Chromatographic Finger Print Analysis of Herbal Drug (*Andrographis paniculata* Nees) by HPTLC Technique

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Abstract

The present study was designed to determine the HPTLC profile of the medicinally important plant *Andrographis paniculata* Nees. The Chloroform: Methanol (85:15) was employed as mobile phase for phyto-constituents. Linear ascending development was carried out in 20 cm x 10 cm twin trough glass chamber (Camag, Mutenz, Switzerland) saturated with the mobile phase and the chromatoplate development for two times with the same mobile phase to get good resolution of phytochemical contents. The developed plate was seen under UV light 254 nm and 366 nm. The methanolic extract of whole parts of *Andrographis paniculata* Nees showed the presence of 12 different types of phyto-constituents with different Rf. values. The developed HPTLC fingerprints will help the herbal drug industry to distinguish the adulterant and standardization of herbal formulations. Such chemo finger printing will act as biochemical markers for this medicinally important plant in the pharma industry and plant systematic studies.

Keywords: Terpenoids, HPTLC profile, Fingerprint, Phytochemistry

Introduction

Natural products derived from food and medicinal plants are the potential sources of antioxidant molecules. Herbal drugs have been in exercise by different civilizations in various parts of the world for centuries to treat a large number of diseases. Today, the plant based medicines are being used worldwide as medication and suggest a broad spectrum of activity since ancient times. But Indian herbal drugs have still low acceptability in the world market due to insufficient scientific validation. International agencies especially WHO emphasized on quality standards of complex herbal formulations through scientific validation of single raw drugs. The drug efficiency depends upon the several active principles and components present in it. Many natural (age, origin) & scientific (methodology of drug formulation) factors influence the proportion of various components in plant material.

The well developed quality standards can be achieved only through systematic evaluation of the plant material using modern analytical techniques including chromatographic ones. TLC and HPTLC are methods commonly applied for the identification, assay and the testing of purity, stability, dissolution or content uniformity of raw materials (herbal and animal extracts, fermentation mixtures, drugs and excipients) and formulated products (pharmaceuticals, cosmetics, nutrients).

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Andrographis paniculata Nees (family: Acanthaceae) popularly known as 'Kalmegh' in trade and widely cultivated in India. It is used in a number of formulations of Ayurvedic, Unani and Sidha system of medicines as ingredients with the name of 'Bhunimba', 'Kalmegh' and 'Nilavempu' respectively. It has been used for centuries in Asia to treat gastro-intestinal track and upper respiratory infection, fever herpes, sore throat, and a variety of other chronic and infectious diseases. Mostly the leaves and roots were used for medicinal purpose.

The key photochemical constituents of the herb are andrographolide other such phytochemical are 14-deoxy-11-oxonedrographplide, 14-deoxy-11,12-didehydroandrographolide, neoandrographolide and deoxyandrographolide (Rajani *et al*., 2000; Cheung *et al*., 2001; Kumaran *et al*., 2003; Raina *et al*., 2007; Kulyal *et al*. 2010; Mishra *et al*., 2010)**.** The plant is reported to posse's antihepatotoxic, antibacterial, antiviral, antimalarial, antihepatitic, antithrombogenic, antiinflammatory, anti snake venom, antipyretic, laxatives, and immunostimulant agent (Chadha, 1985; Madav *et al*., 1995; Handa & Sharma, 1990; Mishra *et al*., 2009; Puri *et al*., 1993; Sharma *et al*., 1992; Srivastava *et al*., 2004; Saxena *et al*., 1998; Patel *et al*., 2008; and Mishra *et al*., 2007). The plant has been reported to possess antipyretic, analgesic, antihepatotoxic, antidiabitic antimalarial, antibacterial, antifertility anti-inflammatory and immunosuppressive properties due to bitter content (Mishra *et al*., 1992; Kapil *et al*., 1993; Saraswat *et al*., 1995; Singhal *et al*., 2003). Most of biological actions of *Andrographis paniculata* has been due to the presence of andrographolide, which is a bicyclic a diterpene lactone. About 26 different poly herbal formulations of this plant are mentioned in Ayurveda as a popular remedy for the treatment of various liver disorders. In traditional Chinese medicine (TCM) *Andrographis* is considered as the herb possessing an important cold property useful to treat the heat of body in fever and to dispel toxins from the body. In Scandinavians countries, it is commonly used to prevent and treat common colds.

The present study communicates the reliable HPTLC finger prints profilethat represent pharmacologically active and chemically characteristics component of the medicinally important plant *Andrographis paniculata* Nees. It will be helpful to authenticate and evaluate the drug in respect of quality evaluation phyto-chemical (active constitutes) identification.

Material and Methods

Plant material and chemicals

Fresh plant material was collected from Ghaziabad (UP), India in the month of January 2011; and the specimens were authenticated with the help of standard floras and pharmacopoeial reference (Anonymous, 2010). The whole plant was shade dried and powdered using the electric homogenizer. 500 gm of the powdered samples were extracted with 500 ml methanol for 8 to 12 hours by using Soxhlet apparatus. There after methanolic extracts of plant material was filtered through Whatman paper no. 42 and the resultant filtrates were concentrated under reduced pressure and finally vacuum dried. The yield of the methanolic extract was 13.2 % w/w. The protocol for preparing sample solutions was optimized for high quality fingerprinting and also to extract the marker compounds efficiently. Since the marker compounds were soluble in methanol, therefore methanol was used for extraction. Preliminary phytochemical screening was done by following the standard method described by Lala (1993) and Kokate et al. (2005). For the experimental work pre-coated silica gel 60 F254 HPTLC plates, standard Andrographolide (Purity: 99% w/w) and analytical reagent (AR) grade chemicals were used.

Chromatographic conditions

HPTLC studies were carried out by CAMAG HPTLC system equipped with Linomat V applicator, TLC scanner 3, Repostart 3 with 12 bit CCD camera for photo documentation, co-controlled by Win CATA-4 software were used. The samples and standard were spotted in the form of bands of width 5 mm with a microlitre syringe on pre-coated silica gel glass plate 60F_{254} (20 × 10 cm with 250 µm thickness using a Camag Linomat IV (Switzerland). The plates were pre-washed by methanol and activated at 60°C for 5 min prior to chromatography. The sample loaded plate was kept in TLC twin trough developing chamber (after saturated with solvent vapor) with respective mobile phase and the plate was developed in the respective mobile phase up to 90 mm. The Chloroform : Methanol (85:15) was employed as mobile phase. Linear ascending development was carried out in 20cm x 10cm twin trough glass chamber saturated with the mobile phase and the chromatoplate development for two times with the same mobile phase to get good resolution of phytochemical contents. The optimized chamber saturation time for mobile phase was 30 min at room temperature (25 ± 2°C). The developed plate was dried by hot air to evaporate solvents from the plate. The plate was photo-documented at UV 254 nm and 366 nm using Photo-documentation

device. Finally, the plate was fixed in scanner stage and scanning was done at 254nm and 366 nm. The plate was kept in Photo-documentation chamber and captured the images under UV light at 254 and 366 nm. Densitometric scanning was performed on Camag TLC scanner III and operated by CATS software (V 3.15, Camag).

Observation

Diverse compositions of the mobile phase for HPTLC analysis were tested in order to obtain high resolution and reproducible peaks. The separation was achieved using Chloroform : Methanol (85:15) as the mobile phase. The methanol extract of whole aerial parts of *Andrographis paniculatata* showed the presence of 12 different types of phyto-constituents with different Rf. values (Figure 1&2 and Table 1&2). The peak area of Rf. value 0.87 (at 254 nm) and 0.53 (at 366 nm) showed highest area. Andrographolide showed single peak at Rf. value at 0.87 (254mm) and 0.91 (366 nm).

Results and Discussion

The chemical analysis of methanol extracts of *Andrographis paniculata* Nees showed the presence of various phytoconstitutents. The isolation and identification of these bioactive compounds can be used to formulate new drugs to treat various diseases and disorders. In recent times during this molecule era in addition to morphological characters in plant taxonomy anatomical, cytological, biochemical and molecular markers are also being used to classify the plants. HPTLC finger printing profile is useful as phytochemical marker and also a good estimation of genetic variability in plant populations. The data generated from the present study would help in the authentication and quality control for *Andrographis paniculata* Nees. Such chemo finger printing will also act as biochemical markers for this medicinally important plant in the pharma industry and plant systematic studies.

Table 1: Peak display of Andrographolide **at 254 nm**

Peak	Start Rf.	Start height	Max Rf.	Max height	Height %	End Rf.	End height	Area	Area %
1.	0.87	2.0	0.89	77.7	100.0	0.91	0.00	1279.0	100.0

Table 2: Peak display at different Rf values of Andrographis paniculata methanol extract at 254 nm

Peak	Start Rf.	Start height	Max Rf.	Max height	Height %	End Rf.	End height	Area	Area %
1.	0.12	0.1	0.13	32.9	2.39	0.16	2.1	424.3	1.26
2.	0.16	2.2	0.18	27.2	1.97	0.2	0.5	516.5	1.53
3.	0.22	1.3	0.25	36.2	2.63	0.29	0.0	33.3	2.46
4.	0.38	0.6	0.42	13.1	0.95	0.44	7.1	326.5	0.97
5.	0.46	5.5	0.48	26.2	1.90	0.5	16.6	617.1	1.83
6.	0.5	17.1	0.53	152.0	11.04	0.58	32.8	3809.3	11.27
7.	0.56	33.5	0.58	104.1	7.56	0.62	1.2	2492.7	7.38
8.	0.63	0.0	0.66	252.4	18.34	0.71	6.1	6095.9	18.04
9.	0.71	6.2	0.72	25.3	1.84	0.74	5.3	321.3	0.95
10.	0.74	5.9	0.77	194.6	14.14	0.8	57.4	4650.2	13.76
11.	0.81	57.8	0.83	98.1	7.12	0.84	87.8	2291.0	6.78
12.	0.84	89.8	0.87	414.8	30.13	0.91	9.5	11412.5	33.78

Table 3: Peak display of *Andrographolide* at 366 nm

Peak	Start Rf.	Start height	Max rf	Max height	Height %	End Rf.	End height	Area	Area %
1.	0.91	3.6	0.93	15.4	100.0	0.95	0.7	305.8	100.0

Table 4: Peak display at different Rf values of *Andrographis paniculata* at 366 nm

Peak	Start Rf.	Start height	Max Rf.	Max height	Height %	End Rf.	End height	Area	Area %
1.	0.12	0.1	0.13	27.5	3.88	0.16	0.3	321.0	1.7
2.	0.2	4.2	0.25	83.4	11.77	0.29	0.3	2247.4	11.88
3.	0.43	4.3	0.47	30.5	4.3	0.49	23.7	964.0	5.09
4.	0.49	24.0	0.53	205.2	28.98	0.56	42.6	5633.3	29.77
5.	0.56	43.1	0.58	66.7	9.43	0.63	13.1	2377.6	12.56
6.	0.63	13.4	0.66	189.6	26.77	0.71	0.3	5183.0	27.39
7.	0.71	0.4	0.72	33.3	4.70	0.74	4.9	450.1	2.38
8.	0.75	3.3	0.79	33.9	4.79	0.81	12.8	1010.6	5.34
9.	0.82	12.0	0.86	15.6	2.20	0.87	2.7	375.5	1.98
10.	089	0.3	0.92	22.5	3.08	0.93	0.6	360.6	1.91

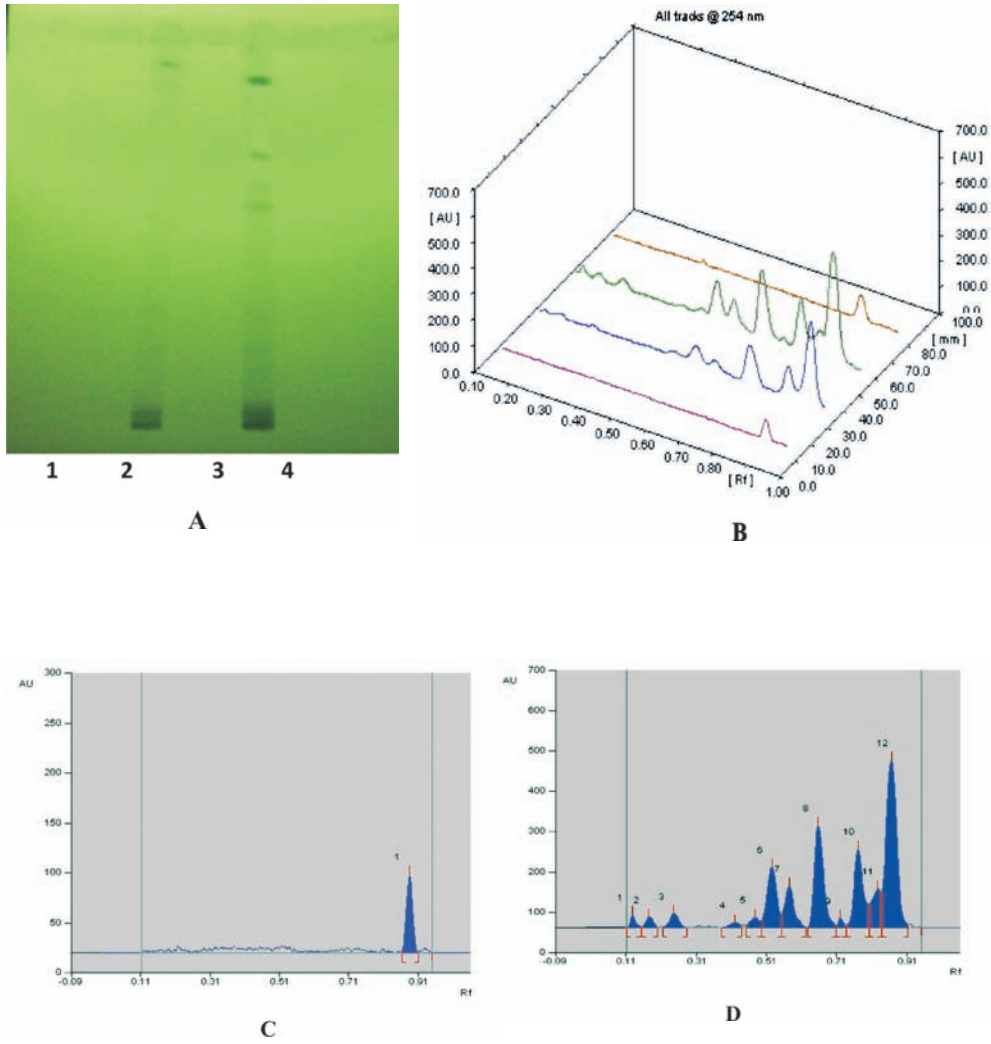

Fig.-1. HPTLC fingerprinting profile under UV 254 nm

A. HPTLC fingerprints profile of methanol extract and reference standard (Andrographolide)
B. Overlay Chromatogram
C. Chromatogram of Andrographolide
D. Chromatogram of methanol extract

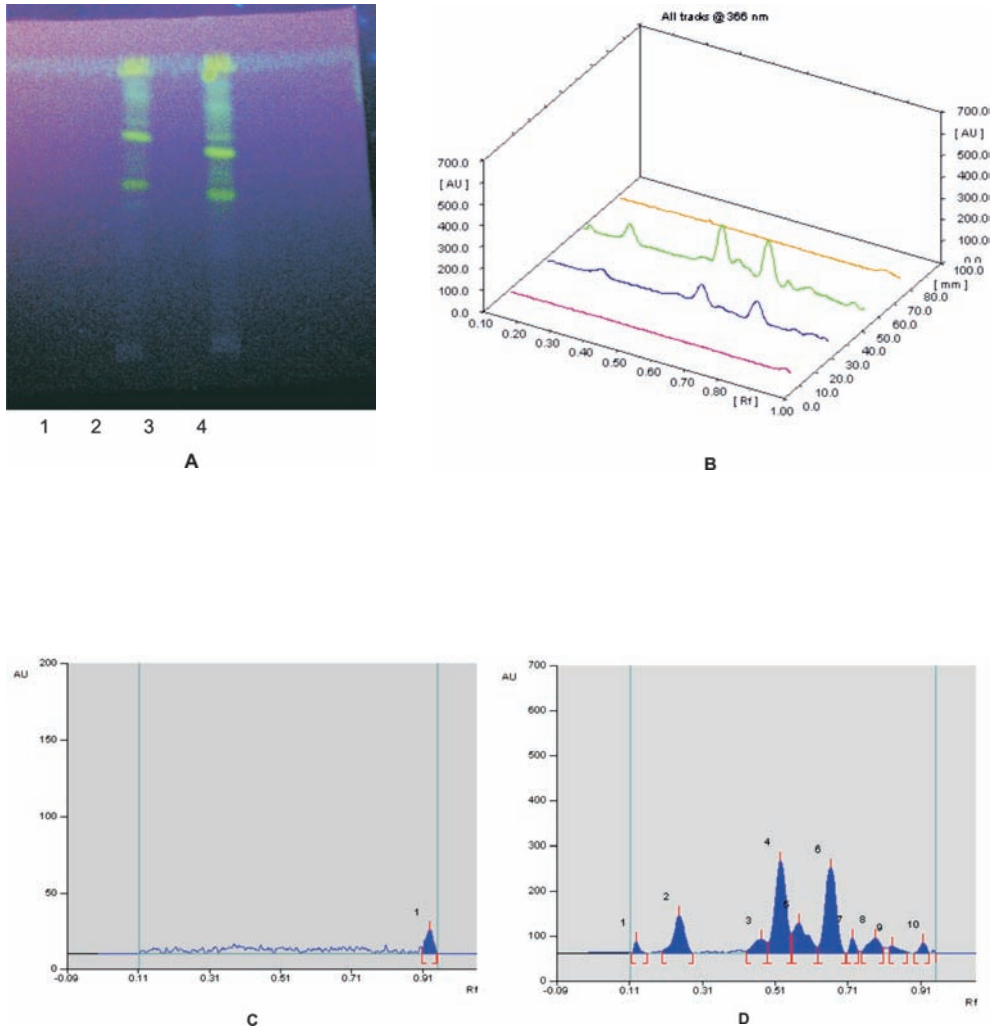

Fig. 2. HPTLC fingerprinting profile under UV 366 nm

A. HPTLC fingerprints profile of methanol extract and reference standard (Andrographolide)
B. Overlay Chromatogram
C. Chromatogram of Andrographolide
D. Chromatogram of methanol extract

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