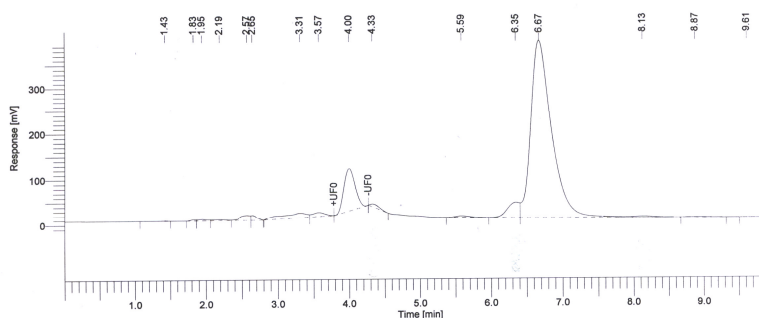


**Figure 5. A representative chromatogram of pure commercial ANG**



**Figure 6. A representative chromatogram of *Andrographis paniculata* chloroform extracts (APCE)**

aortic rings were significantly challenged the norepinephrine induced contractions (Figure 2).

**The effect of chloroform extract of AP on norepinephrine-induced contraction of isolated aortic strip preparations**

Pretreatment with chloroform extract of *Andrographis paniculata* reduced the maximal norepinephrine induced contraction in rat aortic rings from normal Sprague Dawely rats (SD rats). ( $R_{max} = 98.07 \pm 1.69 \%$ ,  $EC_{50} = 7.249 \pm 0.05$ ) (Figure 3), DMSO ( $R_{max} = 99.58 \pm 1.99\%$ ,  $EC_{50} = 7.17 \pm 0.05$ ), chloroform extract 25  $\mu\text{g}/\text{mL}$  ( $R_{max} = 74.38 \pm 2.10 \%$ ,  $EC_{50} = 7.0 \pm 0.07$ ), 40  $\mu\text{g}/\text{mL}$  ( $R_{max} = 41.63 \pm 1.19 \%$ ,  $EC_{50} = 7.0 \pm 0.07$ ), 80  $\mu\text{g}/\text{mL}$  ( $R_{max} = 21.25 \pm 0.79 \%$ ,  $EC_{50} = 6.87 \pm 0.09$ ), and 160  $\mu\text{g}/\text{mL}$  ( $R_{max} = 18.20 \pm 0.9 \%$ ,  $EC_{50} = 6.24 \pm 0.09$ ) rats.

**HPLC analysis**

**Linearity**

The calibration curve demonstrated good linearity and correlation coefficient over the given 10-100  $\mu\text{g}/\text{mL}$ . the mean linear regression equation from six replicate curve was  $y = mx + c$  with a correlation coefficient of 0.9996. The calibration curve is showed in Figure 4.

**Accuracy, precision and limit of detection**

Both Intra-day and inter-day accuracy and precision was given by relative error (% RE) and relative standard deviation (% R.S.D). The intra-day accuracy and precision of ANG were  $\leq 1.74$  and  $\leq 3.46$ . The inter-day

accuracy and precision ANG were  $\leq 0.58$  and  $\leq 2.29$ . The limit of detection was found to be (10  $\mu\text{g}/\text{mL}$ ) ANG was found to be  $\leq 0.13$  and  $\leq 2.17$ . The inter-day accuracy and precision for limit of quantification for ANG was found to be  $\leq 1.94$  respectively.

**DISCUSSION**

The present study shows that petroleum ether, chloroform and methanolic extracts of *Andrographis paniculata* produce concentration-dependent relaxation of aortic rings pre-contracted by norepinephrine. The  $EC_{50}$  and  $R_{max}$  values of these extracts suggest that the chloroform extract of *Andrographis paniculata* was more potent when compared to petroleum ether and methanolic extract of AP. The dose dependent contraction inhibition effects of AP were reversible, indicating that it does not cause tissue damage or tissue tolerance.

The phytochemical constituents of AP that have been identified include the diterpene lactones (6) and flavonoids (7-8). The *Andrographis paniculata* chloroform extract (APCE) inhibited in a concentration-dependent manner the contractile response induced by the norephenephrine in rat thoracic aorta. The presence of diterpene lactones and flavonoids in the extracts could significantly contribute for its vasorelaxant activity.

HPLC determination of the commercially available pure ANG was performed with a Nucleosil C18 (ODS) column

and UV detection was done at 223 nm with mobile phase of methanol: water, 65:35 (v/v). Earlier method (10) described a method using a mobile phase of chloroform: methanol (9:1, v/v) and detection at 254 nm. Hence, this method is a modification and was attempted because of the strong peak and better resolution of the compound at 223 nm. This was also confirmed from  $\lambda_{\max}$  of 223 nm obtained from absorption scan results of ANG.

Andrographolide (ANG) exhibited good linearity in the range from 10-100  $\mu\text{g/ml}$  in the calibration curves with a correlation coefficient of 0.999. The LOQ of ANG was found to be 10  $\mu\text{g/ml}$  within acceptable limits of accuracy and precision. Both intra-day and inter-day accuracy and precision were carried out at three different concentrations and were found to be within limits. The validation result shows that the developed HPLC method is specific, sensitive, and within acceptable accuracy and precision. Hence this method can be applied for routine analysis of ANG and can be used in routine quality control of *Andrographis paniculata* and standardization purposes by small scale local herbal industries in plant extracts. The system suitability parameters were found to be within acceptable limits. The ANG, peak was identified in the APCE by comparison of the retention time of commercially available pure ANG with a retention time ( $R_t$ ) of 4.0 min, (Figure 5 and 6) under similar chromatographic conditions.

In conclusion, the results of this study indicated that chloroform extract of *Andrographis paniculata* shows a potent inhibitor of norepinephrine induced contraction on rat aorta. Further studies on mechanism involved in vasorelaxation are under investigation.

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