

# Deciphering the role of *Andrographis paniculata* in pharmaceutical field and chromatographic evaluation of *Andrographis paniculata* in the solution of methanol and hexane through HPTLC

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**Abstract-** This article explores the medicinal potential of *Andrographis paniculata* (Kalmegh) in Ayurveda, focusing on its hepato protective and anti febrile properties. The paper emphasizes the importance of herbal medicines in primary health care due to their availability, compatibility, and affordability. Additionally, it discusses the rich herbal medicinal wealth in India and the historical significance of Ayurveda.

The study delves into the pharmacological actions of *Andrographis paniculata* including anti-inflammatory, anti-diabetes, anti-viral, and immune stimulating properties. The main objective is chromatographic evaluation using High-Performance Thin Layer Chromatography (HPTLC) to analyze the chemical composition of *Andrographis paniculata*. The HPTLC method is detailed, which is used in qualitative analysis of *Andrographis paniculata*.

The article concludes with the qualitative analysis of *Andrographis paniculata*, comparing chromatograms in different solvents and wavelengths. Methanol is identified as the superior solvent providing a clearer chromatogram. Chemical constitution analysis reveals the number of visible chemicals at different wavelengths, offering insights into the herb's composition. The study provides valuable information for

pharmaceutical quality control and the analysis of medicinal plant.

**Key words:** *Andrographis paniculata*, HPTLC, Pharmacological, Ayurvedic

## Introduction

Ayurveda is a medical science that gives us a profuse knowledge of Dravyas or drugs which we get from plants, animals and minerals. The medicinal plants are the potential source of Ayurvedic medicines and are a core component at primary health care level due to their availability, compatibility and affordability. India is perhaps the richest nation with regard to herbal medicinal wealth (about 15000-20000 plants have been found to have good medicinal value and it also has the oldest medicinal system in the form of Ayurveda. Ayurvedic classics have mentioned many efficacious herbs to treat a variety of ailments. One such herb is being Kalmegh i.e. *Andrographis paniculata* (Burm.f.) Wall. ex Nees is a potent hepatoprotective and antifebrile herb, It can be easily grown in major types of soil and uptakes minimal amount of water in the several areas and well recognized medicinal plant widely utilized in Thailand, China, India, and several other Asian countries.

*Andrographis paniculate* Burm. Nees (AP), commonly known as 'King of bitters', is an indigenous South Asian herb belonging to the Acanthaceae family. It is widely used traditionally in managing diabetes, hypertension, cancer, rheumatoid arthritis and other inflammatory conditions. In Indian and Chinese traditional medicine, AP has been used to address coughs, colds and influenza, indicating its potential effectiveness in managing respiratory tract diseases.

According to phytochemical research, *A. paniculata* encompasses a wide range of bioactive components of pharmacological significance including high quantity of flavonoids, quinoids, xanthones, tannins, alkaloids and other compounds (Hossain et al., 2014)

### Pharmacological properties and the therapeutic actions of Kalmegh

In Ayurveda, the therapeutic actions of any drug is based on its properties such as Rasa, Guna, Virya, and Vipaka. According to Ayurvedic Pharmacopeia of India the properties and actions of Kalmegh are listed below.

**Rasa (Taste)** – Tikta (Bitter)

**Guna (Qualities)** – Laghu (Light for digestion),

**Ruksha (Dryness)** **Veerya (Potency)**- Sheet (Cold)

**Vipaka (Metabolic Property)**- Katu (Transforms into Pungent / Spicy taste after digestion)

**Karma (Actions)**- Kaphapitta shamaka (reduces vitiated kapha and pitta dosha), Dipana (appetizer), Pachana (digestive), Yakrut uttejaka (stimulates liver), Jwaraghna (antipyretic), Krimighna (wormicidal), Raktashodhak (purifies blood), Shothahar (reduces oedema), Svedajanana (stimulates sweating).

### Chemical Constituents

Andrographolide, neo andrographolide, and rograpanin, 14-Deoxy-11, 12-didehydro andrographolide, are the principal chemical constituents found in the plant.

#### These chemical constituents play an important role in pharmaceutical field.

- Andrographolide**- Widely used as a bitter tonic, for snake bite and for the treatment of hepatitis.
- Neoandrographolide**- Mainly used for treating acute bacterial dysentery, acute gastroenteritis, upper respiratory tract infection, acute tonsillitis, pharyngitis.
- Andrograpanin**- Andrograpanin, a bioactive compound from *Andrographis paniculata*, exhibits anti-inflammatory and anti-infectious properties.
- 14-Deoxy-11, 12-didehydro andrographolide**- a diterpenoid in *Andrographis paniculata* (Burm.f.) Nees, acts as a bioactive phyto nutrient that can treat many diseases.

Analysis of pharmaceutical compounds and drugs is commonly used in all the stages of drug discovery and development process. These analytical techniques provide more accurate and precise data, not only supporting drug discovery and development but also postmarket surveillance. Pharmaceutical analysts work regularly to improve the reliability of existing techniques to cope up the demands for better chemical measurements. Modern pharmaceutical analysis is mainly dominated by costlier instrumental analysis. Hence, many analysts focus is on developing newer applications, discoveries and new methods of analysis to increase the specificity and sensitivity of a method.

Analytical methods used in drug analysis are diversified and are still being improved to find better solutions to satisfy manufacturers and institutions that test drug quality. Official documents dealing with the problem of QC of pharmaceutical products recommend diversified analytical techniques, with

chromatographic methods playing a significant role in pharmaceutical analysis. The usage of HPTLC is well appreciated and accepted all over the world. Many methods are being established to standardize the assay methods. HPTLC remains one step ahead when compared with other tools of chromatography.

### **HPTLC (High performance thin layer chromatography)**

HPTLC (high-performance thin layer chromatography) is sophisticated form of TLC, which provides superior separation efficiency. The HPTLC concept includes validated methods for qualitative and quantitative analysis, and fulfills all quality requirements for use in fully regulated environments.

**HPTLC Principle-** The HPTLC works on the same principles as TLC such as the principle of separation is adsorption. The mobile phase or solvent flows through the capillary action. The analytes move according to their affinities towards the stationary phase (adsorbent). The higher affinity component travels lower towards the stationary phase. A low-affinity component travels rapidly toward the stationary phase. On a chromatographic plate, then, the components are separated.

**HPTLC Application Fields-** HPTLC plates offer superior separation performance for quantitative evaluation of highly complex samples in

1. Pharmaceutical quality control
2. Analysis of medicinal plants and herbs
3. Analysis of pesticide mixtures

#### **Instrumentation**

- a) Linomats
- b) Chromatographic chamber
- c) Visualizer
- d) Scanner

**The Advantages of HPTLC are as follows -**

1. More than one analyst works on the system simultaneously.
2. HPTLC can be sharable, as it is not devoted to any sample.
3. The pre-coated plates of HPTLC are available at low prices.
4. There is less maintenance cost as compared to the equipment.
5. HPTLC has no risk of contamination, since the use of the freshly prepared mobile phase and stationary phase.
6. Mobile phases are not required for filtration and degassing such as HPLC.
7. It is highly sensitive, reproducible and precise as compared with a thin layer chromatography

### **Material and Methodology**

**Material required** – Dried kalmegh, oven, mixer grinder, conical flask, measuring cylinder, spatula, methanol, hexane, whatman filter paper, Aluminium foil, chloroform : methanol – 90 :10 (For mobile phase), silica gel plate, dryer, HPTLC

#### **Procedure for chromatographic evaluation of *Andrographis paniculata***

We followed following steps for chromatographic evaluation of *Andrographis paniculata*.

**1. Sample drying** – Firstly, dried the given sample of the *Andrographis paniculata* in the oven for 25 – 30 minutes and after that put it out from the oven.

**2. Powdering of the sample**– Made a powder of the given sample in mixer grinder.

**3. Sample preparation** -For the preparation of sample, took 2 gm powder of the kalmegh in the conical flask with the help of spatula and then mixed it with a 20ml hexane, using as solvent. Same process repeats for the preparation of the other

sample, mixed the 2 gm powder of the 4.solvent), separately in the other conical flask. Closed the conical flask with the help of aluminium foil. Kept the above mixture for overnight. Filtered the above mixture by using whatman filter paper.

**5. Sample application**– Used the linomat, filled the mixture in syringe and then pressed the run button, then sample started to apply in silica gel plate.

**6. Put the plate in Mobile phase of HPTLC** – Put the above silica gel plate in mobile phase (chloroform: methanol – 90: 10) untill solution was reached at the marked point.

**7.Plate drying** –Plate was dried with the

kalmegh in the methanol (using as help of dryer.

**8.Putting the plate in visualizer**–Put the Plate invisualizer and then saw the chromatograms in win CATS Software in computer (which was attaches to a HPTLC Instrument) in a 254nm and 366nm wavelength.

**9.Report reading**– Lastly, read the report, in computer.

After reading report carefully, we did qualitative analysis of *Andrographis paniculata* and also compared the chromatogram of the both samples in n-hexane solution and methanol solution in 254nm and 366nm wavelength.

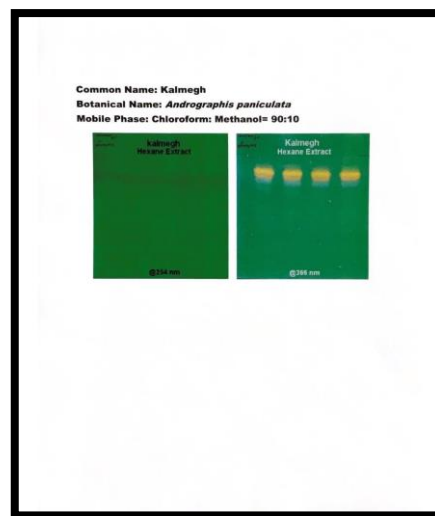
## Result



### Conclusion

After seeing above result, we could do qualitative analysis of *Andrographis paniculata* and concluded the following things:

**Best solution** – Methanol is the better solution than hexane because methanol gave good result, more visible and clear chromatogram than hexane.



**Methanol gives best chromatogram at 366 nm wavelength.**

**Chemical constitution** – After seeing above result, we can count number of the chemicals which are visible in chromatogram of kalmegh at different wavelengths with hexane and methanol as given here under.

**With hexane**

Wavelength	No. of the chemical
At 254 nm	Not visible clearly
At 366 nm	Only 4 are visible

**With methanol**

Wavelength	No. of the chemical
At 254 nm	Only around 4-5 are visible
At 366 nm	Around 15 are visible

**Disclaimer Statement**

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

**References**

1. Sulaiman, I.; Okwuofu, E. O.; Mohtarrudin, N.; Lim, J. C. W. and Stanslas, J. An *Andrographis paniculata* Burm. Nees extract standardized for three main Andrographolides prevents house dust mite-induced airway inflammation, remodeling, and hyper reactivity by regulating Th1/Th2 gene expression in *Journal of ethnopharmacology*, 2024,319,117082.
2. Harikrishnan, A.; Veena, V.; Kancharla, R.; Chavan, S.; Rajabathar, J. R.; Al-Lohedan, H. and Arokiyaraj, S. Anti-breast cancer activity of bioactive metabolites from *Andrographis paniculata* through inhibition of PI3K activity in triple negative breast cancer (MDA-MB-231) cells. *Journal of Molecular Structure*, 2023, 1294, 136506.
3. Paramasivam, V.; Paulpandian, P.; Venkatachalam, K.; Hussain, S.; Kangal, A.; Al Farraj, D. A. and Balaji, P. Cytotoxicity and Antimicrobial efficiency of gold (Au) nano-particles formulated by green approach using *Andrographis paniculata* leaf extract. *Journal of King Saud University-Science*, 2023, 35(5):102687.
4. Siripongboonsitti, T.; Ungtrakul, T.; Tawinprai, K.; Auewarakul, C.; Chartisathian, W.; Jansala, T. and Mahidol, C. Efficacy of *Andrographis paniculata* extract treatment in mild to moderate COVID-19 patients being treated with favipiravir: A double-blind, randomized, placebo-controlled study (APFaVi trial). *Phytomedicine*, 2023, 119: 155018.
5. Priya, L. and Pillai, P. U. Invitro effect of Colchiploidyon andrographolide enhancement in *Andrographis paniculata* (Burm. f.) Wall. ex. Nees. *South African Journal of Botany*, 2023.
6. Huong, Q. T. T.; Nam, N. T. H.; Duy, B. T.; An, H.; Hai, N. D.; Ngan, H. T. K. and Nguyen, H. H. Structurally natural chitosan films decorated with *Andrographis paniculata* extract and selenium nanoparticles: Properties and strawberry preservation. *Food Bioscience*, 2023, 53, 102647.