Quality control of marketed products of Andrographis paniculata by HPLC analysis
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Abstract
Andrographis paniculata is one of the most important medicinal plants having several pharmaceutical properties. Various pharmaceutical products and raw powders of this herb are sold in the market. The high demand for A. paniculata poses the problem of adulteration in their products. Therefore, it becomes necessary to check the quality in terms of their major bioactive compounds. In the present study, several marketed products were analyzed for their quality assessment i.e. for the presence of desired bioactive constituent, andrographolide. Ten tested products of A. paniculata showed the presence of andrographolide.

A validated High Performance Thin Layer Chromatography (HPLC) method has been used for the quantitative estimation of andrographolide. andrographolide was detected at a retention time (Rt) of 17.8±0.05 and was quantified using a linear regression equation. The amount of andrographolide in the marketed products was in the range of 0.01 to 7.08 %. The chromatographic fingerprints were developed in all marketed products in the study. Thus, the present study dealing with the detection and quantification of andrographolide through HPLC analysis would have enough significance in the quality control of marketed products of andrographis paniculata.

Keywords: Andrographis paniculata, andrographolide, HPLC analysis, herbal products, quality control.

Introduction
Andrographis paniculata (Burm. f.) Wall. ex Nees is one of the most used medicinal herbs across the globe. The herb is used both in traditional and modern systems of medicine. Traditionally, it has been used in many countries including India. It has been used against snake bite, diabetes, bug bites, malaria, fever and dysentery. It has many pharmaceutical activities like hepatoprotective, anti-inflammatory, anticancer, anti-diarrheal, etc. The plant extract is known to show the presence of many secondary compounds like lactones, diterpenoids, flavonoids, diterpene glycosides etc. The major bioactive constituent of Andrographis paniculata by which the plant has its widespread medicinal and pharmaceutical activities is andrographolide, a diterpenoid lactone. There are more than 100 products in the market as raw extract, tablets, syrup and tincture. There are at least 26 ayurvedic formulations in which A. paniculata is one of the constituents in Indian pharmacopoeia

The demand for A. paniculata and its products is increasing day by day due to their wide spectrum usability in the field of health care. The high demand for this plant increases the risk of adulteration in its marketed products and ayurvedic formulations. Another factor is the stability of bioactive compounds in the marketed products because the instability of the products results in alteration of quality, efficacy and safety. The changes in environmental parameters like temperature and humidity affect the stability of the compound. The prime cause of adulteration of the herbal products is the high demand and shortage of supply. The risk of adulteration necessitates checking the quality of the herbal products sold in the name of A. paniculata, either in the raw extract or in any ayurvedic formulation. Among many chromatographic techniques, HPLC has been widely used for the detection and quantification of andrographolide in A. paniculata and its products.

Therefore, the present study was designed for qualitative and quantitative analysis of andrographolide in different marketed products of A. paniculata by High Performance Thin Layer Chromatography (HPLC).

Material and Methods
Chemicals required and sample preparation: The HPLC grade methanol and water were procured from Merck Life Science Pvt. Ltd., Mumbai, India. Reference standard andrographolide (purity >99%) was procured from Natural Remedies Pvt. Ltd., Bangalore, India. Ten marketed products of A. paniculata were procured from the market. There were tablets, tinctures and raw powders which were taken for study. The samples were dissolved in methanol and placed in the water bath for 20 minutes in 60°C. They were then filtered and stored in HPLC vials for analysis. The standard chemical i.e. andrographolide was also dissolved in methanol for HPLC analysis.

HPLC Conditions and Method validations: The HPLC conditions were the same as described in the work of Champati et al. The HPLC method was validated for andrographolide according to the guidelines set by International Conference on Harmonization (ICH). The method was validated in terms of linearity, limits of detection (LOD) and quantification (LOQ), precision, stability and recovery test. The linearity was tested by injecting 4 different concentrations of andrographolide in HPLC. The recovery test was done using the standard addition method where a known amount of standard was
added to the analyzed samples and then re-analyzed. By injecting the standard solutions three times in one day, the precision was ascertained. The stability of the standard was assessed by injecting the same solution for two days at 24-hour intervals (0, 24 and 48 h). The limit of detection (LOD) is the minimum amount of analyte which can be detected in HPLC and the limit of quantification (LOQ) is the minimum amount of analyte which can be quantified in HPLC.

Results and Discussion
Method validation of quantitative analysis: The standard compound andrographolide shows good linearity in the range of 10-80 mg/L with a good coefficient of determination (R²) of 0.999. The regression equation used for the quantification of andrographolide was:

\[ y = 37.386x + 0.15 \]

where ‘y’ is the response area and ‘x’ is andrographolide concentration.

The LOD and LOQ of andrographolide were found to be 1.99 and 6.03 mg/L respectively. The precision and stability of the andrographolide showed a relative standard deviation of 1.75 and 1.58 %, respectively which satisfies the acceptable range. The mean recovery rate was 98.91% for andrographolide in the present study. Therefore, the HPLC used in the study is valid for the use of estimation of andrographolide in the samples. The HPLC Chromatogram of and chemical structure of andrographolide are given in fig. 1.

Estimation of andrographolide: The presence of andrographolide in the analyzed marketed products was done with the help of standard injection in the HPLC. Ten products analyzed were found to have andrographolide in the HPLC method in our study. The samples were leveled as APMP-1 to APMP-10 (Table 1). Two levels of qualitative analysis were done to identify andrographolide in the marketed samples. First the retention time of andrographolide was matched between samples and standard. Secondly, the spectra matched between samples and standard compounds.

The andrographolide showed the absorption maxima at 223 nm. The samples in which the HPLC chromatogram showed the presence of andrographolide based on retention time and absorption spectra were selected for quantitative analysis. The quantitative analysis was done with the help of a calibration curve. The peak area was taken for the calculation. The analysis showed that the amount of andrographolide varied from 1.05-7.08 % in monoherbal (single ingredients) formulations and 0.01-0.02 % in multi-ingredient or polyherbal formulations. The detailed content information is given in table 1. The comprehensive chromatogram of both single ingredient or raw powder marketed products and multi-ingredient or polyherbal formulations also differs little by peak pattern and peak number. The chromatograms are given in fig. 2 and fig. 3.

Intentional adulterations to earn more profit are harmful to the safety of the drug and people lose their trust in Ayurvedic medicines due to their inefficacy. Andrographis paniculata has been used as a home remedy in many countries. The processed form of this herb is sold in the market which may result in taking substituted, counterfeit and adulterated herbal products. The use of HPLC is most widely accepted and recommended by various regulatory bodies in testing the efficacy, quality and safety of the herbal products. Several studies have been carried out to access the quality of commercial herbal products. The variability of the bioactive constituents was observed in the commercial products of Withania somnifera through HPLC analysis. The study concluded many folds variation of withaferin A and emphasized stringent phytochemical standardisation of herbal products.

Similarly, the marketed products of Aloe vera and Eurycoma longifolia were accessed through HPLC analysis. The HPLC method validated in the study was efficient in detecting and quantifying the andrographolide in monoherbal tinctures (APMP-1 and APMP-2), raw powdered products (APMP-3 to APMP-8) and polyherbal formulations (APMP-9 and APMP-10). The chromatograms of APMP-1 to APMP-8 mimic to the chromatograms of the Andrographis paniculata plant from the natural population.

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Product Type</th>
<th>Amount of andrographolide in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>APMP-1</td>
<td>Tincture (Monoherbal)</td>
<td>4.96</td>
</tr>
<tr>
<td>APMP-2</td>
<td>Tincture (Monoherbal)</td>
<td>7.08</td>
</tr>
<tr>
<td>APMP-3</td>
<td>Raw Powder (Monoherbal)</td>
<td>1.05</td>
</tr>
<tr>
<td>APMP-4</td>
<td>Raw Powder (Monoherbal)</td>
<td>1.20</td>
</tr>
<tr>
<td>APMP-5</td>
<td>Raw Powder (Monoherbal)</td>
<td>1.52</td>
</tr>
<tr>
<td>APMP-6</td>
<td>Raw Powder (Monoherbal)</td>
<td>1.17</td>
</tr>
<tr>
<td>APMP-7</td>
<td>Raw Powder (Monoherbal)</td>
<td>1.21</td>
</tr>
<tr>
<td>APMP-8</td>
<td>Raw Powder (Monoherbal)</td>
<td>2.58</td>
</tr>
<tr>
<td>APMP-9</td>
<td>Tablet (Polyherbal)</td>
<td>0.01</td>
</tr>
<tr>
<td>APMP-10</td>
<td>Tincture (Polyherbal)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

APMP=Andrographis paniculata marketed products
This is due to the fact that these products were prepared by processing *Andrographis paniculata* without mixing any other herbal materials. The chromatogram of polyherbal formulations is different from monoherbal formulations and raw powdered products. This might be due to the presence of other herbal constituents in the polyherbal formulations and for which the chromatograms deviate from the chromatograms of monoherbal products and plants of the natural population. The other herbal constituents in the polyherbal formulations may give rise to additional peaks which are not found in the pure herbal material.
Conclusion

The present study would be helpful for quality testing of marketed products of Andrographis paniculata in terms of the presence of andrographolide by HPLC. The validated HPLC method used in the present investigation will efficiently detect and quantify the amount of andrographolide both in the monoherbal and polyherbal formulations. The HPLC chromatograms of the marketed products developed in the study could be used as reference chromatograms to access the quality of herbal products of Andrographis paniculata.

Acknowledgement

The authors would acknowledge the support and encouragement of Prof. (Dr.) S.C. Si, Dean, Centre of Biotechnology and Prof. (Dr.) M.R. Nayak, President, Siksha ‘O’ Anusandhan (deemed to be University). The authors would also acknowledge the financial support by the Department of Biotechnology, Ministry of Science and Technology, Govt. of India (Grant No. BT/PR21604/TRM/120/139/2016).

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(Received 04th October 2022, accepted 07th December 2022)