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Quantification of Andrographolide in Kalmegh (*Andrographis paniculata* Nees.) Influenced by Time of Planting and Harvesting

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ABSTRACT

The present study was conducted to know the influence of planting date and harvesting time on herbage yield, andrographolide content and andrographolide yield of Kalmegh (Andrographis paniculata) at Vegetable Research Station, Rajendranagar, Hyderabad. The experiment was laid out in Factorial RBD with three replications. There are 12 treatment combinations comprising of four dates of plating (1st July, 16th July, 1st August and 16th August) and three stages of harvesting (Pre flowering stage, Flowering stage and Pod setting stage). From the results, it is evident that different planting and harvesting treatments have significant influence on dry herbage yield, andrographolide content and andrographolide yield of kalmegh. The crop planted on 1st August recorded maximum values for dry herbage and andrographolide yields. Andrographolide content was maximum with the crop harvested at flowering stage, however highest dry herbage and andrographolide yields were, recorded with the crop harvested at pod setting stage. Planting on 1st August and harvesting at pod setting stage were found superior to other planting and harvesting treatments with highest Andrographolide yield in kalmegh.

Key words: *Andrographis paniculata, Dry herbage yield, HPLC, Andrographolide content, Andrographolide Yield.*

INTRODUCTION

Andrographis paniculata Nees. Commonly known as kalmegh is an important medicinal plant belonging to family Acanthaceae. It is one of the most widely used plants in ayurvedic formulations and was recommended in Charaka Samhita dating to 175 BC for treatment of jaundice along with other plants in multi plant preparations[3]. It is most popular as a remedy for a number of ailments related to hepatoprotection, digestion, vermicidal, analgesic, anti-inflammatory, anti-bacterial, anti-typhoid, hypoglycemic, besides immune enhancement[2,4-8]. The plant is also a well known drug used for treating fever, diabetes, snake bite, common cold and a variety of ailments[7].

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The major bioactive constituents of *Andrographis paniculata* are a group of diterpene lactones. The entire plant contains several derivatives of diterpene lactones out of which andrographolide (bitter constituent) and neoandrographolide (non bitter constituent) are important. Andrographolide being secondary metabolite is often influenced by the environmental and seasonal factors and its distribution in between leaves and whole plant. Inspite of its multifarious medicinal values, the crop is not widely grown on commercial scale in India. At present it is being collected at different stages of growth from the forest areas resulting in a lot of variation in the active principle content of the herb. The quality of the drug required by the industry is however, inconsistent due to wide variation in active principle content of the herb at different growth stages. The present study was undertaken with the objective of maximizing herbage yield and andrographolide yield through identification of suitable planting date and harvesting stage.

MATERIAL AND METHODS

A field experiment was conducted during kharif season at Vegetable Research Station, Rajendranagar, Hyderabad. The experiment was laid out in Factorial RBD with 3 replications. There are 12 treatment combinations comprising of four dates of plating (1st July, 16thJuly, 1st August and 16th August) and three stages of harvesting (Pre flowering stage, Flowering stage and Pod setting stage). The seeds of variety CIM-Megha procured from CIMAP, Regional Research Station, Hyderabad were used for sowing. Forty five day old healthy and uniform seedlings were transplanted according to dates of planting. The crop was grown as per the standard package of practices and harvested according to different planting and harvesting treatments. Andrographolide content was estimated using HPLC analysis followed by Parasher *et al*[6]. The data collected on growth and analytical parameters was subjected to statistical analysis outlined by Panse and Sukhatme[7].

Plant Material

The crop was harvested at different stages of growth *viz.*, pre-flowering, flowering and pod setting stages by cutting the stem close to the ground. The plant material was dried under shade, powdered using blender and stored in air tight bottles.

Chemicals and Reagents

Solvents used for chromatographic analysis were HPLC grade Methanol and HPLC grade water.

The standard solution was prepared by dissolving 10 mg of standard andrographolide (99%, pure, Sigma) in 10 ml methanol 100% (v/v).

Sample preparation for estimation of Andrographolide

The powder (1g) of plant material was refluxed for 1 hour with methanol (50 ml) on a water bath. The mare was filtered and subjected for another two cycles of refluxes (1 hr each) with methanol (50 ml). The combined filtrates were evaporated under vacuum to dryness. The residue was dissolved in methanol (25 ml) and filtered through a 0.45 µm (Nylon) filter into HPLC vials.

Calibration

2 mg of andrographolide standard (98% pure sigma Aldrich) was placed in a 5ml volumetric flask and dissolved in MeOH (stock solution). Standard solution was stored at 4°C for further analysis and was stable for at least 30 days (confirmed by re-assaying the solution). Within the range of concentrations injected (40.0-2.0 µg/ml) the detector response was linear. All data were recorded and processed by millennium 32 software from waters (Milford, MA, USA).

Analytical method

HPLC analysis was performed on a Waters HPLC system, equipped with a 2996 photodiode array detector (Waters Pvt. Ltd). For all separation X Bridge TM C_{18} column (4.6 mm ×250 mm, 5.0 µm particle size) was used. The mobile phase consisted of water (A) and a mixture of MeOH and reagent alcohol in ratio of 1:1 (B) which were applied in the following gradient elution from 35A/65B in 25 min to 45A/55B. Each run was followed by a 5 min wash with 100 B and an equilibration period of 10 min. The separation temperature was kept constant at 25ºc, flow rate and sample volumes were monitored at 223 nm. Peaks were assigned by spiking the sample with authentic sample followed by comparision of UV spectra and retention time.

Andrographolide yield

The andrographolide yield was calculated by using the formula

$$\text{andrographolide yield (Kg ha}^{-1}\text{)} = \frac{\text{andrographolide content (\%) x dry herbage yield (Kg ha}^{-1}\text{)}}{100}$$

RESULTS AND DISCUSSION

Dry herbage yield (kg ha^{-1})

Planting on 1st August (D_3) resulted in maximum dry herbage yield (3804.7 kg ha^{-1}) (Table 1) this could be attributed to higher growth parameters (higher fresh and dry herb weight through increased plant height, more number of branches and leaves in the treatment) due to favourable weather conditions which might have influenced the plants to grow taller by increasing cell division and cell elongation. Among different harvesting treatments, maximum dry herbage yield is obtained at pod setting stage of harvest (H_3). This could be attributed to higher fresh herb weight as a result of higher growth parameters and also lignifications of tender shoots and branches at advanced stage of harvesting. The results are in conformity with the previous studies[1,6-10] in kalmegh, planting on 1st August and harvesting at pod setting stage (D_3H_3) recorded maximum andrographolide yield which might be due to synergistic effect of planting date and harvesting stage.

Andrographolide Content

The data on andrographolide content of kalmegh as influenced by dates of planting and is stages of harvesting is presented in (Table 1) and (Figure 2). Different dates of planting did not exhibit significant effect on andrographolide content of herbage at harvest. Significant differences were observed among different stages of harvesting for andrographolide content of herbage. An increase in andrographolide content from pre flowering stage of harvesting (H_1) to flowering stage (H_2) was noticed and there after a decline at pod setting stage of harvest (H_3) was observed. Maximum andrographolide content (2.55) was recorded at flowering stage of harvest while pod setting stage of harvest (1.90) recorded minimum andrographolide content.

Highest andrographolide content at flowering stage could be attributed to more number of leaves at that time which were reported to contain more andrographolide than stems and branches[7]. Higher andrographolide content of herbage at flowering stage was also reported by Seema Nemade *et al*[9]. Lower andrographolide content of herbage at pod setting stage might be due to lower dry weight of leaves and higher dry weight of stems and branches which were reported to contain less andrographolide. Similar findings were also reported by Singh *et al*.[10] in kalmegh.

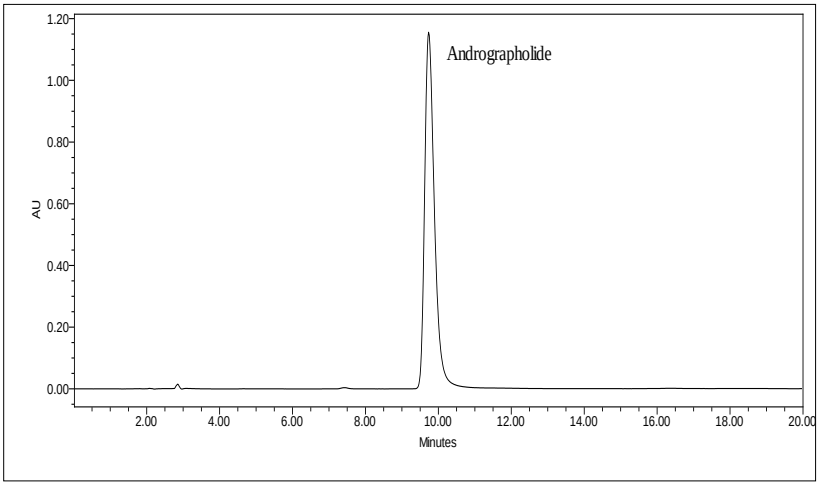

Fig. 1: HPLC chromatogram of Andrographolide standard

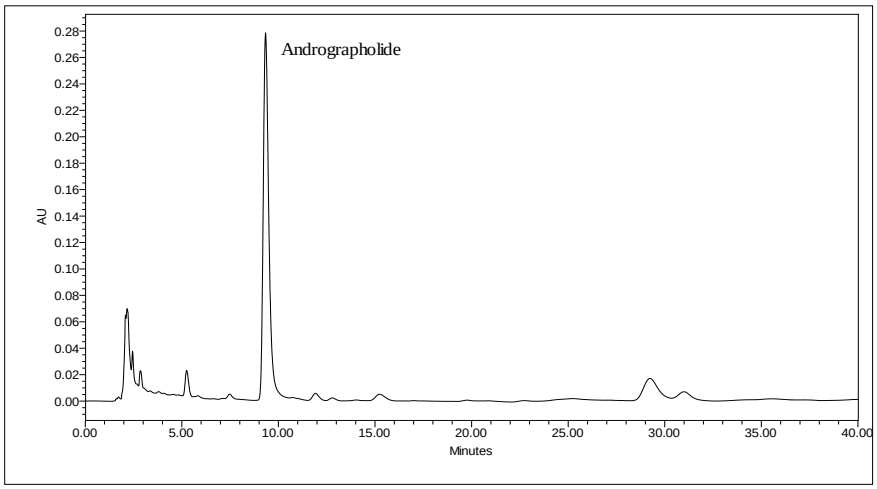

Fig. 2: HPLC chromatogram of methanolic extract of D_2H_2

Table: 1 Dry herbage yield and Andrographolide content and yield of kalmegh influenced by dates of planting and stages of harvesting

Treatments	Dry herbage Yield (kg ha-1)	Andrographolide content (%)	Andrographolide Yield (kg ha-1)
Dates of Planting (D)			
D_1	2869.3	2.21	61.49
D_2	3284.4	2.28	72.93
D_3	3804.7	2.25	83.39
D_4	3011.5	2.23	65.46
SEm±	49.8	0.029	0.89
CD at 5%	146	N.S	2.63
Stages of Harvesting (H)			
H_1	2046.2	2.29	46.79
H_2	3036.8	2.55	77.33
H_3	4644.4	1.90	88.33
SEm±	43.1	0.025	0.78
CD at 5%	126.5	0.073	2.28
Interaction (D×H)			
D_1H_1	1812.4	2.25	40.78
D_1H_2	2661.8	2.51	66.81
D_1H_3	4133.9	1.86	76.89
D_2H_1	2020.9	2.32	46.89
D_2H_2	3118.8	2.58	80.46
D_2H_3	4713.5	1.94	91.44
D_3H_1	2474.8	2.3	56.92
D_3H_2	3520.5	2.55	89.77
D_3H_3	5418.8	1.91	103.49
D_4H_1	1876.8	2.27	42.6
D_4H_2	2845.9	2.54	72.28
D_4H_3	4311.7	1.89	81.49
SEm±	86.2	0.049	1.55
CD at 5%	252.9	N.S	4.55

Andrographolide Yield (kg ha^{-1})

The data on andrographolide yield of kalmegh as influenced by dates of planting and is stages of harvesting is presented in (Table 2) and (Figure1). The planting date of 1st August (D_3) recorded maximum andrographolide yield

(83.39 kg ha^{-1}) due to more number of leaves and higher dry herbage yield observed with the treatment. Andrographolide yield is significantly influenced by different stages of harvesting. An increase in andrographolide yield was observed from pre flowering stage of harvest (H_1) to pod setting stage of harvest (H_3). Harvesting at pod setting stage recorded maximum andrographolide yield (88.33 kg ha^{-1}), this could be attributed to higher dry herbage yield with the treatment. The results are in accordance with the findings of Singh *et al*[10] in kalmegh. Although the content of andrographolide was higher in flowering stage of harvest, the dry herbage yield at pod setting stage of harvest is significantly higher over flowering stage resulting in higher andrographolide yield at pod setting stage of harvest.

CONCLUSION

Planting of *Andrographis paniculata* on 1st August is recommended for obtaining higher herbage and andrographolide yields and is best harvested at pod setting stage for higher herbage and andrographolide yields. Planting on 1st August and harvesting at pod setting stage is recommended for obtaining maximum herbage and andrographolide yields in kalmegh.

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