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Qualitative and Quantitative Standardization of *Andrographis paniculata* by TLC Technique and UV Method

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Abstract

Background: *Andrographis paniculata* Wall. commonly known as Kalmegh and widely used for the treatment of the liver complications in Indian sub-continent. It is important ingredient of several traditional herbal formulations. **Materials and Methods:** Hence we planned to developed quick qualitative and quantitative standardization method for its quality control by using TLC and UV-visible spectroscopic methods for its better therapeutic efficacy. **Results:** TLC and UV-visible spectroscopic method has been developed for identification and quantification of andrographolide in *A. paniculata* herb samples. The developed TLC resulted in well resolved spots of different constituents of herb along with andrographolide standard. The developed UV-visible spectroscopic method was used for the estimation of andrographolide in A. paniculata herb samples. **Conclusions:** These quick standardization methods are used as analytical tool for the routine analysis of *A. paniculata* herb samples for achieving better therapeutic effects.

Key words: *Andrographis paniculata*, Kalmegh, Andrographolide, Standardization, TLC and UV.

INTRODUCTION

Medicinal plants have been identified and used traditionally throughout the world from beginning of the human civilization the primary health care of 70-80% of the world's population is based on the use of medicinal plants derived from natural sources especially from medicinal plants. Due to lack of quality, safety and efficacy data on natural products, people are unable to utilize their benefits. Due to this scientific awareness a scenario has created to undertake the research activities like standardization of natural products and medicinal plants and to develop the scientific methods for the manufacture of quality and safe herbal medicines.[1] The quality control and quality assurance is necessary when dealing with the natural products, intended to be released in market as drug constituents or as test substances in basic pharmacological experiments.[2] Quality refers to the intrinsic value of drug (the amount of medicinal principles or active constituents present). Chromatographic finger printing of the herbal medicines for their general profile or marker compounds is done by Thin Layer Chromatography. Chemical assay of the constituents or marker compounds is carried out using the latest analytical techniques of UV and visible spectrophotometric methods or HPLC/GC-MS for known chemical constituents.[3]

Andrographis paniculata Wall. belonging to family Acanthaceae and is widely used in Indian traditional medicine especially for treatment of liver complications.[4,5] In recent years, commercial preparations of this plant is used in Indian herbal medicines such as Kalmeghasava and Kalmeghnamay Haub, which are used for the treatment of liver complications.[6,7] *A. paniculata* mainly contains diterpenoids, diterpene glycosides, lactones, flavonoids and flavonoid glycosides.[8] The pharmacological actions of Kalmegh is mainly due to presence of andrographolide (Figure 1), neoandrographolide and kalmeghnin.[8-10] Several studies proven its hepatoprotective role in animal models.[9,11,12] *A. paniculata* and its major component andrographolide reported in several studies that it has anticancer,[13] antidiabetic,[14,15] antimalarial,[16] cardioprotective,[17] anti-inflammatory[18] and antioxidant activities.[19] As this plant is important ingredient of several traditional herbal formulations, hence we developed quick qualitative and quantitative standardization method for its quality control by using TLC and UV-visible spectroscopic methods for its better therapeutic efficacy.

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MATERIALS AND METHODS

Plant Material

Different samples (A-D) of *Andrographis paniculata* aerial parts were procured from the Khari Bawli drug market, New Delhi, India. The collected samples were identified and authentic samples were chosen for study purpose and voucher specimens (PRL/2013/09-13) of the plant were kept for future reference.

Chemicals

All chemicals procured of analytical grade. TLC plates (Silica gel 60 F_{254}) purchased from Merck, Mumbai, India. Andrographolide was purchased from Natural Remedies Pvt Ltd, Bangalore, India. Anisaldehyde, methanol, sulphuric acid and potassium hydroxide were purchased from S.D. Fine Chemicals, Mumbai, India.

Extraction Method

Aerial parts of samples (A-D) of *A. paniculata* were dried in an oven below 45°C. The dried samples were grinded separately in a grinder. Weigh about 10g of powder of *A. paniculata* samples and separately extracted in ultrasonic bath at 200 W ultrasonic power (Toshniwal, India) using ethanol (250 ml) at 50°C temperature for 30 min. The extracts were filtered separately and concentrated under vacuum using a rotary evaporator (Buchi, Switzerland).

Preparation of Standard Solution of Andrographolide

Weigh about 10 mg of andrographolide reference standard and transferred to 100 mL volumetric flask. About 10 mL methanol was added mixed well using ultrasonic bath and final volume was made up to 100 mL with methanol. This gives stock solution with concentration of 100 µg/mL for andrographolide. From stock solution we prepared dilutions ranging from 10 to 100 µg/mL for making calibration curve.

TLC Studies of Methanolic Extract of *A. paniculata*

Apply the test samples of *A. paniculata* (A-D) and reference standard andrographolide by using capillary tube on a precoated TLC plate. For the development of TLC, we tried several mobile phase combinations. Chloroform: ethanol (8: 0.5 v/v) was selected as mobile phase. The TLC plate was placed in a TLC chamber (twin-trough chamber, CAMAG) previously saturated with solvent used as mobile phase and TLC plate allowed to run a distance of 8 cm with mobile phase. The developed plate was dried in air and then sprayed with anisaldehyde-sulphuric acid reagent. The sprayed plate was heated in hot air oven at 105°C for 5 min. The TLC plate were also run simultaneously with reference solution of andrographolide (100 µg/mL) concentration.

Estimation of Andrographolide by UV Method

Preparation of Calibration Curve

Various concentrations ranging from 10-100 µg/mL were made from the standard stock solution of andrographolide and were analysed in triplicate. The absorbance of standard andrographolide were measured at 223 nm by using UV-visible spectroscope (Shimadzu, Japan). Linearity curve plotted between mean absorbance and concentration and was treated by linear least-square regression analysis

Application of developed UV Method

The test samples (A-D) of *A. paniculata* were measured at 223 nm by using UV-visible spectroscope and absorbance were obtained under the same conditions as that of standard andrographolide are recorded and the percentage of andrographolide present in samples were calculated.

Statistical analysis

Values are expressed as mean ± SD. Statistical analysis including linear regression analysis and calculation of means and Standard Deviation (SD) were performed with Microsoft Excel 2010.

RESULTS AND DISCUSSION

The safety and efficacy of herbal drugs depend upon purity of drug samples used in herbal formulation.[20] The purity of herbal samples was determined by using specific standardization methods described by WHO, it included physico-chemical methods, phytochemical methods and biological method.[21,22] Recently, chemical fingerprinting by TLC, HPTLC, HPLC, GC-MS etc. and spectroscopic techniques, are strongly recommended for the purpose of chemical standardization and quality control purposes of individual herbs and herbal products.[23]

Andrographolide (Figure 1) is a labdane diterpenoid that is major phytoconstituents of the *A. paniculata*, which has a broad range of therapeutic applications including anti-inflammatory, hepatoprotective, antidiabetic, antimalarial, cardioprotective, antioxidant and anti-platelet aggregation activities. Since andrographolide has multiple therapeutic activities, hence it is very important to develop its standardization protocols. In this paper we developed quick standardization of *A. paniculata* by using thin layer chromatography and UV-visible spectroscopic methods.

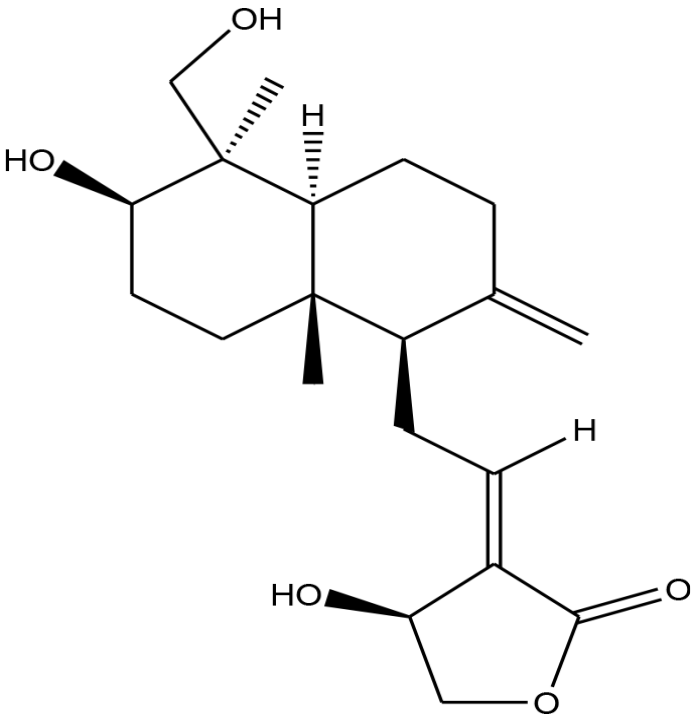


Figure 1: Chemical structure of Andrographolide.

Table 1: Comparative TLC profile of *A. paniculata* herb samples (A-D) and standard Andrographolide.

S. No.	Test samples	R_f values								
1	Sample A	0.06	0.17	0.20	0.37	0.49	0.60	0.70	0.76	0.85
2	Sample B	0.06	0.17	0.20	0.37	0.49	0.60	0.70	0.76	0.85
3	Andrographolide	-	0.17	-	-	-	-	-	-	-
4	Sample C	0.06	0.17	0.20	0.37	0.49	0.60	0.70	-	-
5	Sample D	0.06	0.17	0.20	0.37	0.49	0.60	0.70	-	-

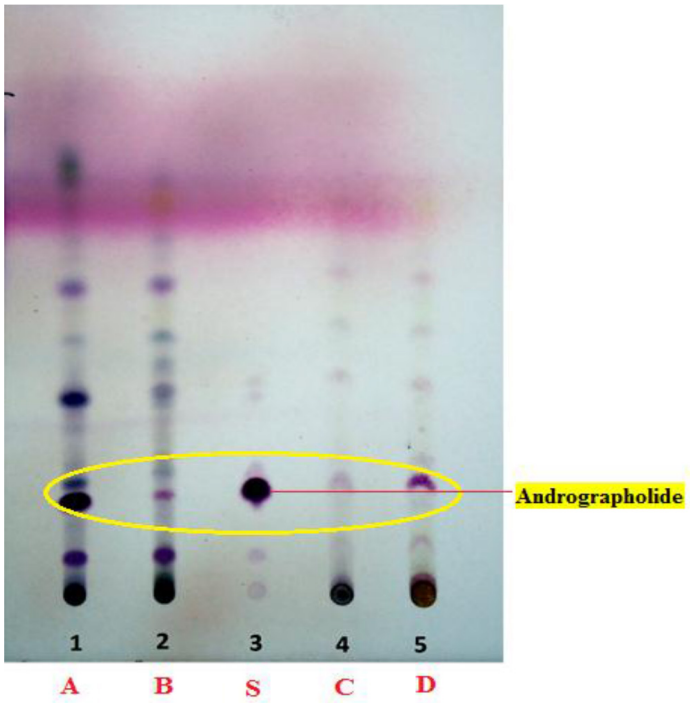


Figure 2: TLC plate showing spots of *A. paniculata* samples (A-D) and reference andrographolide (S) after spraying with Anisaldehyde-sulphuric acid reagent.

Standardization of *A. paniculata* Herb Samples by TLC Technique

TLC was development by using chloroform: ethanol (8: 0.5 v/v) as mobile phase. The developed TLC plate resulted in well resolved spots of phytoconstituents. The TLC plate shows well resolved spot at R_f 0.17 corresponding to andrographolide reference solution in all test samples of Kalmegh (Figure 2 and Table 1). From the Figure 2 and Table 1 it is evident that *A. paniculata* sample A and B shows nine spots and sample C and D only showing seven spots. Hence from the TLC studies the it is clear that sample C and D are inferior quality herbs instead of these herb samples having main phytoconstituents andrographolide.

Quantitative Analysis of *A. paniculata* Herb Samples by UV Method

The calibration curve absorbance versus concentration (µg/mL) was found linear in the range of 10-100 µg/mL (Table 2 and Figure 3). The regression equation was y = 0.02543 x + 0.33299 with correlation coefficient (r^2) of 0.99697.

Table 2: Calibration curve for standard Andrographolide.

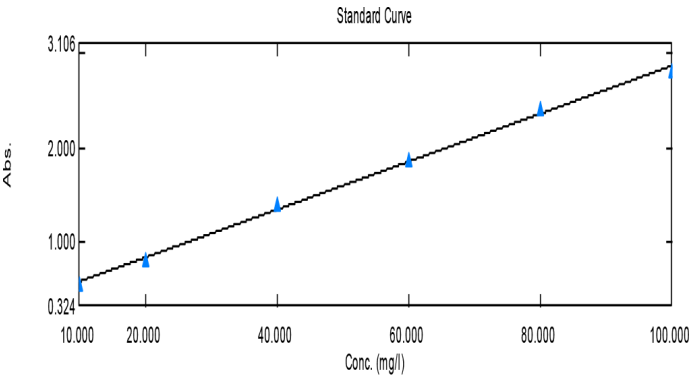
S. No.	Andrographolide conc. (µg/mL)	Absorbance (at 223 nm)
1	10	0.556±0.06
2	20	0.810±0.14
3	40	1.412±0.13
4	60	1.878±0.08
5	80	2.412±0.18
6	100	2.814±0.21

Each value represents a mean of triplicate analyses ± SD

Table 3: Andrographolide content in *A. paniculata* herb samples A-D.

S. No.	Test samples	Andrographolide content (w/w %)
	A. paniculata sample (A)	0.9662±0.03
	A. paniculata sample (B)	0.5608±0.04
	A. paniculata sample (C)	0.1838±0.02
	A. paniculata sample (D)	0.6154±0.01

Each value represents a mean of triplicate analyses ± SD.



y = 0.02543 x + 0.33299
Correlation Coefficient r2 = 0.99697

Application of UV-visible Spectroscopic Method

The proposed UV-visible spectroscopic method was applied for the estimation of andrographolide in *A. paniculata* herb samples (A-D). The content of andrographolide in ethanolic extract of *A. paniculata* herb samples (A-D) on dry weight basis of plant material is summarized in Table 3. The quantitative results showed that the *A. paniculata* herb samples (A) having more content (0.9662 % w/w) which is quite evident from qualitative TLC results.

CONCLUSION

A thin layer chromatographic and UV-visible spectroscopic method has been developed for identification and quantification of andrographolide in *A. paniculata* herb samples. The developed TLC resulted in well resolved spots of different constituents of herb along with andrographolide. The developed UV-visible spectroscopic method was successfully applied for the estimation of andrographolide in different samples of *A. paniculata*. These quick standardization methods are used as analytical tool for the routine analysis of *A. paniculata* herb samples for achieving better therapeutic effects.

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CONFLICT OF INTEREST

None.

ABBREVIATIONS

TLC: Thin layer chromatography; **HPLC:** High performance liquid chromatography; **HPTLC:** High performance thin layer chromatography; **GC-MS:** Gas chromatography mass spectroscopy; **SD:** Standard deviation; **Mg:** Micro gram.

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