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A Rapid Method for Isolation of Andrographolide from *Andrographis paniculata* Nees (Kalmegh)

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ABSTRACT

A simple and rapid method for isolation of andrographolide from the leaves of Andrographis paniculata *is reported. This involves extraction of the leaf powder by cold maceration in a 1:1 mixture of dichloromethane and methanol and isolation of andrographolide directly from the resulting extract by recrystallisation. The identity of the compound was confirmed through IR, UV, mass and melting point, and co-chromatography with a reference standard on TLC. The purity of the compound was confirmed by TLC, UV absorption spectrum, HPLC and differential scanning calorimetry, the latter of which gave the melting point of andrographolide as 235.3°C.*

INTRODUCTION

Andrographis paniculata (Acanthaceae), commonly known as kalmegh, is widely used in Indian systems of medicine as a stomachic, tonic, antipyretic, alterative, anthelmintic, febrifuge and cholagogue; for liver disorders, general debility and colic pains (Nadkarni, 1954; Bentley & Trimen, 1983; Gupta et al., 1990; Aminuddin et al., 1997). The leaf forms an ingredient of many patented Indian herbal proprietary preparations (e.g., *Kalmeghasava* and *Kalmeghnamay Haub*) for the treatment of liver ailments (Handa et al., 1986; Chaudhri, 1996; Evans, 1996).

Keywords: Andrographis paniculata Nees., andrographolide, Kalmegh.

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Andrographolide, neoandrographolide and kalmeghnin present in the plant have been reported to be the active principles (Handa et al., 1986; Choudhury et al., 1987). Some of the other chemical constituents include andrographanin, andrograpanoside, 14-deoxy-12-methoxyandrographolide, and deoxyandrographolide (Fujita et al., 1984). The leaf extract, as well as andrographolide, have been shown to protect against alcohol and CCl_4-induced hepatotoxicity and CCl_4-induced microsomal lipid peroxidation (Choudhury et al., 1987; Chander et al., 1995). Andrographolide has also been reported to have activity against *Plasmodium berghei* NK 65 (Misra et al., 1992).

We report a simple method for isolation of andrographolide from the leaves of *A. paniculata* and details of its characterization and purity check by IR, UV, LCMS, HPTLC, HPLC and DSC.

MATERIALS AND METHODS

Plant Material

Fresh leaf material of *A. paniculata* was obtained from a local medicinal plant farm in the month of November and authenticated. The leaf material was air-dried under shade for a day and then in a hot-air oven below 60°C. It was powdered to 40 mesh and stored in an air-tight container at 15–20°C until further use.

Extraction and Isolation

Three samples of leaf powder, 50 g each, were subjected to extraction with 3 different solvents:

- One sample of leaf powder was extracted exhaustively with a 1:1 mixture of dichloromethane and methanol by cold maceration. The extract was filtered and the solvent removed under vacuum

(extract: 4.8g) – (AP I denotes andrographolide sample isolated from this extract).

- The other two samples were macerated overnight, one in methanol and the other in 95% alcohol and filtered. The marc was packed into a Soxhlet and extracted with the respective solvents (10 cycles in each case). The extracts were collected separately and the solvents removed under vacuum (MeOH extract: 5.4 g; 95% alcohol extract: 5.5 g) – (AP II and AP III denote andrographolide samples isolated from the above methanolic extract and 95% alcoholic extract, respectively).

The dark green crystalline mass obtained was washed, separately, with toluene several times until most of the colouring matter was removed from the residue. Then the toluene was completely removed from the residue. The crystalline material left behind (yield 1.9–2.0 g) was dissolved in hot methanol and cooled in a refrigerator for crystallisation. The process was repeated several times until colourless plates of constant melting point (uncorrected) of 230–231°C (cc. Merck Index, 1989) were obtained (melting point was taken in a Melting Point Apparatus, TOSHNIWAL, Bombay).

Thin-Layer Chromatography

For TLC experiments, precoated plates of silica gel $60F_{254}$ (E. Merck) were used and spotting was done on CAMAG LINOMAT IV Automatic TLC spotter. For purity assessment of the isolated compounds and for recording UV spectrum of the compound, the plates were scanned on a CAMAG TLC Scanner 3.

Methods Adopted for Testing the Purity of the Andrographolide Isolated

The purity of the compound isolated was checked by the following:

1. By carrying out TLC in different solvent systems (Puri et al., 1993; Chander et al., 1995) and co-chromatography along with reference standard andrographolide (obtained from Regional Research Laboratory, Jammu-Tawi, India).
2. By recording chromatogram and UV absorption spectrum of the compound developed on TLC plate in the solvent system containing chloroform: methanol:ethyl acetate (8:1.5:1) on a CAMAG TLC Scanner 3
3. By carrying out HPLC of the isolated compounds on a μ Bondapack C-18 (10 μm) column (3.9 × 300 mm), using a JASCO HPLC system, with a mobile phase containing methanol and water (1:1), at a flow rate of 1 ml/min and the eluted compound detected at 223 nm (method given in Anonymous, 1998, with some modifications).
4. By recording the melting point of the compound on a differential scanning calorimeter (DSC 7, PERKIN ELMER)

UV, IR and MS

The sample along with the reference standard of andrographolide was spotted and developed in a solvent system containing chloroform:methanol:ethyl acetate (8:1.5:1). The UV absorption spectrum was recorded on a CAMAG TLC Scanner 3. UV absorption spectrum of the sample and the reference standard, in methanol, were also recorded on UV/VIS spectrophotometer (JASCO model 7850). IR spectra were recorded on a BUCK SCIENTIFIC IR Spectrophotometer (model 500). Atmospheric pressure ionisation with ion spray mass spectra of molecular ions were obtained on a PE SCIEX API 165 MS with WATERS LC.

RESULTS AND DISCUSSION

The yield of crude andrographolide isolated, using the three different solvents, was found to be about the same (1.9–2.0 g). Preliminary TLC evaluation of the three extracts showed andrographolide to be the major component. Isolation of this compound in pure form was achieved by repeated washing of the crystalline matter off the green colouring material with toluene and repeated recrystallization from methanol and final washing of the crystals with cold methanol. The purity of the sample at every stage of recrystallization was monitored through TLC. Among all the solvent systems tried, the one containing chloroform: methanol:ethyl acetate (8:1.5:1) was found to be suitable for both extracts and pure andrographolide (R_f: 0.65).

Purity of the Andrographolide Isolated

The purity of andrographolide isolated was established by the following:

1. TLC of the isolated sample, carried out in different solvent systems (some of them reported, Puri et al., 1993; Chander et al., 1995), showed a single spot with its R_f value varying from 0.2 and 0.9 in different solvent systems:

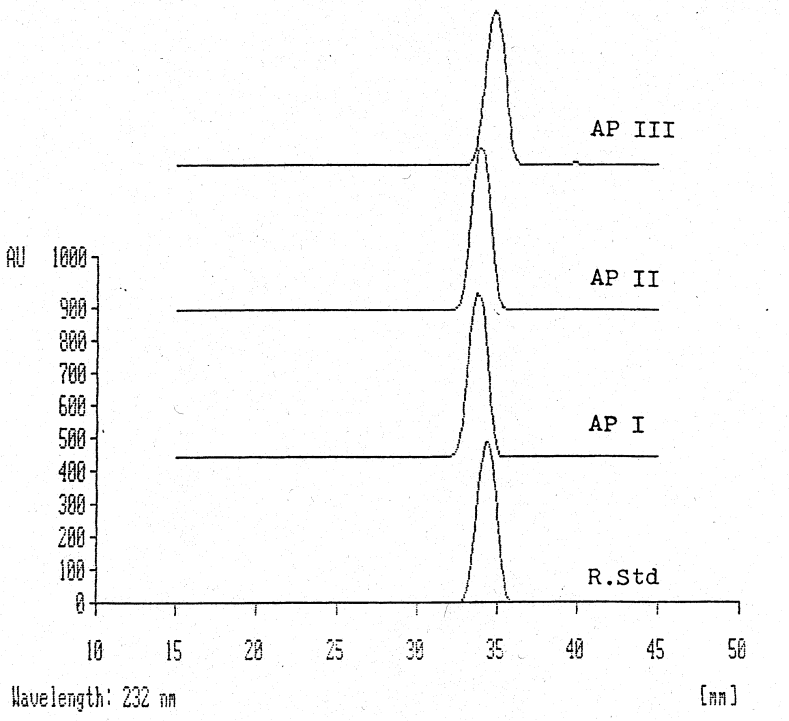

Fig. 1. TLC chromatogram of andrographolide isolated (AP I, APII and AP III) and reference standard (R. Std).

Solvent system	*R_f of Andrographolide*
Chloroform:methanol:ethyl acetate (8:1.5:1)	0.65
Chloroform:methanol (9:1)	0.9
Chloroform:ethyl acetate (6:4)	0.2
Chloroform:acetone:formic acid (7.5:1.65:0.85)	0.8

2. UV absorption spectrum, recorded (on CAMAG TLC Scanner 3) at start, middle and end positions of the band for purity of the sample, completely over lapped, and gave an absorption maxima (λ_{max}) at 232 nm (Fig. 2). Further, the TLC chromatogram also showed a single peak (Fig. 1).
3. The HPLC analysis of the isolated compound gave a single peak (Fig. 3) with a retention time of 4.8 min, as with the standard andrographolide.
4. The analysis by DSC gave a single sharp peak with a melting point of 235.3°C and it is comparable with that of the standard compound (Fig. 4). To the best of our knowledge, this is the first report on the accurate melting point recorded for andrographolide on DSC (cc with the m.p. of 230–231°C obtained on melting point apparatus, TOSHNIWAL).

The isolated sample of andrographolide was characterised by its spectral data (UV, IR and mass) and found that it matched well with that of the reference standard andrographolide. The UV and IR spectra of the isolated sample and the reference standards were superimposable.

Spectral Data

UV: λ_{max} = 232 nm (CAMAG TLC Scanner 3), λ_{max} in MeOH = 222 nm (JASCO UV/VIS spectrophotometer).

IR: 3340–3200 (OH), 1725 (lactone), 1667 (double bond), 909 (external CH_2).

LCMS: Mobile Phase: MeOH + 2 mM NH_4OAc; 351 $(M + H)^+$, 368 $(M + NH_4)^+$, 373 $(M + Na)^+$ (cc. Mol. wt. 350.46, Merck Index, 1989).

The above spectral data of the isolated sample and the reference standard confirm the identification of the isolated compound as andrographolide (IR comparable with the reported data of Fujita et al., 1984). The reported activities of andrographolide, as antihepatotoxic, analgesic, antipyretic, antiulcerogenic, anti-inflammatory and immunostimulant agent (Puri et al., 1993; Chander et al., 1995; Saraswat et al., 1995; Madav et al., 1995, 1996), justify the development of this simple and rapid method for its extraction and isolation. Since in Ayurveda texts it has been recommended to collect the plant material in the months of

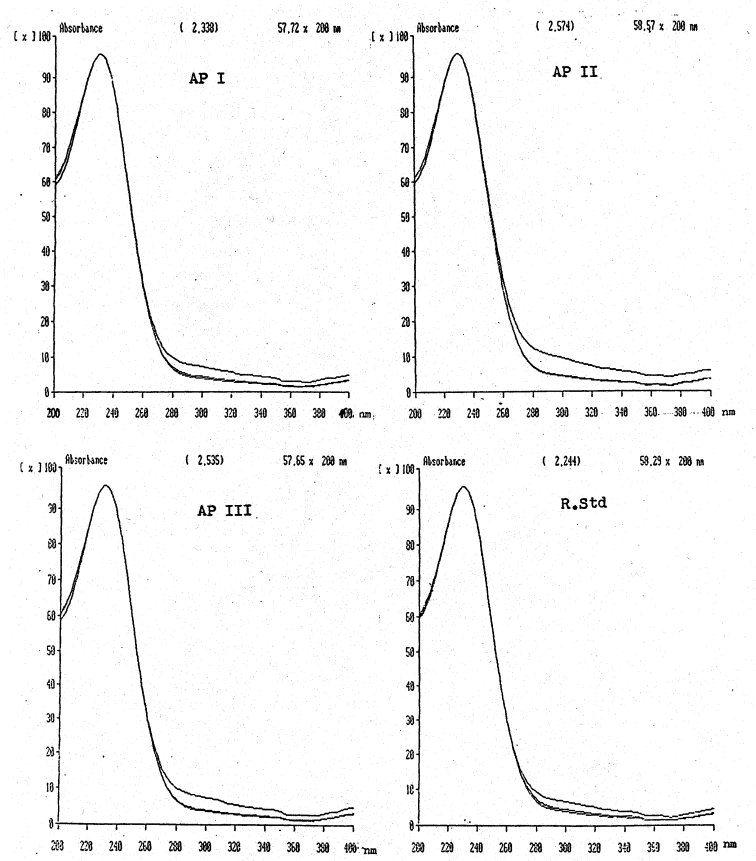

Fig. 2. Absorption spectra of andrographolide isolated (AP I, APII and AP III) and reference standard (R. Std), in the UV range, taken on a CAMAG TLC Scanner 3.

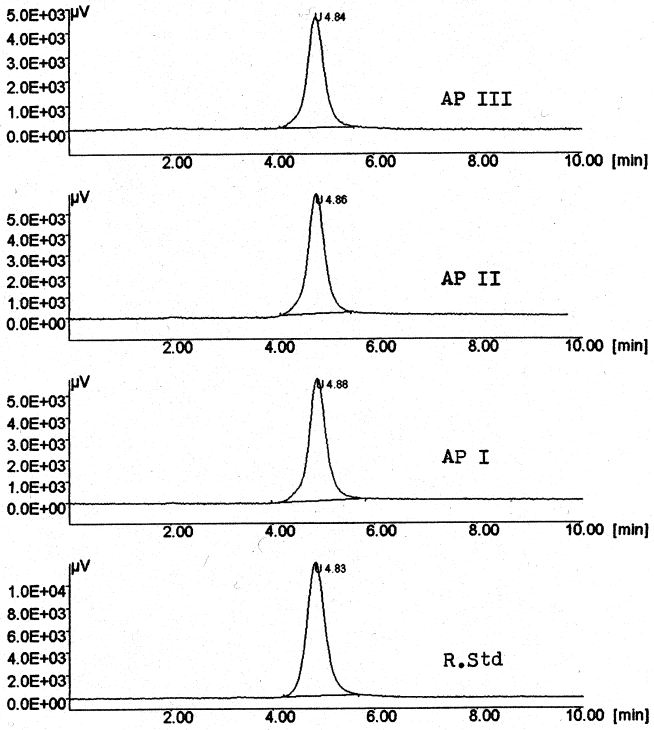

Fig. 3. HPLC of andrographolide isolated (AP I, APII and AP III) and reference standard (R. Std).

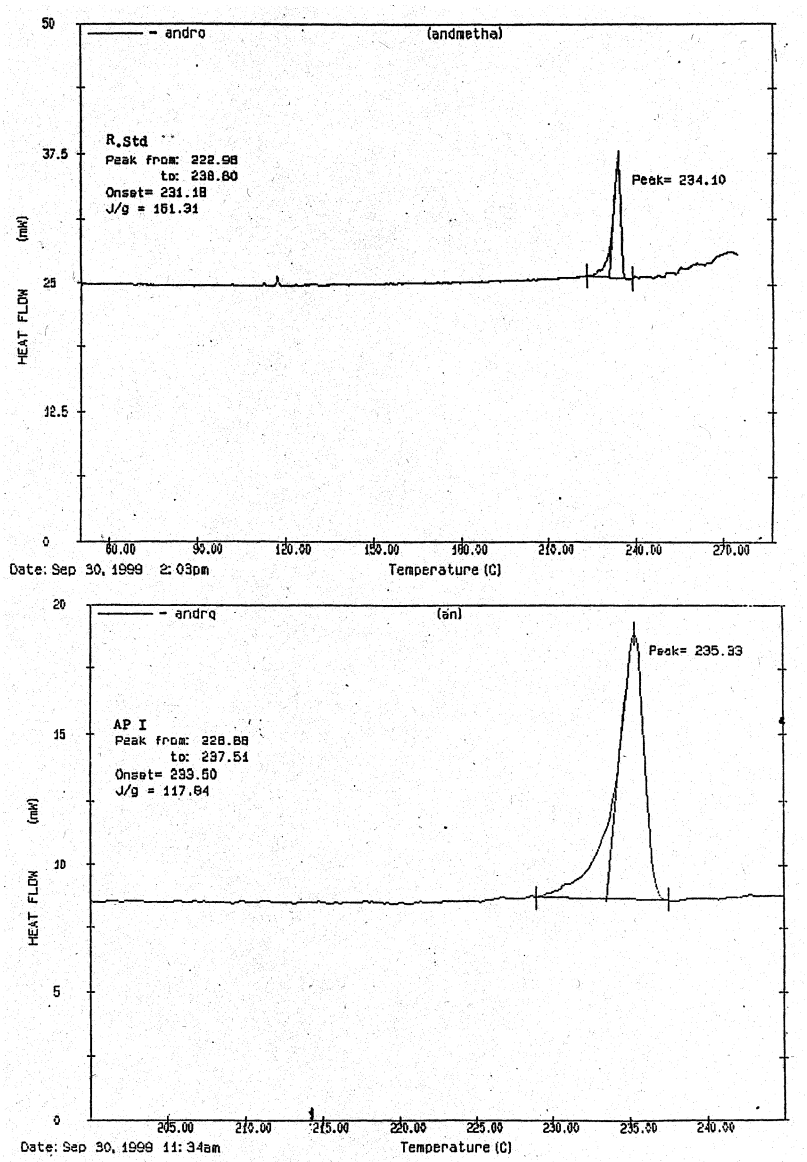

Fig. 4. DSC curve for andrographolide isolated (AP I) and reference standard (R. Std).

October-November, we collected the sample in November and obtained a high yield of andrographolide.

CONCLUSION

Of the three solvents used for the extraction of *A. paniculata* leaf, the mixture of dichloromethane and methanol (1:1) was found to be comparable in its extractive efficiency as far as the amount of andrographolide is concerned, with the conventional solvents, *viz*., methanol and alcohol. Furthermore, this extraction eliminates the requirement of heating and Soxhlet extraction.

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