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# Analysis of Andrographis Paniculata (Kalmegh) Extracts Through Hptlc

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**Abstract:**

*Andrographis paniculata* important bioactive constituent is andrographolide, mostly present in the leaves of plant and isolation procedure is easy from crude plant, present in form of crystalline solid. The geographical abundance of plant is Bengal and constituents consisting of lactones and flavones.

**Aim: The quantitative determination of extracts of** *Andrographis paniculata* (Kalmegh) by the use of High-Performance Thin Layer Chromatography method for determining of phytochemical compounds and FTIR for characterization.

**Material and methods:** *A. paniculata* fresh leaves were collected and dried; and grounded to a powdered form with the help of milling machine. The evaluation of extracts was done by calculating the % extraction yield, Lost on Drying, ash content, Chemical identification, Phytochemical screening of the extract like alkaloid, saponin, glycoside, phytosterol, terpinoid, tannis, flavanoids, anthraquinone derivatives. Further analytical method development for AGL(Aandrographolide) was done by High-Performance Thin Layer Chromatography (HPTLC) and Fourier-transform infrared spectroscopy (FTIR) studies were done for extract of Andrographolide.

**Results and discussion:** The % yield of extract was higher in 95 % ethanol (v/v) as the yield of *A. paniculata* in ethanolic extract of 95%, 70%, and 50%, were 23.6, 9.62 and 6.56 gm respectively Loss on drying result of *Andrographis paniculata* is not more than 10% and ash content is 7.63. Alkaloids, Flavonoids, Saponin, Terpenoid, Tannin, Glycosides, Phytosterol, and Proteins, were present and absence of Anthraquinone derivatives according to common phytochemical methods.

**Keywords:** Andrographolide, Ash content, FTIR, HPTLC, Phytosterol, Proteins, Phytochemical screening

## 1. INTRODUCTION:

Andrographolide ($C_{20}H_{30}O_5$) is a bioactive constitutes of *Andrographis paniculate*, it presents in the plant leaves. The isolation process from the crude extracts of plant is considered as an easy process, where it furnished as a solid crystalline form (family: *Acanthaceae*). The geographical presence is mainly present in Bangal.[1] It is commonly known as a "Kalmegha" and "king of bitter" name in Bengal and another name is Bhuineem. It has Major bioactive molecule is ent-labdanediterpenoid lactone. The Kalmegh is main traditional drug in South Asia. It is used as a substitute of quinine in the England.[2] In the Ayurveda and Unani system, it is mainly used for infants, andrographolide mainly contain in leaves and root's part and it is used for different medicinal purposes. It is potent constitute used more than twenty-six Ayurveda formulation as pe the report published in the Indian Pharmacopeia (IP).

[3]*A. Paniculata* used as antiviral[4], antimalarial[5], hepatoprotective[6], cardiovascular[7], anticancer, and immunostimulatory activities.[8]

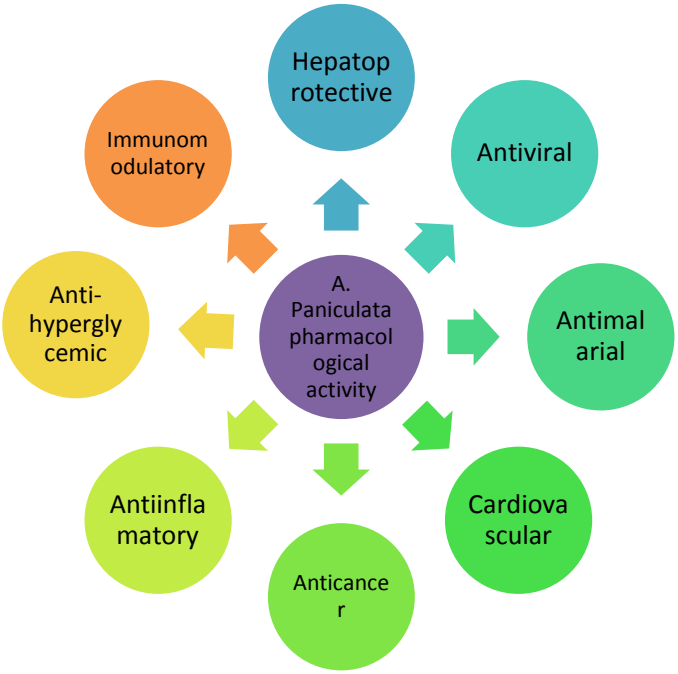



Fig 1: Uses of Andrographispaniculata[9]

**Morphology of plant:** [10]

Andrographolide plants named as *Andrographis paniculata* belongs to a family Acanthaceae and kingdom Plantae, the part used is dried leaves. Usually, the height of plant is 0.3 to 1.0 M and found geographically in topical Asian countries, Shri Lanka, India, Thailand, Malaysia and Indonesia. The stem of the plant is 3-6 mm thick, smooth and leaves are opposite, Lanceolate in shape, roots are woody, simple and fusi-form. The plant is bitter in taste with dark green colour and having characteristics odor. Chemical constituents present are lactones and flavones. Which further subdivided as lactones consists of Andrographolide (Depicted in figure 2) 8-methylandrograpanin, 3-hydrodeoxy andrographolide and flavones as Wogonin, 7-o-mehylwogonin. The non-bitter compounds present are neoandrographolide, homoandrographolide, andrographosterol, andrographon, andrographane, andrographosterin; andrograpanin; stigmesterol; α-sitosterol; andrographin ($C_{18}H_{16}O_6$); and dihydroxy-di-methoxyflavone.

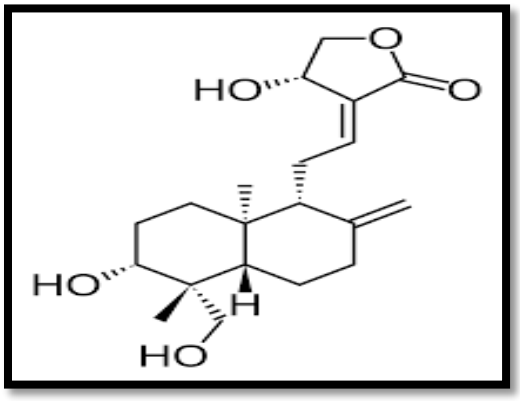


Fig 2: Andrographolide Chemical Structure

## 2. MATERIALS AND METHODS[11-14]

**Plant Material:**

*A. paniculata* fresh leaves were collected in November from FRI Dehradun and verification and authentication were done. Leaves were air-dried for a day under shade further dried in a hot air oven at a temperature less than 60ºC. Passed through 40 number mesh to get the powdered form and store in an airtight container at 15–20°C for further use.

**Chemical Requirement:**

The excipients utilized, such as Synth Haldwani and Nainital, were of analytical quality

**Method of Extraction:**

Harvesting of plants were done between the eight to twelve weeks of transplantation. The l part, which is in dried form, was ground into a powder with the help of a milling machine. The obtained dried powder was divided into four equal parts, then weighted and ethanolic extraction was done at a concentration of 95, 70, and 50% ethanol v/v respectively along with reflux on a water bath for three repeated days maintain the temperature at 40℃. The extract obtained was filtered and evaporated under vacuum with the help of a rotary evaporator to get the dried form of extract and further dried by putting it in the oven at 60℃ to obtain the constant weight of the extract. The final weight was done and percentage yield was calculated by using the formula, Where, $W_1$= Weight of the extract after dried, $W_2$= Weight of the plant powder.

$$\%\boldsymbol{Yield(extract)} = \frac{(\boldsymbol{W1 \times 100})}{\boldsymbol{W2}}$$

**FTIR Spectrum**

FTIR spectra of plant extract and standard were done by SHIMADZU instruments model no. 01236 from Devsthali Vidyapeeth, Analytical laboratory, is used for collecting data related to the component structure and analytical tool for accessing compound purity as well. To obtained spectrum by IR is easy and quick method. The radiation obtained by absorption of organic molecules is open to IR radiations and if radiant energy matches with energy of particular molecular vibrations, absorption takes place. Thus, IR spectra of each and every bond will be formed. FTIR spectra of Standard Andrographolide and extracted andrographolide were determined. [14]

**Determination of Lost on Drying**

Powder material weighted around 1 gm was taken (W1) and transferred into a shallow capped weighing bottle, that was prior heated to 105℃ for 30 minutes and weight again (W2). This was done by at least 0.5 – 1.0 g of material. Drying was done at a temperature between 105-110ºC for 3 hours in an oven and then cooling of the sample was done at RT in a desiccator and weight was taken. The total time duration from the oven to the weighing point was usually about 30 minutes. The results are expressed in a range or mean± standard deviation.

**Loss on drying = (Initial sample weight - Sample weight after drying)/ Initial sample weight ×100**

**Ash content:**

Powder material weighted around 2 gm. was taken ($W_1$) and transferred it into the prior ignited and tarred silica dish (W2). Spreading of extract evenly and ignition was done in a muffle furnace, further value determination was done by using at least 0.5–1.0 g of material in a furnace and heating done up to ignition temperature of 650-700ºC.Repeat the procedure to obtain two constant weights. The results were evaluated and expressed as range or mean value± standard deviation

$$\textbf{Total ash}\left(\%\frac{\mathbf{w}}{\mathbf{w}}\right) = (\textbf{weightof ash}) \times \textbf{100/weight of sample}$$

**Phytochemical screening of the extract:**[15-16]

**Alkaloids Test:** The small quantity of extract was taken in 1 ml diluted HCL and stir properly then filter the mixture. The filtrate obtained was treated with Dragandroff's reagent, organic precipitate appearance confirms the presence of the alkaloid in the extract.

**Saponin Test:**

Two gm of the powdered extract was mixed with 20 ml of distilled water and boil the solution in a water bath, filter the solution and obtained filtrate was mixed again with 5 ml distilled water and vigorously shaking to get stable persistent froth. The obtained froth was mixed with 3 drops of olive oil and shaking was done; emulsion formed confirms the presence of saponin in the sample.

**Glycosides Test:**

A portion of the extract was hydrolyzed with HCl (5 ml) for some hours in a water bath and the hydrolysate was considered for Fehling's test. Fehling's solution i.e., mixture of 1ml Fehling's solution A and 1 ml Fehling's solution B, 2 ml was taken and added with extract around 2 ml, mixed both solutions, and boil them. If yellow to red color precipitates appears confirms the presence of reducing sugars.

**Test for Proteins:** A portion of sample extract was dissolved with water around 5 ml and a Xantho protein test was performed. In 3 ml of sample extract, 1 ml nitric acid was added, a white precipitate was obtained, the solution was further heated for 1 min and cooled under tap water. Alkaline preparation was done by the addition of excess NaOH 40%, orange precipitation confirms the presence of protein.

**Phytosterol, tannins, flavonoids and terpenoids Test**

For Phytosterol testing, Salkowski test was done. In this test, 1 ml of concentrated sulphuric acid was added to the 1g plant extract and allowed to stand for 5 minutes. After shaking, formation of golden yellow color in the lower layer indicates the presence of phytosterols. For Flavonoids test, the extract was treated with concentrated sulphuric acid. Appearance of yellowish orange show the presence of anthocyanins, yellow to orange color show the presence of flavones, and orange to crimson show the presence of flavanones. For terpenoids (Salkowski test), 5 g of each extract was mixed in 2 ml of chloroform, and concentrated $H_2SO_4$ (3ml) was carefully added to form a layer. A reddish-brown coloration of the inter face was formed to show positive results for the presence of terpenoids. Tannin's test was about 0.5 g of the dried powdered sample was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added and observed for brownish green or a blue-black coloration. Borntrager's test for anthraquinone derivatives was done; about 100 mg of air-dried herb was extracted with 5 ml of chloroform by shaking and warming over a water bath. To about 2 ml of the supernatant, 1ml of dilute 10 %v/v ammonia solution was added, followed by shaking. A pink or red color in the aqueous layer indicated the presence of fully oxidized anthraquinone derivatives.

**Analytical method development for Andrographolide by HPTLC:** Fixed concentration strength 42 µg/mL (60 µL) spot of standard along with different was spotted by applicator (Camag, Linomat V, Anchrome, India) TLC plate was developed by placing the TLC in saturated glass chamber (10×10, Cammag, Anchrome, India) covered with the lid, Saturation of glass chamber was done prior 30 min. of TLC development mobile phase. Development of TLC plate was done at a distance of 8 cm above the location of the sample application. The TLC plate was ejected from the chamber and processed for

air drying at RT. HPTLC fingerprint profile was captured by Camag TLC visualizer, under UV at 254 nm and 366 nm. [17]

**Mobile phase:** Toluene: Ethyl Acetate: Formic Acid prepared in 5:4.5:0.5observed at 256nm at Camag TLC Scanner.

## 3. RESULTS AND DISCUSSION:

### Chemical Identification of andrographolide in plant sample:

The initial chemical tests of the plant were done with the well determined manners of the plant extract**;** the results are depicted in the table 1 with the relevant results.

Table 1: Chemical identification of Andrographolide in plant sample

| **Test** | **Identification** |
|---|---|
| Dried extract (0.5 mg) dissolved in 5 ml methanol treated with 2,4-dinitrophenylhydrazine around 1 ml and 2M HCl around 100 ml was added to this. | Yellowish orange color appearance confirms andrographolide presence |
| 5 mg of dried extract and 10 % alcoholic KOH | Kept 15-minute, red colored appeared, red color changes to yellow showing andrographolide presence in extract. |

Plant sample was treated with distinct chemicals confirms andrographolide presence in the plant extract. The confirmation of andrographolide were further taken for the extraction process as per reported below given protocols.

**Extraction**

The obtained dried powder was divided into four equal parts, then weighted and ethanolic extraction was done at a concentration of 95, 70, and 50% ethanol v/v respectively. The % yield of extract was calculated and the % yield of extract found to be higher in 95 % ethanol. The quantity of AGL in ethanolic extract 95, 70, and 50%, were 23.6, 19.24 and 6.56 g. The standard curve with the $R^2$ value high was determined in the alcoholic solvent; it is depicted in the Fig 3.

Table 2: Andrographolide extracts % yield in different concentration of ethanol

| **Solvent %** | **Sample weight used (gm)** | **Extract weight (g)** | **% Yield (extract)** |
|---|---|---|---|
| 95 | 50 | 11.587 | 23.6 |
| 70 | 50 | 9.62 | 19.24 |
| 50 | 50 | 3.28 | 6.56 |

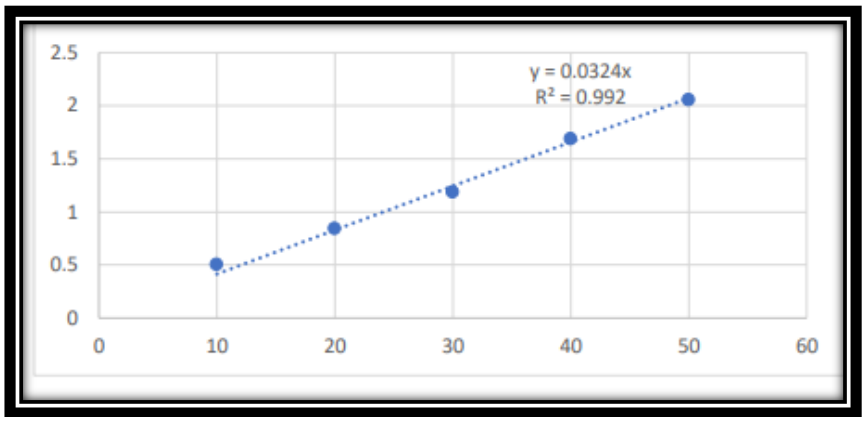



Fig 3: Standard curve of AGL at224 nm in methanol

The standard curve of 0.5mg extract in distinct concentration of ethanol was described in above table. 0.5mg extract in 10 ml methanol abs=1.3056 (40.8 µg/mL)

**FTIR**

The FTIR of the plant extract was determined with some of the character peaks as below-given table 3 and the fingerprint regions value (Figure 4), which confirm the presence of the chief constituents of the extract.

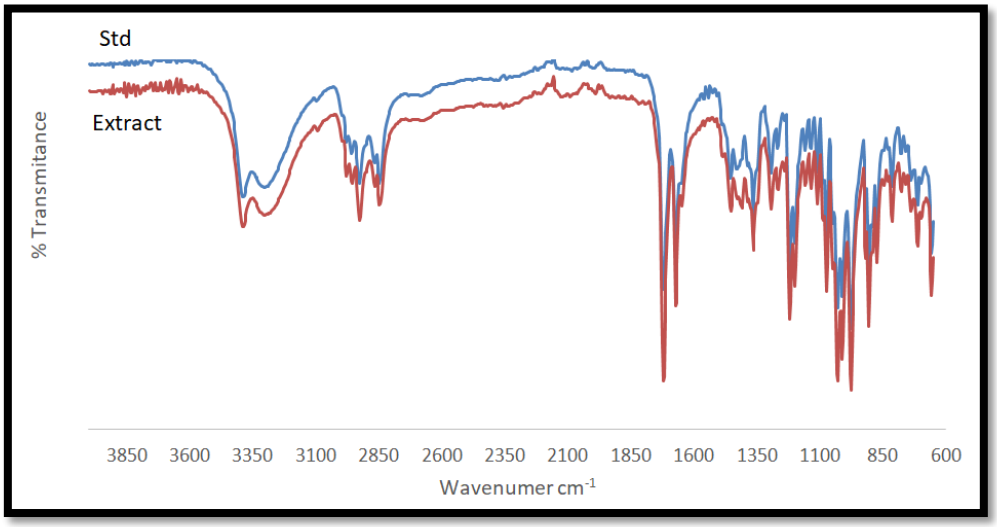



Fig 4: FTIR spectra of Standard Andrographolide and extracted andrographolide

Table 3: FTIR spectra of Standard Andrographolide and extracted andrographolide

| **S.No.** | **Wavenumber $Cm^{-1}$** | **Interpretation** |
|---|---|---|
| 1 | 3498 | O-H Stretch |
| 2 | 3390 | |
| 3 | 3025 | $Sp^3$ C-H Stretch |
| 4 | 2957 | |
| 5 | 1831 | C=O Stretch |
| 6 | 1784 | C=O Stretch |
| 7 | 1568 | $Sp^3$ C-H banding |
| 8 | 1474 | $Sp^3$ C-H banding |
| 9 | 1329 | $Sp^3$ C-H banding |

| | | |
|---|---|---|
| 10 | 1184 | C-O- Epoxy and Oxirane rings |
| 11 | 1143 | Cyclic ether C-O stretch |
| 12 | 1066 | Cyclic ether C-O stretch |
| 13 | 1005 | $Sp^2$ C-H Stretch |
| 14 | 766 | $Sp^2$ C-H Stretch |

The result of the IR spectra from the functional region to the finger print regions, which is overlapping with reported, gives the confirmatory information of the chemical constitute, which helps to proceed in the next step of the research as given below.

**Determination of Lost on Drying:**

*Andrographis paniculata* at 105°C was found to be 9 %. It is depicted in table 4

Table 4: *Andrographis paniculata* loss on drying

| **Initial weight** | **Weight after drying** | **% LOD** |
|---|---|---|
| 1g | 0.91g | 9 |

**Total Ash of sample**

Total Ash value of *A. paniculata* was found 7.63%.

**Phytochemical screening of the extract**

The extract of plants has gone through screening of phytochemical constituents that are secondary metabolites present in plant-like Alkaloids, Flavonoids, Saponin, Terpenoide, Tannin, Glycosides, Phytosterol, and Proteins, and absence of Anthraquinone derivatives as per common phytochemical screening tests. The test performed was examined on the visual appearance of change in color, precipitate formation on the addition of particular specific reagents. The test was performed for the presence of distinct phyto-constituents mentioned in Table 5. The present study shows the presence and absence of phytochemical compounds in each solvent extract. [18]

Table 5: Results of Anthraquinone derivatives, common phytochemical screening test

| **S.No,** | **Components (Ethanolic extract)** | **Result** |
|---|---|---|
| 1 | Alkaloids | Present |
| 2 | Saponin | Present |
| 3 | Glycosides | Present |
| 4 | Proteins | Present |
| 5 | Phytosterol | Present |
| 6 | Flavonoids | Present |
| 7 | Terpenoids | Present |
| 8 | Tannins | Present |
| 9 | Anthraquinone | Absent |

**Selection of Detection Wavelength**: Once chromatogram was developed, bands were scanned at a between 200-400 nm range. Chromatogram shows that AGL absorbance observed at 256 nm. Thus, in

the followed experiment the detection of wavelength was kept 256 nm.

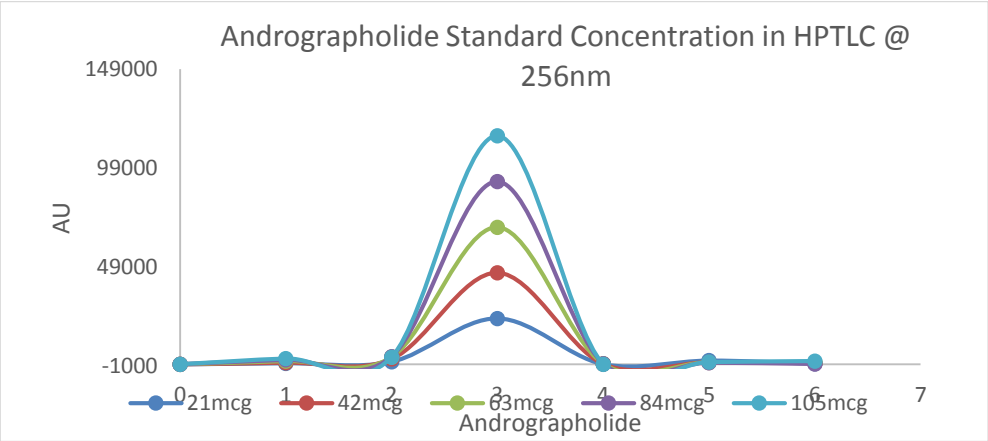



Fig. 5: HPTLC AUC AGL

The selections of mobile phase were done through the Toluene: Ethyl Acetate: Formic Acid, which was prepared in 5:4.5:0.5 and showed good resolution. Well defined spot was obtained after saturation of chamber for 20 minutes at room temperature. The identification of AGL was confirmed by comparing the chromatogram of extract with the standard one.

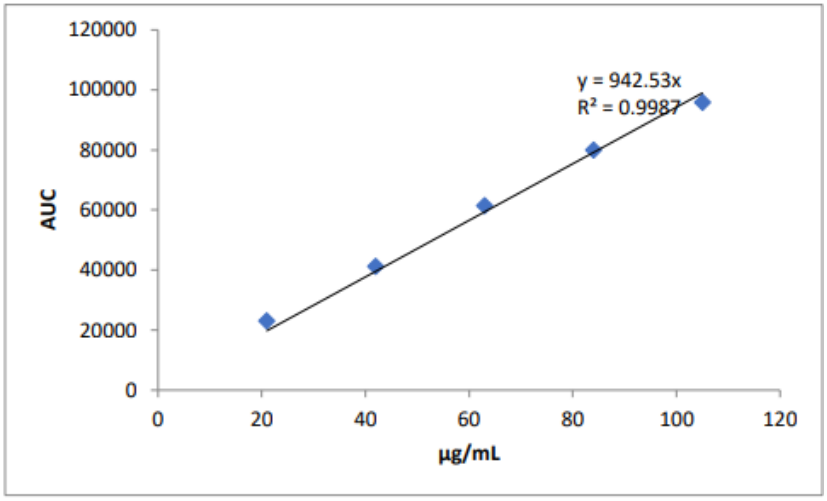
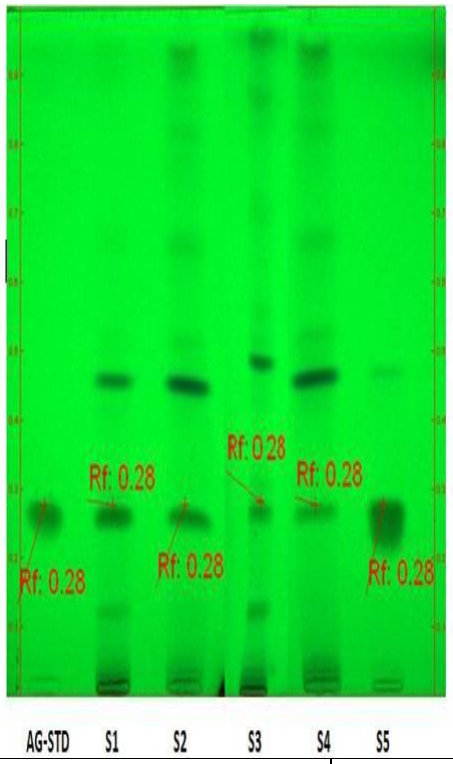



Fig 6: HPTLC AGL standard curve

The confirmation of the plant and extract through the physical and chemical analysis methods lead us to the quantitative analysis of the extract to get the amount of the AGL. The mobile phase and flow of the mobile phase was optimized first, to get the best yield of the product. The HPTLC image where the $R_f$ value is shown with other parameters to get the desire chemical with the optimized mobile phase combination as given in the table 6.

Fig 7- Rf value of the Standard and extract

Table 6: HPTLC AGL AUC and extract AUC




| **Conc.(µg/mL)** | **AUC** |
|---|---|
| 21 | 23066.2 |
| 42 | 41139.1 |
| 63 | 61417.9 |
| 84 | 79874 |
| 105 | 95905.4 |
| | |
| Extract<br><br>(1mg in10mL) | 76816.1<br>AUC |
| | 81.5µg/mL |

The quantity of the AGL was calculate as per given below table 7 and found with the good yield, the optimized HPLTC method found the robust and accurate with the high sensibility.

Table 7: weight of extract and AGL amount

| **Extract weight** | **AGL weight** |
|---|---|
| 40 | 32.4 |
| 60 | 48.6 |
| 80 | 64.8 |

The reliable and robust procedure was found with the 80% of the conversion from the extract, it is considerable improved with ease from other reported methods.

## 4. CONCLUSION

*A. paniculata* fresh leaves were collected and evaluated the extracts was done by calculating the % extraction yield, lost on Drying, ash content, Chemical identification, Phytochemical screening of the extract like alkaloid, saponin, glycoside, phytosterol, terpinoid, tannis, flavanoids, and anthraquinone derivatives. The analytical method development for AGL(Aandrographolide) was done by High-Performance Thin Layer Chromatography (HPTLC) and Fourier-transform infrared spectroscopy (FTIR) studies were done for extract of Andrographolide. All results obtained it was concluded that for estimating phytochemical constituents in the extract of *andrographis paniculata* (Kalmegh), the developed HPTLC method is simpler, faster, sensitive, new, and reproducible. This method helps in the regular analysis of drugs in pharmaceutical dosages without any interference of excipient and having good sensitivity. HPTLC methods are also suitable methods used for determining the chemical constituents present in plant extracts. The result obtained from the present study also supports the presence of phytoconstituents in ethanolic extract of AGL and could be used in treating several disorders.

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**Author Contributions:** Conceptualization, AT, PU and Formal Analysis, GJ and Investigation, GJ, and Methodology, GJ and Writing and editing AT, GJ. All author read and agreed to published version of manuscript

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