Abstract

*Andrographis paniculata* is an herbal medicine which is widely used in traditional system of medicine especially in Ayurveda for the treatment of many diseases in Asian countries. Recently, some new biological activities of *Andrographis paniculata* such as cytokine induction or deduction, T cell activation suppression, a potential cancer therapeutic agent and its activity against chronic and infectious diseases have been reported. However the pharmaceutical potential of *Andrographis paniculata* extracts has not been determined. The main aim of the study was to study and characterize the major active chemical present in the different parts of *Andrographis paniculata* (Burm. f.) Nees (Family –Acanthaceae) for pharmaceutical potential. To fulfill the aim of the study, fractionation of different parts using different solvents, detailed study of Physico-chemical properties, physiological variation and study of the phytochemical profile of *Andrographis paniculata* was carried out. Anti-oxidant activity and antimicrobial activity of different extracts was also analyzed. %w/w content of the active component Andrographolide for pharmaceutical potential was determined in different parts, at different stages of life cycle and in different locations by HPTLC and HPLC analysis.

Various solvents (acetone, chloroform, ethanol, hexane, methanol, petroleum ether and water) were used to prepare extracts of different samples and for fractionation. Maximum yield of crude of leaves and whole plant material (drug) of different samples was obtained in methanolic extracts followed by the aqueous extracts. Study of Physico-chemical properties reveals that melting point of whole plant material (drug) and leaves was 228.5°C, 26°C respectively. Results of extractive value determination show that alcohol soluble extractive value was maximum and Pet. ether soluble extractive value was least. Among all samples studied alcohol soluble extractive value of L₄ (Leaves) sample was highest (23.42%). Results of physiological variation clearly indicate that both Chlorophyll a and Chlorophyll b were more in young plants than matured ones. Results also show that as Chlorophyll content decreases carotenoids and anthocyanin content increases. Preliminary phytochemical analysis of *Andrographis paniculata* shows presence of flavonoids, phenols, alkaloid, glycosides, saponins and tannins. Hot methanolic leaves extract was rich in phytochemical constituents. It was found that level of polyphenols and flavonoids content in the methanolic extract of *Andrographis paniculata* leaves was higher than ethanol and aqueous extracts.
The methanolic extracts of leaves of *Andrographis paniculata* showed promising anti-oxidant activity. Reducing power of the methanolic extracts of the leaves of *Andrographis paniculata* was found significant but less as compared to the standard ascorbic acid. The methanol extracts of leaves of *Andrographis paniculata* recorded the highest scavenging activity of 54% at 0.7mg/ml and 52% at 0.9mg/ml. Results also suggest that there is a direct co-relation between the total polyphenols extracted and anti-oxidant activity. Study of antimicrobial activity of different extracts indicates that for *S. aureus* and *E.coli* inhibition zone was maximum in chloroform extract of leaves while for *B. subtilis, P. aeuriginosa, A. niger* and *C. albicans* it was maximum in leaves methanolic extracts. In petroleum ether extracts, inhibition zone was negligible.

Whole plant material at different stages, from 30 days of plantation up to maturity of the crop was studied by HPTLC. The results observed under UV light showed a good separation for all compounds. The spots for Andrographolide in samples were confirmed by comparing the Rf and spectra of the spot with that of standard biomarker of Andrographolide. Rf for Andrographolide was found to be 0.31. The average Andrographolide content varied from 0.42% to 2.02% in the sample studied. The maximum Andrographolide content (2.02 %) was found in the sample D harvested after 110 days of plantation i.e. just before flowering. Quantitative analysis was undertaken by HPLC. Retention time of pure Andrographolide was 2.871 minutes. The maximum content of the Andrographolide was observed at 110 days after sowing (1.86%) whereas minimum was found at 30 days after sowing (0.81%). Leaves were found with highest 2.23% where as in flowers negligible percentage (0.01%) of Andrographolide was determined. Percentage of Andrographolide was found to be in the range of 1.03 – 2.60% in location variation study. The study revealed that in different growth stages, Andrographolide content showed significant variation and different parts of plant of *Andrographis paniculata* contain different percentage of Andrographolide. Maximum potency of Andrographolide was obtained in leaves harvested just before flowering.