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Research Article

Seasonal effect on foliar photosynthetic pigments, nutrients and crude alkaloids of *Andrographis paniculata* (Burm.f.) Wall ex Nees. (Kalomegh)

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Abstract: Seasonal effects on foliar photosynthetic pigments, nutrients and crude alkaloids of *Andrographis paniculata* (Burm. f.) Wall ex Nees. were studied on four seasons of the year. Maximum foliar photosynthetic pigments and nutrients were observed in monsoon (June-August) and minimum in winter (December-February) respectively. Whilst maximum crude alkaloids were determined in leaf of early monsoon (March-May) and minimum in stem of winter. R_f values of extracts of crude alkaloids varied with five different concentrations of solvent systems used in the experiment. R_f values ranged from 0.985-0.213 where maximum number of R_f observed in benzene: chloroform: acetic acid and minimum in methanol: water in all treatments irrespective of growing seasons and age of plants under the present experimental conditions. Present study concludes that monsoon (June-August) is suitable for efficient photosynthesis as well as nutrient accumulation in kalomegh and early monsoon (March-May) is the perfect time for harvesting kalomegh to be obtained maximum alkaloid for medicinal use.
Keywords: Kalomegh, Seasonal effect, pigments, nutrients, crude alkaloid.

INTRODUCTION

The medicinal value of drug plants is due to the presence of some chemical substance or substances in the plant tissues that produce a definite physiological action on the human body. However, Wall *et al.* [1] in their work screened more than 4000 plant species and found alkaloid distribution only in 10% plants. But only 5% of world's plant species have examined so far and 95% still remain to be examined for alkaloids [2].

Andrographis paniculata (Burm.f) Wall ex Nees. is a medicinal plant of seasonal herb [3]. It is commonly known as Bitter plant-kalomegh and it belongs to the family of Acanthaceae [4]. Andrographolide is the main active bitter principle of the species [5]. The structure of andrographolide is 3α,14,15,18-tetrahydroxy-5β, 9βH, 10α-labda-8 12,-dien-16-oic acid. The molecular formula and weight of andrographolide is $C_{20}H_{30}O_5$ and 350.4(C 68.54%, H 8.63% and O 22.83%) respectively [6].

Phytochemical studies have shown that the leaves of *Andrographis paniculata* (Burm.f) Wall ex Nees. contain several diterpenoid lactones such as andrographolide, neoandrographolide, deoxyandrographolide- 19-β-D-glucoside, deoxyandrographolide and dehydroandrographolide together with other types of natural compounds [7]. Numerous pharmacological activities of the *Andrographis paniculata* (Burm.f) Wall ex Nees. extract have been reported including antimicrobial, antimalarial, anticoagulant, antifertility, antihelmintic, hypotensive, anti-inflammatory and antispasmodic activities [8, 9, 10]. Andrographolide protects liver tissues from alcohol induced toxic effect and accelerates digestion processes and helps in the absorption of carbohydrates [11]. The biological activities of pure andrographolide have also been reported to be antimicrobial [12], antiviral [13, 14], anti-malarial [15], immunostimulatory [16, 17], anti-inflammatory and anticancer activities [17, 18, 19, 20]. Lin *et al.* [21] showed free radical scavenging ability of Andrographolide. A recent study exhibited that andrographolide attenuated concanavalin A-induced liver injury and inhibited hepatocyte apoptosis [22]. Another recent study thoroughly showed that andrographolide has strong hypolipidemic effects and protects the cardiovascular system without significant liver damage in mice and rats [23, 24].

Ayurvedic scholars established fundamental rules for medicinal plant identification, collection and storage of drugs. According to Ayurveda, habitat an ecological factors have influence on pharmacological activity of plant based drugs. Season has impact on availability of active principles in medicinal plants. According to principles of Western Herbal Medicine, Therapeutic efficacy varies during different times or

seasons of the year [25]. It is self-explanatory that ancient physicians were aware about relation between period of collection and distribution of active principles. Usually people are habituated to collect Kalomegh plants at mature stage. Literature review reveals that no specific study has yet been done on the dynamics of alkaloids in Kalomegh plant with seasons and habitats in Bangladesh. The present study was under taken to assess the seasonal effect on foliar photosynthetic pigments, nutrients and crude alkaloids of *Andrographis paniculata* (Burm.f.) Wall ex Nees. on four different growing seasons.

MATERIALS AND METHODS

Andrographis paniculata (Burm.f.) Wall ex Nees. was used to culture in garden soil (sandy loam) on four seasons. Fresh seeds were collected from Bangladesh Forest Research Institute (BFRI), Chittagong. Seedlings were raised in the germination pots (9″ ht. x 9″dia.) and transplanted later in sandy loam soil contained in plastic pots (2.5L) on first 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). Sown seeds and growing seedlings were watered with tap water at 2-3days interval depending on season and age of seedlings. Seedlings of about 4 weeks old were used in transplantation. Weeding and mulching were done 2-3 times during the whole period of growth. The experimental plant samples (stem and leaf) were collected just a week before the initiation of flowering from the experimental pots. Each plant was cut off at 2.5 cm. above the ground. Stem and leaf 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 alkaloid contents.

Foliar photosynthetic pigments were determined by Wettstein method [26]. For this purpose fresh leaves (3^{rd} and 4^{th} pairs) were collected from the plants. Foliar nitrogen was determined by Micro-Kjeldahl Distillation Method [27]. Foliar phosphorus was determined Spectrophotometrically (Blue Color Method) [28]. Foliar potassium (K), calcium (Ca) and sodium (Na) were measured by Flame Photometer Method [28]. Crude alkaloids contents in the dry powdered plant parts were estimated following Bisset and Phillipson method [29] and separation of crude alkaloids by thin layer chromatography (TLC) on silica gel [30, 31] with uniform thickness (0.5mm) using five different solvents viz. Methanol: water (60:40); Benzene: n-hexane (80:20); Benzene: n-hexane: ethyl acetate (10:8:9); Benzene: chloroform (13:2); and Benzene: chloroform: glacial acetic acid (9:9:1). There were three replications for each set of experiment. Experiments were designed on CRD method. Statistical analyses were done according to student's t- test.

RESULTS AND DISCUSSION

The results (Fig.1) reveal that the foliar photosynthetic pigments varied with the change of seasons. Chlorophyll-a, Chlorophyll-b, Carotenoids and total pigments ranged from 9.55mgg^{-1} (Monsoon) to 6.13 mgg^{-1} (Winter); 8.75 mgg^{-1} (Monsoon) to 5.55 mgg^{-1} (Winter); 5.65 mgg^{-1} (Monsoon) to 2.53 mgg^{-1} (Winter); and 23.96 mgg^{-1} (Monsoon) to 14.22 mgg^{-1} (Winter) respectively and showed the following trend as M>EM>LM>W. ANOVA of foliar total pigments showed significant value (P>0.001) with seasons (Table-1). In case of plucked shoots of clonal agrotypes of tea, photosynthetic pigments were found to be changed with plucking seasons and maximum value was obtained in monsoon [32] which is similar to this finding.

Foliar nutrients viz. Nitrogen, Phosphorus, Potassium, Calcium and Sodium are shown in Fig. 2. Foliar Nitrogen, Phosphorus, Potassium, Calcium and Sodium contents ranged from 10.35 mgg^{-1} (Monsoon) to 5.24 mgg^{-1} (Winter); 2.55 mgg^{-1} (Monsoon) to 1.48 mgg^{-1} (Winter); 17.32 mgg^{-1} (Monsoon) to 8.27 mgg^{-1} (Winter); 6.15 mgg^{-1} (Monsoon) to 4.74 mgg^{-1} (Winter); and 5.83 mgg^{-1} (Monsoon) to 4.07 mgg^{-1} (Winter) respectively and showed the following trend as M>EM>LM>W. ANOVA of foliar N, P, K, Ca and Na showed significant value (P>0.001) with seasons (Table-1). Chanprasert and Pecharably [33] reported that the foliar nutrients of *Andrographis paniculata* (Burm.f) Wall ex Nees. leaves changed with seasons as well as growing conditions.

The value of crude alkaloid contents (stem, leaf, and total shoot biomass mg^{-plant} parts on dry weight basis) varied with the change of seasons (Fig. 3 and 4). Crude alkaloid contents in leaf, stem and total shoot dry biomass ranged from 21.78 mg^{-plant} (EM) to 3.64 mg^{-plant} (Winter); 17.60 mg^{-plant} (EM) to 2.71 mg^{-plant} (Winter) and 39.38 mg^{-plant} shoot dry wt. (EM) to 6.35 mg/total shoot dry wt. (Winter) respectively and showed the following trend as EM>M>LM>W. Maximum crude alkaloids were determined in leaf of early monsoon. ANOVA of foliar total crude alkaloids showed significant value (P>0.001) with seasons (Table-1).

The constituent and active principles vary quantitatively at different seasons of the year and the majority of plant materials are usually best collected during the dry season, when the herbs are at peak maturity and concentration [25]. The best harvesting time was observed at 120 days after sowing to get higher biomass containing maximum andrographolide content [34]. Patarapanich *et al.* [35] reported that the total alkaloid content in the leaves of *Andrographis*

paniculata varied according to the location and seasonal factors.

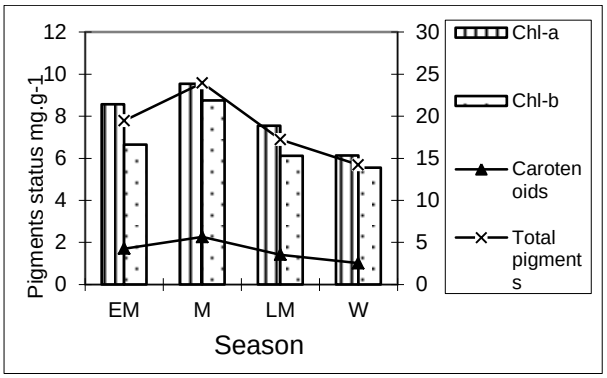

Fig. 1: Change of foliar photosynthetic pigments of *Andrographis paniculata* on four growing seasons.

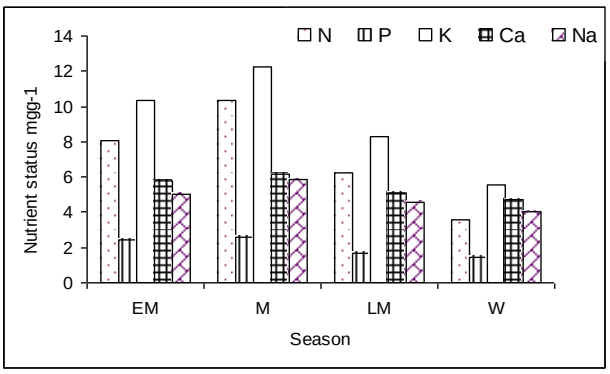

Fig. 2: Change of foliar nutrient status of *Andrographis paniculata* (Burm.f) Wall ex Nees. on four growing seasons.

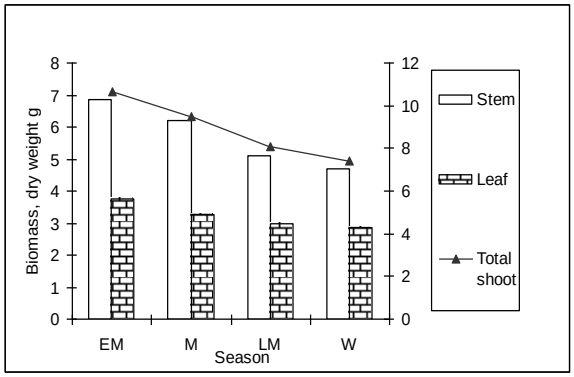

Fig. 3: Change of biomass in the stem and leaf of *Andrographis paniculata* on four growing seasons.

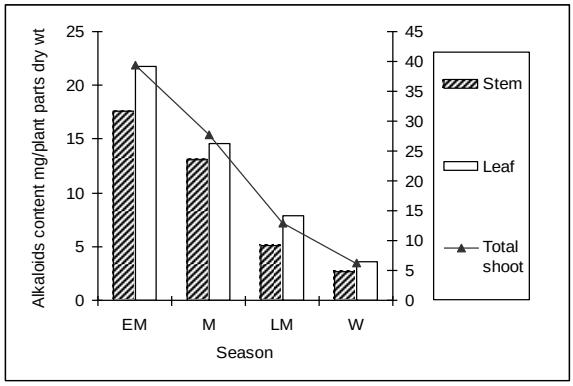

Fig. 4: Change of crude alkaloid contents in the stem and leaf of *Andrographis paniculata* on four growing seasons.

He also showed that the total alkaloid content in *Andrographis paniculata* varied significantly during one year time course and was found to be highest at 11.81% w/w in April and lowest at 7.68% w/w in October.

The number of R_f and their values were different with different solvent systems (Table-2). Presence of more than one alkaloid in the same plant organ is reported by different workers [36, 37, 38]. The number of R_f was maximum in benzene: chloroform: acetic acid (9:9:1) and minimum in methanol: water (60:40). Patarapanich *et al.* [35] found maximum R_f value in benzene: chloroform mobile phase. Alamgir *et al* [39] also considered benzene: chloroform: acetic acid solvent system to be better in separating the crude alkaloids into higher number of components.

Table-1: Analyses of variance of total pigments, nutrients and crude alkaloids content in leaf of *Andrographis paniculata* (Burm.f) Wall ex Nees. on four growing seasons.

Source of variance	Degree of freedom	F-values						
		Total pigments	N	P	K	Ca	Na	Total alkaloids
Season	3	411.97*	5.95*	6.12*	5.03*	6.00*	5.51*	264*
Error	8	-	-	-	-	-	-	-

Legend: * denotes significant at 1% level

Table-2: R_f values of the crude alkaloids extract in leaf of *Andrographis paniculata* in different solvent systems during four growing seasons.

Season	R_f value in different solvent				
	(i) Methanol: Water (60:40)	(ii) Benzene: n-hexane (80:20)	(iii)Benzene: n-hexane: ethyl acetate (10:8:9)	(iv) Benzene: chloroform (13:2)	(v) Benzene: chloroform: glacial acetic acid (9:9:1)
EM	0.985	0.951 0.831 0.712	0.953 0.453 0.361 0.213	0.954 0.912 0.551	0.984 0.965 0.724 0.517 0.448
M	0.945	0.851 0.732	0.852 0.551 0.361	0.851 0.452	0.954 0.752 0.546 0.453
LM	0.981	0.642 0.541	0.854 0.353	0.954 0.862 0.521	0.852 0.732 0.545 0.321
W	0.965	0.853	0.854 0.453 0.334	0.854 0.734	0.842 0.741 0.452

CONCLUSION

Present study concludes that monsoon (June-August) is suitable for efficient photosynthesis as well as nutrient accumulation in kalomegh and early monsoon (March-May) is the perfect time to harvest kalomegh to obtain maximum alkaloid for herbal medicinal use.

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