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Research Article

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Development of quality standards of *Andrographis paniculata* Wall ex. Nees whole plant

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ABSTRACT

India being a rich and varied flora of medicinal plants since the Vedic period. The present study deals with the scientific validation of medicinal plants - Andrographis paniculata Nees. (Kalmegh or green chiretta) with special reference to its pharmacognostical investigations. Kalmegh or green chiretta and is known to have medicinal properties, also even used as home remedies in the rural and the remotest parts of the India. The Andrographis paniculata used as anti-diabetes, antioxidant, anti-inflammatory, antidiarrhoeal, antiviral, antimalarial, hepatoprotective, anticancer, anti-human immunodeficiency virus (HIV), immune stimulatory and antisnakebite activity.

Keywords: *Andrographis paniculata* Nees., phytoconstituents, pharmacological investigations, Standarization

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INTRODUCTION

Andrographis paniculata is an annual herbaceous plant in the family Acanthaceae, native to India (cultivated in gardens from Chitrakoot, Satna, M.P., Lucknow, UP Asam and Bengal) and Sri Lanka. It is widely cultivated in Southern and Southeastern Asia, where it has been traditionally used to treat infections and some diseases. Mostly the leaves and roots were used for medicinal purposes. The *Andrographis paniculata* used as pills, malaria, stomachs and antheelmintic as a domestic remedy in griping, irregular, stools, loss of appetite, flatulemce and dirrhoea of children; is also anthelmintic, sluggish liver, neuralgia gouty dyspepsia, fevers, influenza, anti-diabetes, antioxidant, anti-inflammatory, antidiarrhoeal, antiviral, antimalarial, hepatoprotective, anticancer, anti-human immunodeficiency virus (HIV), immune stimulatory and antisnakebite activity [1-2].

Unlike other species of the genus, *A. paniculata* is of common occurrence in most places in India, including the plains and hilly areas up to 500 m, which accounts for its wide use. Since ancient times, *A. paniculata* is used in traditional Siddha and Ayurvedic systems of medicine as well as in tribal medicine in India and some other countries for multiple clinical applications.

The fresh juice of the leaves mixed with spices such as cardamom (*Elettaria cardamomum*), clove (*Syzygium aromaticum*), cinnamon (*Cinnamomum zeylanicum* Blume.) etc., dried and made into pills for use as a household remedy for the minor digestive ailments of children. The plant also enjoys considerable reputation as a febrifuge,

alternative and bitter tonic, and is often used as a substitute for *Chiratta* (*Swertia chirata*). The central Indigenous drugs common found it to be of some value in dysentery and malaria [3-4].

EXPERIMENTAL SECTION

Preparation of the Curna

The samples used were of pharmacopoeial quality. Samples were washed, dried and grinded and individually passed through sieve of 180 μm separately then weighed separately, mixed in specified ratio and passed through 355 μm to obtain a homogenous blend. It was stored in an airtight container to protect from light and moisture. Sample of *Andrographis paniculata* was collected from Herbal garden,Arogyadham, Chitrakoot, Satna, Madhya Pradesh.

Physicochemical analysis

Physicochemical analysis such as the percentage of ash values and extractive values, loss on drying at 105° C and pH of filtrate of 10% w/v aqueous solution were carried out according to the official methods prescribed in Indian Pharmacopoeia [5] and the WHO guidelines on quality control methods for medicinal plant materials [6-7]. Fluorescence analysis was carried out by the method of Chase and Pratt [8].

Preliminary phytochemical screening

Preliminary phytochemical evaluation was carried out by using standard procedure [9-10].

High Performance Thin Layer Chromatography (HPTLC)

For HPTLC, 5 g of coarsely powdered drug was taken in 250 ml Stoppard conical flask & extracted with 100 ml ethanol for 24 hours by maceration technique with occasional shaking. The extract ((25 ml of) thus prepared was further diluted to 100 ml. A portion from this extract (25ml) was concentrated on a water bath and used for HPTLC. Similarly ethanol extract was prepared for the both the samples. HPTLC of the extracts of all the test and reference samples was carried out on Silica Gel 60 F254 precoated plates (0.2 mm thickness; from Merck India Limited). Camag Linomat 5 applicator was used for band application and Desaga Video documentation Unit 3 was used for documentation of fingerprints profile. The mobile phase used was Toluene: Ethyl acetate (6:4). The plate was developed over a distance of 10 cm in a saturated development chamber **(**Twin trough chamber **(**10×10 cm with SS lid, and visualized under visible light, UV (254nm and 366nm). The plates were also visualized after spraying with 5% methanolic-sulphuric acid followed by heating at 105^0C for 5-7 min [11-12].

Test for microbial limits

Following tests were carry out as per standard methods [13-14] to determine the microbial load in five batches of *Psoralia corylifolia* curna, a formulated compound drug powder of pharmaceutical substances

1.Enumeration of *Staphylococcus aureus* /gm
2.Enumeration of *Salmonella sp./gm*
3.Enumeration of *Pseudomonas aeruginosa/gm*
4.Determination of *E.coli*
5.Determination of total bacterial plate count (TBC)
6.Determination of Yeast and mould.

The microbiological tests were determined using specified agar and enrichment media from Himedia and Privet Limited Mumbai.

RESULTS AND DISCUSSION

Powder in brown in colour, odour less; taste distinctive very tikhta. The powder completely passes through 355μm and not less than 50 percent through 180 μm.

Physiochemical analysis

Physicochemical analysis was subjected to various analytical parameters average value of total ash content 16.65%, acid insoluble ash 4.61%, alcohol soluble extractive 9.33%, water soluble extractive 23.65%, hexane soluble extractive 10.9%, acetone soluble extractive 8.01%, methanol soluble extractive 10.43% and loss on drying(LOD) at 105 ^{0}C 9.69 % (Table 1).

Table 1. Physiochemical analysis of *Andrographis paniculata*

S.N.	Parameters	Results
1.	Loss on drying at 105 ^{0}C	9.69 %
2,	Total ash content	16.65%
3.	Acid insoluble ash	4.61%
5.	Alcohol soluble extractive	9.33%
6.	Water soluble extractive	23.65%
7.	Methanol soluble extractive	10.43%
8.	Hexane soluble extractive	10.90%
9.	Acetone soluble extractive	8.01%

Preliminary phytochemical screening

The preliminary phytochemical test was performed on the extracts of plant of *Andrographis paniculata*. They show the presence of the alkaloid, resin, saponin, tannin, carbohydrate and flavonoids (Table 2).

Table 2: Preliminary phytochemical screening of extracts of *Andrographis paniculata*
(+ present, -absent)

S.N.	Parameters	Results
1.	Alkaliods	+
2.	Carbohydrate	+
3.	Flavonoids	+
4.	Resins	+
5.	Saponins	+
6.	Tannins	+
7.	Protien	-
8.	Steroids	-

Fluorescence analysis

The fluorescence analysis is a tool for the determination of constituents present in the plant that gives an idea on its chemical nature. Therefore fluorescence analysis of the powder was carried out and data has been presented in (Table 3).

Table 3: Fluorescence analysis of *Andrographis paniculata*

Powder + Reagents	Observation at 254 nm	Observation at 366 nm
Only Powder	Green colour	Whitish Green
Powder + Iodine water	Light Yellow	Greenish Yellow
Powder + 50% KOH	Yellow	Dark Yellow
Powder + 1N NaOH in Methanol	Yellow	Greenish Yellow
Powder + Acetic acid	Yellow	Green
Powder + 50% HNO_3	Yellow	Black
Powder + Conc.H_2SO_4	Yellow	Yellow
Powder + 1N HCL	Yellow	Yellowish Green
Powder + 1N NaOH	Light Yellow	Light Yellow
Powder + 50% H_2SO_4	Yellow	Green

Table 4. R_f values of test solution of *Andrographis paniculata* at day light (before derivatization)

R_f value	*Andrographis paniculata*		
	Ethanol extract	Methanol extract	Hexane extract
R_f1(Yellow)	0.48	0.48	NA
R_f2(Yellow)	0.51	0.51	0.51
R_f3 (Light Green)	0.59	NA	NA
R_f4 (Yellow)	0.69	NA	NA
R_f5 (Light Green)	0.73	NA	NA
R_f6(Yellow)	0.92	NA	0.92
R_f7 (Yellow)	0.95	NA	NA
R_f8 (Black)	NA	NA	NA

NA-Spot not appeared

High Performance Thin Layer Chromatography (HPTLC)

The TLC plates were examined under ultra violet light at 254 nm; at 366 nm and at visible light for both before and after derivatization with 5% methanolic-sulphuric acid reagent (Figures 3). The R_f values and colour of the bands obtained were recorded (Table 4-7).

Table 5. R_f values of test solution of *Andrographis paniculata* at 366 nm (before derivatization)

R_f value	*Andrographis paniculata*		
	Ethanol extract	Methanol extract	Hexane extract
R_f 1(Light Red)	O.O8	0.08	NA
R_f 2(Blue)	NA	0.27	0.27
R_f 3 (Light Red)	0.41	NA	NA
R_f 4 (Sky Blue)	0.48	0.48	NA
R_f 5 (Blue)	NA	NA	0.50
R_f 6(Light Red)	NA	0.52	0.52
R_f 7 (Light Red)	0.60	NA	NA
R_f 8 (Light Red)	0.69	0.69	NA
R_f 9 (Light Red)	0.73	0.73	NA
R_f 10(Light Red)	0.77	NA	NA
R_f 11(Light Red)	0.87	0.87	0.87
R_f 12(Light Red)	NA	0.91	NA

Table 6. R_f values of test solution of *Andrographis paniculata* at day light (after derivatization)

R_f value	*Andrographis paniculata*		
	Ethanol extract	Methanol extract	Hexane extract
R_f 1(black)	0.10	0.10	NA
R_f 2(Brown)	0.52	0.52	0.52
R_f 3 (Dark brown)	0.64	0.64	0.64
R_f 4 (Black)	0.95	NA	NA

Table 7. R_f values of test solution of *Andrographis paniculata* at 366 nm (after derivatization)

R_f value	*Andrographis paniculata*		
	Ethanol extract	Methanol extract	Hexane extract
R_f 1(White)	0.10	0.10	NA
R_f 2(Sky Blue)	0.14	0.14	0.14
R_f 3 (Sky Blue)	NA	0.23	NA
R_f 4 (Light Red)	0.25	NA	NA
R_f 5 (Sky Blue)	0.27	0.27	NA
R_f 6(Light Red)	0.47	0.47	NA
R_f 7 (Yellow)	NA	NA	0.50
R_f 8 (Light Red)	0.52	0.52	NA
R_f 9 (White)	NA	NA	0.55
R_f 1(White)	0.62	NA	NA
R_f 2(Red)	NA	NA	0.65
R_f 3 (Light Red)	0.72	0.72	NA
R_f 4 (Light Blue)	NA	NA	0.75
R_f 5 (Light Red)	0.84	NA	0.84
R_f 6(White)	NA	NA	0.92
R_f 7 (Light Red)	0.94	NA	NA
R_f 8 (White)	0.96	NA	NA

The microbial profile of the *Andrographis paniculata* sample was found satisfactory. Total bacterial plate counts (average 25 cfu/g), Yeast and Moulds (average 2 cfu/g) counts were reported less than the limit set by WHO and pathogenic bacteria, i.e. *Salmonella sp., Pseudomonas aeruginosa*, *Staphylococcus aureus* and *E. coli* were found to be absent (Table 8 and Figures 2.1-2.6).

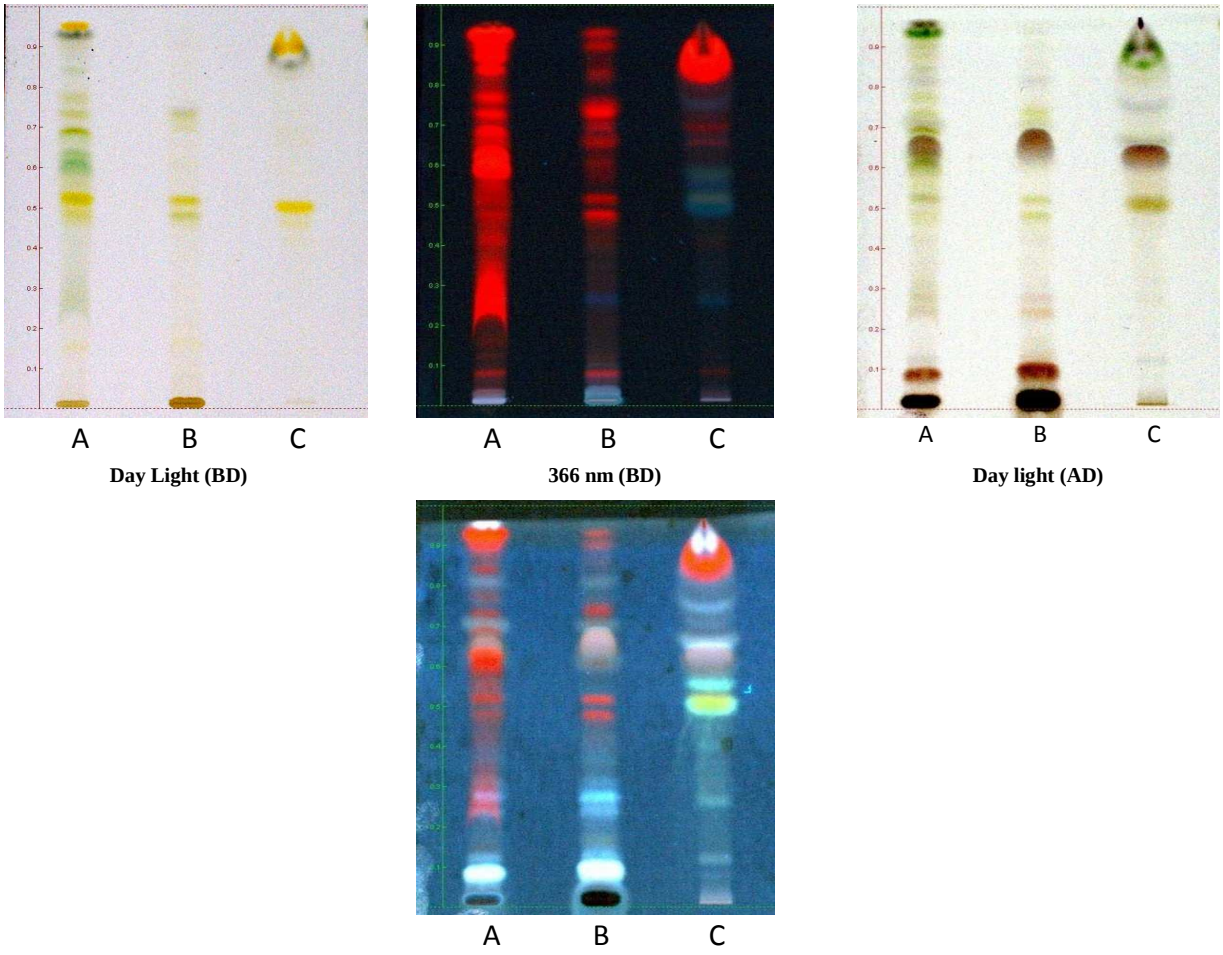

Figure 1. HPTLC Finger prints of test solution of *Andrographis paniculata* at 254nm, 366nm and visible light (before derivatization -BD and after derivatization-AD)

Test Solution of Andrographis paniculata in Herbal Garden, Arogyadham Chitrakoot, Satna, Madhya Pradesh A-Ethanolic extract, B-Methanolic extract, C-Hexane extract.

Table 8. Microbiological limit test in *Andrographis paniculata*

Parameters	*Psoralia corylifolia*	Permissible Limits API part II
Staphylococcus aureus /g	Absent	Absent
Salmonella spp. /g	Absent	Absent
Pseudomonas aeruginosa /g	Absent	Absent
E.coli	Absent	Absent
Total bacterial plate count (TBC)	25 cfu/g	10^5 /gm
Total Yeast & mould.	2 cfu/g	10^3 /gm

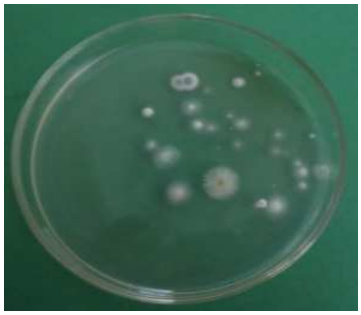

Figure 2.1. Showing Total Bacterial count

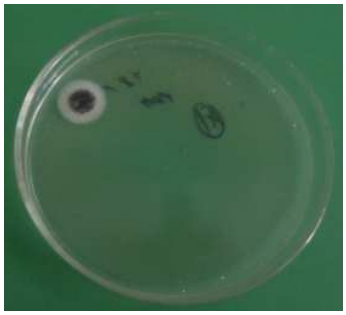

Figure 2.2. Showing yeast & moulds

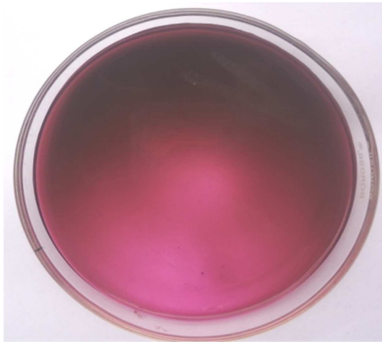

Figure 2.3. E. coli showing negative result

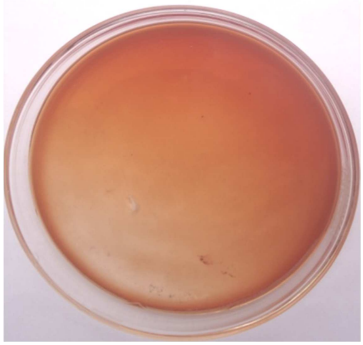

Figure 2.4. Test for *Salmonella* showing negative result

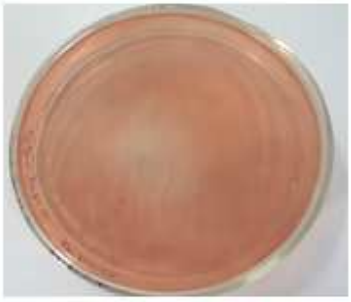

Figure 2.5. Test for *Staphylococcus* showing negative result

Figure 2.6.Test for *pseudomonas* showing negative result

Figure 2. Photographs of Microbiological limit test in *Andrographis paniculata*

CONCULUSION

The Pharmacognostical features of *Andrographis Paniculata* studied in the present study have been utilized in developing standards of this plant which will be useful in the detection of its identity and authenticity. The parameters such as physiochemical analysis, preliminary phytochemical test, fluorescence analysis, microbiology and HPTLC studied add to its quality control and quality assurance for proper identification.

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