Effect of colchicines on enhancement of andrographolide content and quantification

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Abstract

Andrographis paniculata commonly known as, Kalmegh is a wonder drug used in traditional Siddha and Ayurvedic systems and also as a tribal medicine in India and other countries for a number of clinical applications. The therapeutic value of Kalmegh is mainly due to the compound Andrographolide found concentrated in the leaves. The present study was performed to enhance the level of andrographolide in normal diploid cells using colchicine, a polyploidy inducer. One month old healthy Andrographis paniculata plants were chosen for this treatment. Few terminal leaves were excised and wounds were made on the plants. The plants were sprayed with two concentrations of 0.02% and 0.05% colchicine separately. Leaves from control, 0.02% and 0.05% colchicine treated plants were picked after treatment. They were shade dried and powdered separately. Five grams of each powder was extracted using methanol and left undisturbed for 48 hrs. The crude extract was then filtered using Whatmann filter paper and concentrated to 10 ml. This extract was quantified for Andrographolide content using both spectrophotometer and HPLC methods. The results confirmed that the 0.05% and 0.02% concentrations of colchicine treatments showed enhanced levels in the quantity of andrographolide when compared to normal untreated plants. Similarly, 0.05% concentration of colchicine treatment was found to be more effective in enhancing when compared to 0.02% concentration.

Keywords: Andrographis paniculata, andrographolide, colchicines

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Introduction

Andrographis paniculata (Burm. f.) wall. ex. Nees Syn.Justica paniculata is traditionally known as, Kalmegh or Neelavembu and commonly as, King of Bitters. Since ancient times, A. paniculata is used as a wonder drug in traditional Siddha and Ayurvedic systems of medicine as well as in tribal medicine in India and some other countries for multiple clinical applications. Several active compounds reported from the plant are diterpene lactones, flavonoids and polyphenols (Wongkittipong et al., 2000). The prime constituent is the Diterpenoid lactone andrographolide (C_{35}H_{30}O_{3}) which has been mainly attributed for its therapeutic properties (Handa and Sharma, 1990). It is the principal compound found mainly concentrated in the leaves and can be easily isolated from the crude plant extract as crystalline solid. This bitter compound was isolated in pure form by Gorter in 1911. In recent past, the compound is reported for its anti-tumour, anti-HIV and cardio-protective properties. However, it shows a weak anti-microbial activity against bacteria and viruses (Mishra et al., 2009). Andrographolide is soluble in methanol, ethanol, pyridine, acetic acid and acetone, but sparingly soluble in ether and water. Extraction of andrographolide from Andrographis paniculata can be carried out using liquid solvents like methanol, ethanol and dichloromethane-methanol employing crude digestion and soxhlet methods. Similarly, other techniques used for the analysis of andrographolide are thin layer chromatography (TLC) (Puri et al., 1993) and high-performance liquid chromatography (HPLC) (Li and Fitzloff, 2004).

Colchicine, a toxic natural product and secondary metabolite, originally extracted from plants of the genus Colchicum (autumn crocus, Colchicum autumnale, also known as meadow saffron) is used for inducing polyploidy during meiosis by inhibiting chromosome segregation (Blakeslee and Avery, 1937) and therefore colchicine effectively functions as a mitotic poison or spindle poison. While this would be fatal in animal cells, in plant cells it is not only usually well tolerated, but in fact frequently results in plants which are larger, harder, faster growing, higher enzyme content and in general more desirable than the normal diploid parents. Andrographis paniculata being a tropical plant can be generally seen only at few wild sources and at gardens specially maintained by the
traditional users of medicinal plants in traditional system of medicines. It is placed at 17th position among the 32 prioritized medicinal plants of India with a demand of 2,197.3 tonnes (2005-06) and annual growth of 3.1% (Misra et al., 2001). It has an important place in the Indian Pharmacopoeia and is being prominently used in at least 26 Ayurvedic formulae (Nadkarni, 1954). Hence due to the increasing demand for this plant, the present work was performed to enhance the level of andrographolide in normal diploid cells using colchicine, a polyploidy inducer. Furthermore, the amount of Andrographolide in treated and untreated plants was quantified using spectrophotometer and HPLC.

Materials and Methods

Plant material and place of Collection

Andrographis paniculata (Burm. f.) wall. ex. Nees Syn. Justica paniculata belonging to the family Acanthaceae was chosen as the experimental plant material. The healthy plants of Andrographis paniculata taken for the present study were selected and collected from the Botanical garden of the Quaid-e-Millath Government Arts College for Women, Chennai. The collected seeds were shade dried. They were then grown in petridishes lined with moist filter papers. The seeds germinated between 9 and 12 days. They were then transferred to mud pots and watered regularly.

Treatment of Colchicine on Andrographis paniculata plants

One month old healthy Andrographis paniculata plants were chosen for the treatment. Few terminal leaves were excised and wounds were made on the plants. The plants were sprayed with 0.02% and 0.05% concentrations of colchicine separately. Colchicine was sprayed using a sprayer. The treatment was given at seven days interval for three times. Three successive treatments were made. Plants without colchicine treatments were considered to be the control. Triplicates were maintained for reproducibility. Leaves from control, 0.02% and 0.05% colchicine treated plants were picked after treatment. They were shade dried and powdered separately. The powders were extracted using solvent methanol separately and left undisturbed for 48 hrs. The crude extract was then filtered using Whatmann filter paper and used for quantification of andrographolide using spectrophotometer and HPLC.

Quantification using spectrophotometer

The linearity curve was prepared using methanol. Different concentrations of the crude extract (1 to 5 ml) were taken in volumetric flasks. Volume was made up to 10 ml with methanol. The flasks were vortexed and kept aside for 20 min. Resultant colour complex was measured against blank at 494 nm using spectrophotometer. The blank was prepared in a similar manner without sample. The absorbance values were plotted against their respective concentrations to obtain a linearity curve. This was carried out for the control and the treated plants. All the experiments were carried out in triplicate.

HPLC analysis

Sample preparation: Air-dried and powdered plant material (1 g) was extracted with methanol (5 ml, 12 hr), filtered, completely dried under vacuum and 5 ml of acetonitrile was added. Sample was filtered through a millipore filter (0.45 µm) and the successive extracts were used for HPLC analysis.

Chromatographic condition: The extracts were filtered through sartorius RC-membrane syringe filter 0.20 m and 20 u of filtrate was injected into the HPLC. Chromatography was performed using Shimadzu HPLC (Model SPD-10A UV-VIS Detector) and supelcosil LC-18 column (25 cm × 4.6 mm, 5 m) with mobile phase consisting of acetonitrile-water (70:30, v:v). Flow rate was maintained at 1 ml.min⁻¹ with a back pressure of 250 psi and the compounds were read at 230 nm using a UV detector. The total run time was 20 min, but preferably extended up to 40 min (Jain et al., 2000; Kapadi et al., 2010).

Results

The andrographolide content of the samples with the control were quantified using spectrophotometer and HPLC. The results indicate that there is a gradual increase in the optical density of the control, 0.02% and 0.05% colchicine treated as the concentration of the extract increased. Moreover, 0.05% concentration showed increase in andrographolide when compared to 0.02% concentration of colchicine. Thus, higher the concentration of colchicine, greater is the quantity of andrographolide and as the amount of the plant extract increased, the quantity of andrographolide also increased. Hence, by
spectrophotometer it was confirmed that colchicine enhanced the level of andrographolide (Fig.1).

**Fig.1.** Quantification of andrographolide by spectrophotometer

![Graph showing quantification of andrographolide](image)

**HPLC analysis**

Using HPLC, the retention time for treated and untreated control were noted. The andrographolide from untreated control of *Andrographis paniculata* plants, eluted through HPLC with the run time of 15 min, had a retention time of 3.69 min. A Retention time (Rt) value of 3.72 min. for 0.02% concentration of colchicine and 3.73 min. for 0.05% concentration against 3.69 min. for control resulted by HPLC analysis, confirmed the enhancement of the Andrographolide compound in treatments of leaf extracts. This is shown below in Table 2.

**Table 2.** Quantification of andrographolide by HPLC

<table>
<thead>
<tr>
<th>Active compound</th>
<th>Retention time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Andrographis paniculata</em> control</td>
<td>3.69 min</td>
</tr>
<tr>
<td><em>Andrographis paniculata</em> - Treatment 0.02%</td>
<td>3.72 min</td>
</tr>
<tr>
<td><em>Andrographis paniculata</em> - Treatment 0.05%</td>
<td>3.73 min</td>
</tr>
</tbody>
</table>

**Fig. 2.** Control and 0.02% and 0.05% concentration of andrographolide active compound eluted through HPLC

**Discussion**

From the above results, it was seen that both spectrophotometer and HPLC analyses confirmed the enhancement of the quantity of andrographolide through colchicine treatment. The 0.05% concentration of colchicine was found to be more effective than 0.02% concentration. Similar studies were reported by Drazkrewicz et al. (2007), where the enzymes like superoxide dismutase, catalase, and glutathione peroxidase could be enhanced. Maria Drążkiewicz et al. (2002) have also reported that the colchicine pre-treatment resulted in an increase of CAT activity. In a similar study on cotton, colchicine produced better results with comparable morphological differences (Arunee Wongpiyasatid et al., 2003). Certain organs like the leaves were often found to be thickened by Uhlik, 1981. Plants with a higher degree of ploidy are often marked by stunted growth due to chromosomal anomalies that lead to disturbances in the correlation between the sets of chromosomes (Stebbins, 1940, 1950). Thus, it is concluded that both spectrophotometer and HPLC analyses confirmed the enhancement of the quantity of andrographolide through colchicine treatment. Hence, this could be a promising method to meet the increasing demand for this plant and also to enhance the level of andrographolide in normal diploid cells using colchicine, the polyploidy inducer. To conclude, the application of colchicine if used in the correct proportion will help to get better quantity of andrographolide. This methodology will definitely help to reduce the burden of production for medical purposes.

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**References**


