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Short communication

Distribution pattern of andrographolide and total lactones in different parts of *Kalmegh* plant

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Andrographis paniculata Nees. is an important medicinal herb, commonly known as *kalmegh*. It is extensively used in Indian system of medicine for curing various ailments including liver disorders, stomachic, general debility and colic disorders. It is also used as tonic, anthelminitic, cholagogue and anti-inflammatory agent. The active principle of the plant is diterpene lactones of which andrographolide is the major constituent. In addition to it, *A. paniculata* has deoxyandrographolide, neoandrographolide, 14-deoxy-12-methoxyandrographolide and andrographoinin andrographonoside (Handa *et al*., 3) and several other lactones derivatives in minor quantities.

The active principle in raw material is dependent to large extent on the source of the material from which it is obtained. *Kalmegh* is sold as whole herb powder and also purified extract. It was found that many of the market samples analyzed by other workers showed considerable variation for the andrographolide content for the same type material (Shah *et al.,* 9). Since the amount of active principle in the different parts of the herb may vary due to variation in the distribution pattern and time of harvest, it becomes necessary to quantify the amount present in different plant parts. Since the contribution of stems to the final herbage is substantial and amount present in stems and fruit capsules may alter the final concentration of andrographolide yield from the plant sources, it becomes vital to study the distribution of andrographolide and total lactones from individual components of *A. paniculata* herbage to find out the actual content and contribution of active principles to the raw material used for drug purpose.

Several analytical methods HPLC, HPTLC, gravimetric, spectrophotometric and calorimetric have been reported for the quantitative estimation of andrographolides (Burgos *et al*., 2; Shah *et al.,* 9). However, none of the studies quantified the amount of the active principle from different plant parts of the species. Hence, a study was undertaken to find out the distribution pattern of andrographolide in the leaves, stems, seeds and fruit capsules of *A. paniculata* through HPLC and compared with spectrophotometer method of total lactones estimation.

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Samples were taken from 120-day-old *A. paniculata* plants from Experimental Farm of the Directorate of Medicinal and Aromatic Plants Research, Anand, in three replications, with three plants in each replication. Different plant parts *viz*., leaf, stem and capsules were separated from the samples and shade dried. Dried plant material was ground in cyclone mill and powdered material used for the analysis. To study the accumulation pattern of andrographolide in different plant parts at various growth stages, plant parts like stem, leaf and capsule were harvested at 20 days interval from three different plants and analysis were by HPLC. The sample size of 1.0 g dried powder was extracted with mixture of dichloromethane and methanol in the ratio of 1:1 by cold maceration (Rajani *et al*., 6). The extracts were filtered through Whatman filter paper Grade1 and evaporated on rotary evaporator (M/s Hidolph, Germany). The dark green residue was washed with toluene. After complete removal of toluene, samples were re-dissolved in HPLC grade methanol for HPLC analysis and in ethanol for spectrophotometeric estimation. Reference standard andrographolide was purchased from M/s Sigma Aldrich, USA. All the chemicals used in the analysis were of AR grade and all the chemicals except 3,5-dinitro benzoic acid (HiMedia, Mumbai) and absolute ethanol (Merck India). Stock solutions of andrographolide (10 mg dissolved in 100 ml HPLC grade methanol for HPLC analysis and 10 mg in 100 ml ethanol for spectrophotometeric assay) were prepared. Dilution of these solutions was made with the respective solvents to produce the working standards. The HPLC system (Shimadzu, Japan) consisted of LC-10AD pumps and SPD-10A UV-VIS detector along with Aimil chromatographic data station was used for andrographolide estimation with RP-18 column (250 mm × 4.6 mm, 5 µm, Merck). Absorbance at 229 nm was selected for detection on UV-VIS detector.

Calibration curve for andrographolide was constructed by using standard andrographolide (Sigma Aldrich, USA). The analysis was carried out in mobile phase 65:35 of methanol and water at a flow rate 1 ml min^{-1} for HPLC. The calibration curve was obtained by loading different concentrations of andrographolide (15.25 to 122.00 µg ml^{-1}) against peak area. The calibration equitation obtained was Y= 1876.4X +

195.04, linearity $r^2 = 0.997$, which was used for sample analysis.

Analysis was done by loading the samples extracted from the different plant parts. For spectrophotometric determination of total lactones, 3,5-dinitro benzoic acid reagent was prepared by dissolving 1.0 g the sample in 100 ml ethanol and potassium hydroxide reagent was prepared by dissolving 0.5 g of it in 100 ml absolute ethanol and stored in amber coloured bottles and used fresh at the time of analysis.

A single UV-visible beam spectrophotometer (Biomate-3, Thermo Electron Corporation, USA) was used for spectrophotometric determination of total lactones (Aromdee *et al*., 1). Aliquots of 0.10, 0.20, 0.40, 0.60, 0.80 and 1.00 ml of the standard were transferred in test tube and volume was adjusted to 2 ml by ethanol. 0.5 ml each of 3,5-dinitro benzoic acid and potassium hydroxide reagents were added to the aliquots and mixed thoroughly on vortex machine. After 10 min., the absorbance was measured at 536 nm against blank. The standard calibration curve was made by plotting absorbance against concentration which followed linear equation $Y = 0.3565 \times -0.0011$; $r^2 = 0.991$ and was used for the sample analysis. For the sample analysis, the same procedure was followed for plant extracts also. The andrographolide content in plant samples were calculated from the regression equation of standard curve.

The andrographolide and total lactones content in capsule, stem, leaves and total herbage of plant are depicted in Table 1. The study showed that andrographolide percent (w/w) was highest in the leaves (3.16%) by HPLC method which was followed by total herbage (1.24 ± 0.06), stem (0.75 ± 0.00) and lowest in the capsules (0.16 ± 0.010).

The pattern of andrographolide accumulation in different plant parts at different growth stages was also investigated. Andrographolide content in stem was minimum at 40 DAP and increased subsequently with increasing growth stage and peaked at 120 DAP. It declined afterwards towards the end of the maturity period (Fig. 1). Leaves showed accumulation of andrograholide with increasing trend at the initial stages from 40 to 80 DAP and recorded the highest content at 100 DAP. Among the plant parts, leaves showed the highest content of andrographolide at all growth stages. Total herbage showed highest andrographolide content at 80 DAP coinciding with the peak flowering. At the initial stages of capsule formation, it accumulated at higher rate (120 DAP) and showed lower content at harvest.

The accumulation of andrographolide in the leaves was highest which is almost 22 times higher than the amount present in capsule. Whereas, in stem it was only 4 times compared to capsules. The lower concentration in stem becomes critical because at the growth later stages, the contribution of stem to final herbage is more than the leaves (Khristi, 4). Similar study conducted on *Nicotiana tabacum* (Zhao *et al*., 10) also showed that solanesol which is an important secondary metabolite in the species varied greatly in different plant parts *viz.,* leaf, stalk, flower and fruit and its concentration was maximum in leaves. Accumulation of gymnemic acid in *Gymnema sylvestre* showed differential accumulation in mature and tender shoots (Manohar *et al*., 5).

The results obtained from the spectrophotometric analysis also showed the same trend in andrographolide accumulation pattern. The lactones percent in leaf was 7.44 ± 0.267, in total herbage it was 5.55 ± 0.370, in stem, it was 2.63 ± 0.129 and in the capsule it was 1.01 ± 0.066. It was interesting to note that the amount of lactones present in the leaves compared to capsule is almost 7 times as opposed to the andrographolide which was present at 22 times higher than capsule. The ratio of total lactones in stem to capsule is also 2.5 times only compared to above 4 times for the andrographolide content.

Accumulation of phytochemical in growth stages vary within organs and with developmental stage, as the synthesis and accumulation of bioactive principles are regulated by genetic and environmental factors. Studies conducted by Saravanan *et al.* (7, 8) in plants

Table 1. Contents andrographolide and total lactones in various plant parts of *A. paniculata.*

Plant part	Andrographolide (%)	Total lactones (%)
Stem	0.751	2.637
Leaf	3.572	7.301
Total herbage	1.238	5.397
Capsule	0.159	1.071
CV (%)	1.82	4.89
CD (p = 0.01)	0.041	0.26

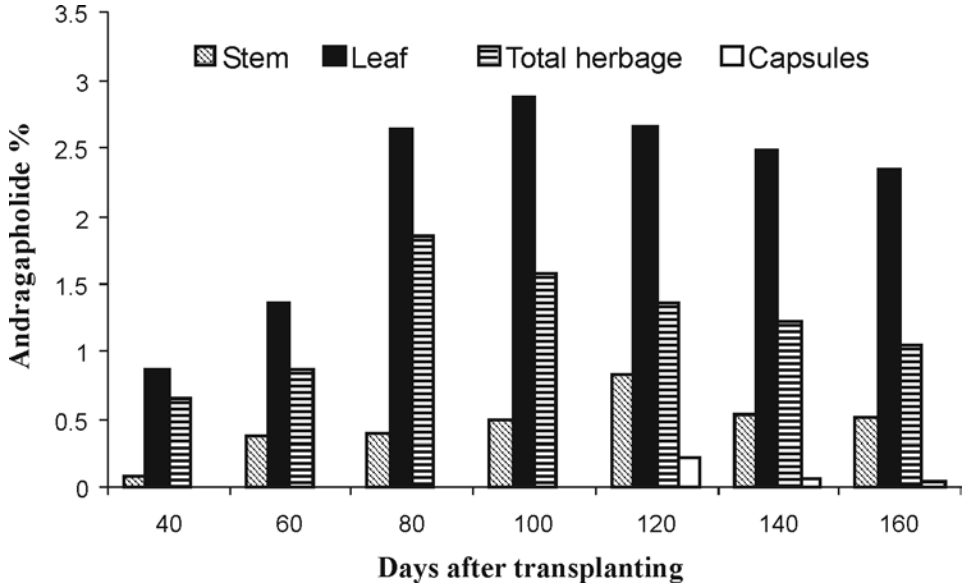

Fig. 1. Changes in andrographolide content (%) in different plant parts at various growth stages of plant**.**

grown at different light intensities and plant population densities showed variation in andrographolide accumulation in plant parts of *A. paniculata* under field conditions. These results showed that the amount of andrographolide present in different plant parts vary widely. The total lactones also showed similar trend for the plant parts studied.

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