

# PRELIMINARY PHYTOCHEMICAL INVESTIGATION AND ISOLATION OF OLEANOLIC ACID FROM *ANDROGRAPHIS SERPYLLIFOLIA* HERB.

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## ABSTRACT

*Andrographis serpyllifolia* herb, a member of the family Acanthaceae, is an important medicinal plant with significant therapeutic activity and is found in several ethnobotanical formulations. *A. serpyllifolia*, is being used in the traditional system of medicine for snake bites, antipyretics, cancer and inflammation. Preliminary phytochemical study showed the presence of various secondary metabolites such as alkaloids, sterols, glycosides, phenolic compounds, tannins, and flavonoids.

Hence, in the present study, oleanolic acid is isolated and characterized first time from *A. serpyllifolia* herb.

**KEY WORDS:** *A. serpyllifolia*, Acanthaceae, phytochemical, oleanolic acid.

## INTRODUCTION

*Andrographis serpyllifolia* is an Indian plant of Acanthaceae family. This plant is grown in South India in the state of Karnataka, Tamil Nadu and Andhra Pradesh. <sup>[1, 2, 3]</sup>

It occurs in hill top regions containing less quantity of soil and humus. The plant bears orbicular or sub-reniform, sessile leaves, presence of much bearded anthers, glabrous capsule which points at both ends, deeply curved spoon shaped retinacula and deeply rugose small seeds. *A. serpyllifolia*, is being used in the traditional system of medicine for snake bites, antipyretics, cancer and inflammation.

The microscopical characters of *A. serpyllifolia* shows presence of numerous diacytic stomata on both upper and lower leaf surfaces, restricted presence of abundant sessile glandular trichomes on the abaxial leaf surfaces, reticulate pollen ornamentation with echinate sulcus outlined with smooth morus and deeply reticulate, highly pitted and convoluted seed testa reminiscent of human brain. These three features may serve as pharmacognostic markers aiding in accurate identification and quality control of this herb. <sup>[4, 5]</sup>

The present study was to assess the phytochemical constituents and isolation of the constituent from ethanolic extract of *A. serpyllifolia*

## MATERIALS AND METHODS

### Collection and authentication of plant material

Whole plant of *Andrographis serpyllifolia* were collected in the month of August, from local region of Tirupati, Andhra Pradesh (India). Herbarium specimens were prepared; identification and authentication were done from Department of Botany, Sri. Venkateswara University, Tirupati, A. P. India. A Voucher specimen number of the plant 1189 has been deposited for future reference. The plant materials were shade dried and coarsely powdered by using mechanical grinder. The powders were passed through sieve no. 40 and stored in airtight container for the extraction.

### Extraction and fractionation

All the solvents used for extraction were of technical grade and distilled before use. The solvents used for column chromatography and preparative TLC were of Analytical grade. The Silica Gel used for column chromatography was Silica Gel G 60-120 (Merk).

### Procedure for extraction and isolation

The air dried whole plant (1000 g) was extracted with ethanol (70 % v/v) and concentrated in rotary evaporator under reduced pressure to get ethanol extract (192 g). Ethanolic extract was chromatographed on silica gel column (70cmX15cm, 60-120mesh, 2 kg) chromatography and preparative TLC.

Column was first eluted with chloroform, then polarity of mobile phase was gradually increased by adding methanol in different concentrations (100:0, 95:5, 90:10, 85: 15, 80:20, 70:30 v/v).

190 fractions each of 40 mL were collected and TLC was performed of each fraction individually and eluates were monitored for the presence of various constituents. Fractions were pooled on the basis of their TLC profile, pooled fractions (27-54) was selected for the isolation of constituent. Further purification was performed by preparative TLC of isolated constituents offered PT1. [6, 7, 8]

### Detection of sterols [9]

#### a) Salkowski test

A small amount of extract was dissolved in chloroform and 2 mL of conc. sulphuric acid was added into this. Formation of red colour in chloroform layer indicated the presence of sterols.

#### b) Liebermann's test

A small amount of extract was dissolved in chloroform, to this few mL of acetic anhydride was added, heated and cooled. After addition of concentrated sulphuric acid, formation of blue colour showed the presence of sterols.

#### c) Liebermann-Burchard test

A small amount of extract was dissolved in chloroform, to this few mL of acetic anhydride was added, heated and cooled. After addition of concentrated sulphuric acid, formation of red, pink blue colour showed the presence of sterols.

## Analytical Methods

Thin Layer chromatography was performed on precoated TLC plates. IR spectra was recorded on FTIR (Shimadzu),  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra was recorded on Bruker (500MHz) in  $\text{CDCl}_3$ . TMS was used as internal standard. ESIMS were measured using a Q-TOF micro mass spectrometer (Waters, USA)

## RESULTS AND DISCUSSION

### Phytochemical screening

Preliminary phytochemical screening of the ethanolic extract of *Andrographis serpyllifolia* revealed the presence of various secondary metabolites such as alkaloids, sterols, glycosides, phenolic compounds, tannins, flavonoids.

### Physical and spectral properties of isolated compound

**Appearance:** White amorphous powder

**Solubility:** Chloroform

**TLC (Rf value):** 0.7

**m.p.** 171-172°C

**MASS:** molecular ion m/z 360

**IR (KBr, in  $\text{cm}^{-1}$ ):** Intense band at  $3296\text{ cm}^{-1}$ , observed for the OH bond vibration of hydroxyl group. Carbonyl stretch was observed at  $1715\text{ cm}^{-1}$ . The corresponding C=C vibrations was shown at  $1449\text{ cm}^{-1}$  was weakly intense band.

**$^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ,  $\delta$ , TMS=0):** 0.077(3H,S), 0.086(3H,S), 0.088(3H,S), 1.11(3H,S), 1.26(3H,S), 1.62(3H,S), 1.63(3H,S), 2.31(1H,m), 3.67(1H, m), 4.6(2H,S), 7.2(2H,S).

**$^{13}\text{C}$  NMR (75.4Hz,  $\text{CDCl}_3$ ,  $\delta$ , TMS=0):** 14.22, 14.78, 15.57, 15.81, 16.00, 16.16, 16.74, 18.31, 19.13, 19.20, 20.77, 22.58, 23.46, 23.77, 23.92, 25.16, 27.14, 28.24, 29.13, 30.17, 31.27, 32.54, 34.12, 36.86, 38.67, 40.37, 50.29, 58.93, 109.58 and 139.08.

**Mass spectral data:** Mass spectrum of isolated compound showed molecular ion m/z 360 [M+1]

$^1\text{H}$  NMR spectrum showed the presence of seven tertiary methyl protons at  $\delta$ 0.077,  $\delta$ 0.086,  $\delta$ 0.088,  $\delta$ 1.11,  $\delta$ 1.26,  $\delta$ 1.62, and  $\delta$ 1.63 on an oleanane skeleton. At  $\delta$  3.81, a doublet of one proton and at  $\delta$  3.89, a doublet of one vinyl proton were assigned to H-18 and H-12, respectively.

$^{13}\text{C}$  NMR spectrum showed seven methyl groups at  $\delta$ 14.22 (C- 23),  $\delta$ 14.78 (C-28),  $\delta$ 15.57 (C-25),  $\delta$ 15.81 (C-26),  $\delta$ 16.00(C-24),  $\delta$ 16.16(C-27), and  $\delta$ 16.74 (C-30). The deshielded signals at  $\delta$ 58.93 was due to presence of hydroxyl group at C-3. The spectral data were similar to the ones reported for oleanolic acid.

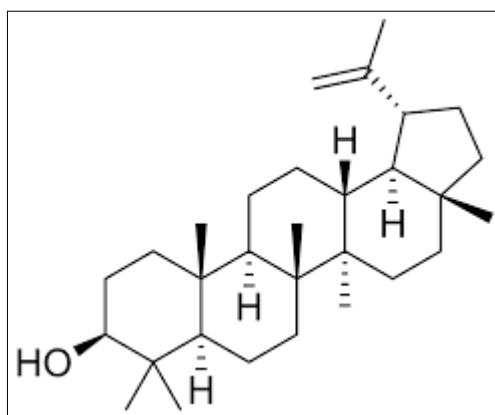


Fig.1: Chemical structure of Oleanolic acid

## REFERENCES

1. Rao CH. V., Azmi L, Gupta S. S., 2014. Phytochemical and Pharmacological Review *Andrographis serpyllifolia*: Potential herbal cure-all. Mintage J. Pharma. Med. Sci. 3(3), 1-3.
2. C. Neeraja P. Hari Krishna C. Sudhakar Reddy C. C. Giri K. V. Rao V. D. Reddy. Distribution of *Andrographis* species in Different Districts of Andhra Pradesh. Proc. Natl. Acad. Sci., India, Sect. B Biol. Sci. (Apr–June 2015) 85(2):601–606.
3. Smitha Jayaram, and Shylaja M. Dharmesh. Antiproliferative Effect of Antioxidative Free and Bound Phenolics from *Andrographis serpyllifolia*. Free Radicals and Antioxidants. Vol 1, Issue 3, Jul-Sep, 2011
4. Suma Krishnaswamy, Bopaiah Ajjikuttira Kushalappa. Systematic Review *Andrographis serpyllifolia* (Rottler ex Vahl) Wight: An Ethno-Pharmaco-Botanical Perspective. Pharmacogn J. 2018; 10(6) Suppl:s14-s26.
5. Vasu Kandati, P Govardhan, Ch Siva Reddy, A Ravinder Nath, R R Reddy. Kandati. In-vitro and in-vivo anti-inflammatory activity of *Andrographis serpyllifolia* (Rottl. Ex Vahl.) Wt. Inter Current Pharma J 2012, 1(8): 199-204.
6. Mukherjee, P.K., 2010. Quality control of herbal drugs: An approach to evaluation of botanicals. 4<sup>th</sup> reprint. New Delhi (India): Business horizons. pp. 417-419.
7. Khandelwal, K., 2000. Practical Pharmacognosy. 2<sup>nd</sup> edition. Pune (India): Nirali Publication. pp. 9-38.
8. Stills W.C., Kahn M., Mitra A., 1978. Rapid chromatographic techniques for preparative separation with modern resolution. Pharmacognosy Reviews. 1: 180-184.
9. Farnsworth, NR.,1966. Biological and Phytochemical Screening of Plants. Journal of Pharmaceutical sciences. 55(3):243-244.