56.7*	31.04*	41.05*

Legend: * denotes significant at 1% level.

Table 2: Analyses of variance of chlorophyll-a, chlorophyll-b, carotenoids, total pigments, alkaloids, polyphenol and antioxidant activity in the leaf of *Centella asiatica* in four different seasons

Source of variance	Degree of freedom	F-values						
		Chl-a	Chl-b	Car.	Total pigments	Alkaloids	Poly-phenol	Anti-oxidant
Season	4	127.5*	46.9*	235*	239.7*	81.32*	104.3*	35.5*
Error	8	-	-	-	-	-	-	-

Legend: * denotes significant at 1% level

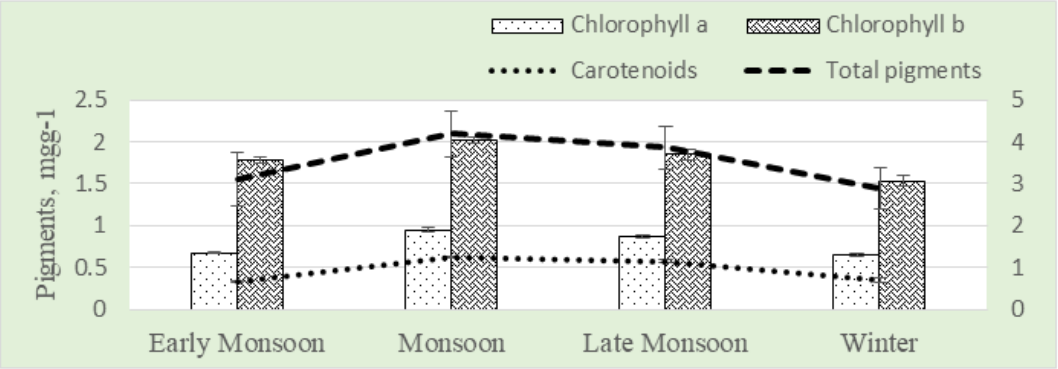

Figure 1: Change of foliar pigments of *Centella asiatica* in four different seasons

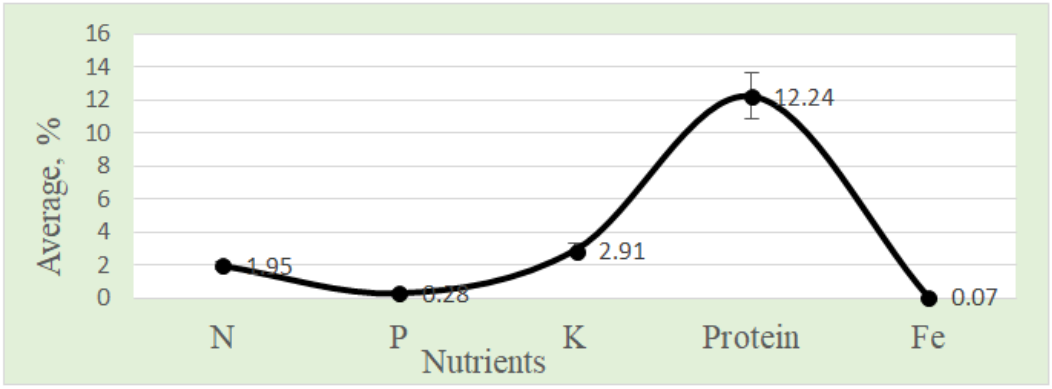

Figure 2: Change of foliar average nutrient contents of *Centella asiatica*.

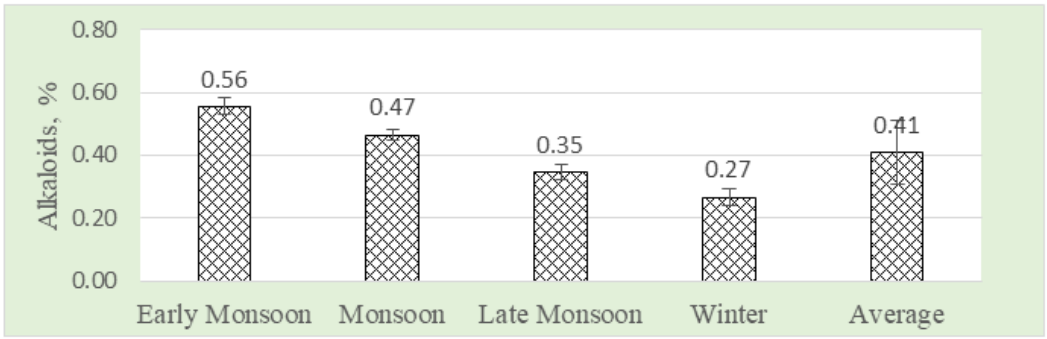

Figure 3: Change of alkaloids in the leaves of *Centella asiatica* in four different seasons.

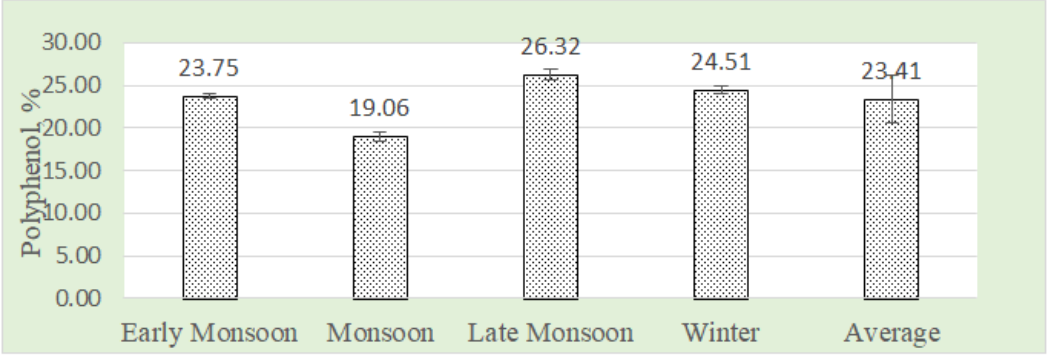

Figure 4: Change of polyphenol content in the leaves of *Centella asiatica* in four different seasons

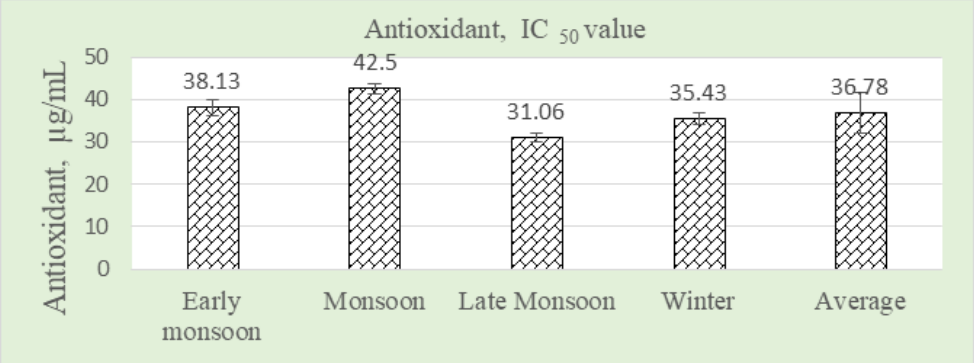

Figure 5: Change of antioxidant activity in the leaves of *Centella asiatica* in four different seasons

The result of nutrients of leaves *viz.* nitrogen, phosphorus, potassium, iron and protein are illustrated in Table-1. Foliar nitrogen, phosphorus, potassium, iron and protein status ranged from 2.216% (Monsoon) to 1.624% (Winter), 0.376% (Monsoon) to 0.273% (Winter), 3.349% (Monsoon) to 2.474% (Winter), 0.089% (Monsoon) to 0.065% (Winter) and 13.852% (Monsoon) to 10.152% (Winter) respectively and showed the following sequence as M> EM> LM> W. ANOVA of foliar nitrogen, phosphorus, potassium, iron and protein was appeared to be significant value ($P<0.01$) with seasons (Table-2). It was noticed that highest foliar nutrients in monsoon in the leaf extract of *Gynura*, *Abrus*, *Vitex* and Kalomegh, altered with seasons and growing status [50-53]. These reports are analogous with present findings.

The average value of the foliar nutrients of *Centella asiatica* are exposed in Fig 2. The average value (average of four seasons) of the nutrients *viz.* nitrogen, phosphorus, potassium, protein and iron status were found to be 1.95%, 0.28%, 2.91%, 12.24% and 0.07% respectively.

The results of alkaloids status of leaf extract are presented in Fig. 3. Foliar alkaloid contents fluctuated from 0.56% (Early Monsoon) to 0.27 % (Winter) and followed the succession as EM> M> LM> W. ANOVA of alkaloids contents showed significant value ($P<0.01$) with seasons (Table 2). Highest alkaloid content was found in the root extract of shatamuli in early monsoon [54]. The maximum caffeine content in tea leaves was achieved in the increasing plucking period (April-June) and minimum in the decreasing plucking period (October-December) [55, 56]. These observations are consistent with the findings.

The results of polyphenol status are displayed in Fig. 4. Foliar polyphenol content altered from 26.32% (Late Monsoon) to 19.06% (Monsoon) following the sequence as LM> W> EM> M. ANOVA of foliar polyphenol was showed to be significant value ($P<0.01$) with seasons (Table 2). Polyphenol status was highest in late monsoon and lowest in monsoon. It was enumerated that highest phenolic status in late monsoon in the root extract of Shatamuli and shoot extract of *Oxalis corymbosa* DC [54, 57]. Which is substantiate to this study.

The results of foliar antioxidant activity (IC_{50} value) are demonstrated in Fig. 5. Foliar antioxidant activity (IC_{50} value) changed from 31.06 µg/mL (Late Monsoon) to 42.5 µg/mL (Monsoon) and maintained the progression as LM> W> EM> M. ANOVA of antioxidant activity was showed to be significant ($P<0.01$) with seasons (Table 2). It is noticed that the antioxidant activity (IC_{50} value) in the leaf extract of *Centrella* was found to be 31.25 µg/mL [58]. The Highest antioxidant activity was observed in late monsoon in the root of shatamuli, in the leaves of rabbiteye blueberry and *Cyclocarya paliurus* (Batalin) Iljinskaja [54, 59]. Which are bear a similarity to the present observation.

CONCLUSION

The present study concludes that monsoon (June-August) is favorable for the photosynthesis and accumulation of nutrients in the leaves of *Centella* and early monsoon (March-May) is suitable for alkaloid synthesis. Considering the amount of all studied components, it is noticeable that late monsoon (September-November) is the right time for harvesting the leaves of *Centella asiatica* to obtain highest polyphenol content and antioxidant activity for medicinal use.

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