Solicitation of HPLC and HPTLC Techniques for Determination of Rutin from *Polyalthia longifolia Thwaites*

Gaurav Mahesh Doshi[1,2], Sandeep Prabhakar Zine[1], Pratip Kashinath Chaskar[1], Hemant Devidas Une[3]

[1]Department of Pharmacology and Pharmaceutical Medicinal Chemistry, Vivekanand Education Society's College of Pharmacy, Mumbai, Maharashtra, [2]Pacific Academy of Higher Education and Research University, Udaipur, Rajasthan, [3]Department of Pharmacology, Y. B. Chavan College of Pharmacy, Rouzabaugh, Aurangabad, Maharashtra, India

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ABSTRACT

Background: *Polyalthia longifolia Thwaites* is an important traditional plant in India. Rutin, an active constituent has been reported to possess good amount of pharmacological as well as therapeutic potential. **Objective:** The aim of the present study was to find out by analytical techniques how much percentage of rutin is present in the plant leaves' ethanolic extract by analytical techniques. **Materials and Methods:** Shade dried leaves of *Polyalthia longifolia* were subjected to cold ethanolic extraction followed by monitoring the isolated rutin high-pressure liquid chromatography (HPLC) and high performance thin layer chromatography (HPTLC) after carrying out preliminary phytochemical screening. **Results:** Extraction yield was found to be 13.94% w/w. Phytochemical screening of the extract showed the presence of flavonoids, steroids, diterpenoids, alkaloids, saponins, tannins and phenolic compounds and mucilage. From the Rf value, the ethanolic extract was found to be having constituent identical to rutin. By HPTLC and HPLC the amount of rutin was found to be 11.60% w/w and 4.03% w/v, respectively. **Conclusion:** The active constituent isolated was found to be equal to rutin.

Key words: High-pressure liquid chromatography, High performance thin layer chromatography, *Polyalthia longifolia*, Rutin, Thin layer chromatography

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INTRODUCTION

In an agricultural country like India, where medicinal plants are abundantly and easily available at relatively low cost, there is every virtue in exploiting such local and traditional medicine of vegetable origin for human and animal use. India has the knowledge and skill to develop its research and development capabilities in herbal research.[1-3] In recent times, focus on plant research has increased all over the world and evidence has been collected to show immense potential of medicinal and local plant utility traditional systems of herbal medicines.[4,5]

Polyalthia longifolia Sonn. *Thwaites* (Order: Magnoliales; Family: Annonaceae) is an evergreen, tall, handsome plant commonly used as an ornamental street tree due to its effectiveness in combating noise pollution. *Polyalthia* is the Greek word for *poly*, meaning much or many and *althia* from althea, meaning to cure. It is large shrub of genes including 120 species. India has 14 species of *Polyalthia*. Among the several species of *Polyalthia longifolia cv. Pendula* grown in India is mostly used in indigenous medicine, whereas, *Polyalthia longifolia var. Pendula* is used mostly in traditional medicine. Leaves are aromatic and used as essential oils.[6,7]

Various chemical constituents identified from the plant leaves are namely, azafluorene alkaloid namely polylongine and three new Aporphine *N*-oxide alkaloids. In addition, leaf oil has been reported to contain a vast number of sesquiterpenes such as allo-aromadendrene, caryophyllene oxide, β-caryophyllene, β-selinene, α-humulene, α-pinene and camphene. Activity guided fractionation of ethanolic extract has revealed the presence of flavonoid components in the extract such as quercetin, quercetin-3-*o*-β-glucopyran oside, kaemperfol-3-*o*-α-rhamnopyranosyl-β-glucopyranosi de, kaempferol-3-*o*-α-rhamnopyranosyl-(1-6)-β-glucopyran oside, rutin and allantoin from butanol fraction. Ethanolic extract has found to contain bulbocapnin, steroids such as β-sitosterol, stigmasterol and campesterol constituents.[8-12]

Address for correspondence:
Asst. Prof. Gaurav M. Doshi, Department of Pharmacology, Vivekanand Education Society's College of Pharmacy, Mumbai, Maharashtra, India.
E-mail: gaurav.pharmacology@gmail.com

MATERIALS AND METHODS

Collection, authentication and extraction

Fresh leaves of *Polyalthia longifolia* (Asapalav) were collected from Mumbai local market in month of April-May and shade-dried. The leaves were authenticated by Agarkhar Research Institute, Pune. A voucher specimen (No. 3/187/2013/Adm. 1692/080) was deposited in the botany department of Agharkar Research Institute, Pune. Further, they were subjected to cold extraction procedure[13] as follows:

Step 1: To 600 gm of leaves powder, 5 L 70% ethanol was added
Step 2: The sample was soaked for 12 hrs in an orbital shaker at 50 revolutions/min
Step 3: The extracts were filtered using Whatman Filter No. 1
Step 4: The concentrate was evaporated to dryness under reduced pressure using rotary evaporator at 40°C
Step 5: The extract was collected and stored in an air-tight amber colored glass container.

This ethanolic extract of *Polyalthia longifolia* leaves was subjected to analytical studies after carrying out preliminary phytochemical screening as described[14,15] by comparing it with standard biomarkers.[16] Standard biomarker and analytical grade solvents used for identification purpose were obtained from Sigma-Aldrich Private Limited, India. The general selection criterion for high-Pressure liquid chromatography (HPLC) and high performance thin layer chromatography (HPTLC) methods is to optimize the separation and identification of the bioactive compounds from the extract and to check the comparison of accuracy of the results obtained by these two widely used techniques. The basic advantage in selecting these powerful visualization techniques was their accuracy, preciseness, specificity, sensitivity and reproducibility.[17]

Analytical studies

Analytical studies are comprised of:

- Thin Layer Chromatography (TLC) for identification of the constituents
- High Performance Thin Layer Chromatography (HPTLC) for quantization and to identify the active constituents
- High Pressure Liquid Chromatography (HPLC) for determining the percentage of the active constituents.

Thin layer chromatography

- Mobile Phase: Methanol: Glacial Acetic Acid: Formic Acid: Water (3: 0.9: 0.9: 0.5)
- Standards: Rutin dissolved in ethanol.
- Extract: 10 mg in 1 ml of ethanol. The sample was filtered before spotting on to the plate.
- Chamber Saturation Time: 30 min
- Visualization: The TLC plate was exposed to ammonia and visualized under UV lamp.

High performance thin layer chromatography

The HPTLC was performed at the Radiant Research Laboratories Private Limited, Bangalore. The analysis was carried out by application of the sample and standard dissolved in methanol on HPTLC plate's silica gel 60 F254 (20 × 10 cm). Spots of extract (6 and 9 µg/l) and standard (3, 6 and 9 and 12 µg/l) were applied on the plates. The PL leaves extract (47.5 mg) and standard (5.16 mg) were dissolved in (ratio of 1:10 in terms of weight in mg) 10ml of the solvent. Scanning of the developed plates was carried out at 333 nm (before) and 550 nm (after) with following details:

- Instrument: CAMAG Linomat 5
- Linomat 5 application parameters
  - Spray gas: Inert gas
  - Sample solvent type: Methanol
  - Dosage speed: 150 nl/s
  - Predosage volume: 0.2 µl
- Sequence
  - Syringe size: 100 µl
  - Number of tracks: 10
  - Application position Y: 12.0 mm
  - Band length: 8.0 mm
- Calibration parameters
  - Calibration mode: Single level
  - Statistics mode: CV
  - Evaluation mode: Area and peak height
- Formula:

$$\text{Percentage of Rutin} = \frac{\text{sample area} \times \text{standard dilution} \times \text{purity}}{\text{standardarea} \times \text{sample dilution} \times 100} \times 100.$$

High pressure liquid chromatography analysis

- Chemicals: Rutin Standard, buffers, methanol, plant extract
- Instrument and other details:
  - HPLC instrument make: Shimadzu LC-10 ATVP
  - Software: Chromtech N 2000 data
  - Detector: UV Wavelength 280 nm
  - Flowrate: 1.5 ml/min
  - Injection volume: 20 µl
  - Column dimensions: RP C-18, 250 × 4.6mm, 5 µ
- Procedure:
  - Mobile Phase: Methanol: Phosphate Buffer (pH 3) in ratio 60:40.
  - 2 mg of standard and 5 mg of sample were dissolved in 2 ml and 5 ml of solvent respectively.

- Formula:

$$\text{Percentage of Rutin} = \frac{\text{sample area} \times \text{standard dilution} \times \text{purity}}{\text{standardarea} \times \text{sample dilution} \times 100} \times 100.$$

RESULTS

Extraction yield

The extraction yield of the dried powder of *Polyalthia longifolia* (PL) leaves for ethanolic extract was found to be 13.94% w/w [Table 1].

Preliminary phytochemical analysis of the plant extract

The plant ethanolic extract was found to be present positive for flavonoids, steroids, diterpenoids, alkaloids, saponins, tannins and phenolic compounds and mucilage [Table 2].

Chromatographic analysis of extract

TLC reports

Rf value (standard Rutin) = 0.78
Rf value (ethanolic extract) = 0.69
By comparing the Rf value [Figure 1], the sample was found to be identical to Rutin as active constituent.

HPTLC reports

The ethanolic extract of PL leaves has shown well resolved spots on the HPTLC plate at Tracks 5 and Track 6 in comparison to standard Rutin at Tracks 1-4 and 7-10. The images were obtained under UV visible wavelength at 333 nm but well resolved spots were obtained only after derivatization at 550 nm. Quantization by HPTLC and by comparing the Rf value at 0.36 (start),0.41(maximum) and 0.44 (end) confirms that the spots resolved were of rutin present in extract identified by comparison with standard biomarker as dark green paste spots [Table 3 and Figures 2-13].

By HPTLC studies, the amount of rutin present in the extract was found to be 11.60% w/w (5.51 mg of rutin present in 47.5 mg of PL leaves extract).

HPLC reports

The ethanolic extract of PL leaves has shown well resolved peak at 280 nm (retention time is 3.8 min) in comparison to rutin at a flow rate of 1.5 ml/min using methanol and phosphate buffer (pH 3) in the ratio of 60:40 when HPLC studies were undertaken [Table 4 and Figures 14 and 15].

Table 1: Percent yield of the extract

Name of the plant, its part, extract name	Yield (%w/w)
Polyalthia longifolia ethanolic extract leaves	13.94

Table 2: Preliminary phytochemical analysis of the plant extract

Test for	Reagent	Observation
Carbohydrates	Molish's reagent	Absent
Reducing sugars	Fehling's reagent	Absent
	Benedict's reagent	Absent
Saponin glycosides	Foam	Present
Flavonoids	Shinoda reagent	Present
Alkaloids	Dragendorff's reagent	Present
	Hager's reagent	Present
	Wagner's reagent	Present
	Mayer's reagent	Present
Tannins and phenolic compounds	5% $FeCl_3$ solution	Present
	Bromine water	Present
	Dilute iodine solution	Present
Mucilage with powdered drug material	Ruthenium red	Present
	Swelling property	Present
Steroids	Salkowski reagent	Present
	Liebermann-Burchard reagent	Present
	Lieberman reagent	Present
Fats and oils	Sudan red III reagent	Absent
	Filter paper test	Absent
	Saponification	Absent

Figure 1: TLC of *Polyalthia longifolia* (PL) ethanolic extract

Table 3: HPTLC analysis of *Polyalthia longifolia* and rutin

Track no	Details of standard and sample (µg/l)	Max. Height	Area
1	Rutin (3)	171.7	3500.1
2	Rutin (6)	298.4	6611.2
3	Rutin (9)	374.8	8687.0
4	Rutin (12)	426.4	10279.3
5	*Polyalthia longifolia* ethanolic extract (6)	342.0	7997.4
6	*Polyalthia longifolia* ethanolic extract (9)	411.1	10184.8
7	Rutin (12)	434.9	10486.6
8	Rutin (9)	380.7	8751.5
9	Rutin (6)	304.7	6649.9
10	Rutin (3)	181.1	3778.9

HPTLC=High performance thin layer chromatography

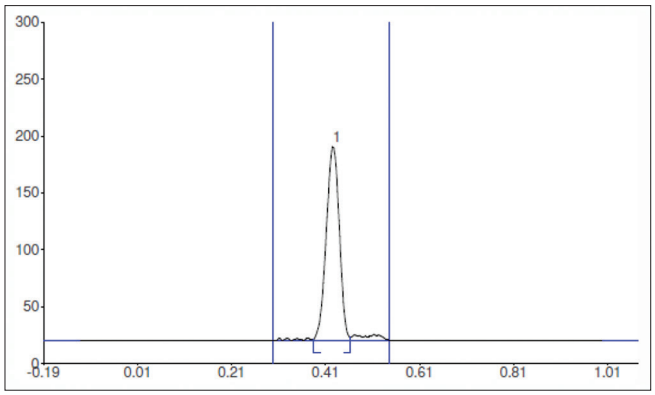

Figure 2: Track 1 - HPTLC peak of standard Rutin

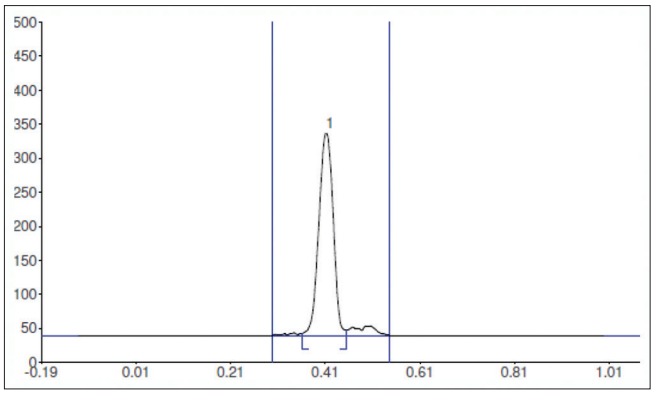

Figure 3: Track 2 - HPTLC peak of standard Rutin

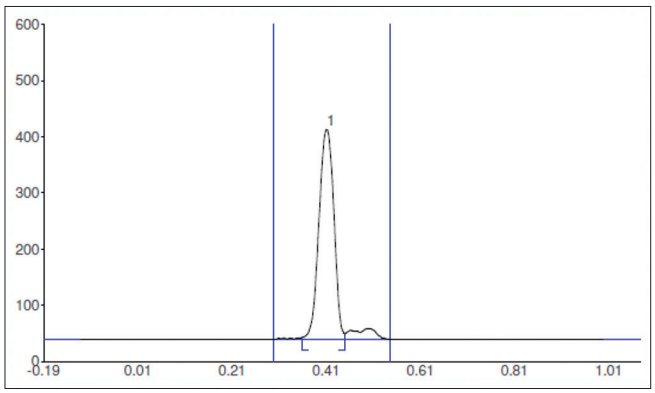

Figure 4: Track 3 - HPTLC peak of standard Rutin

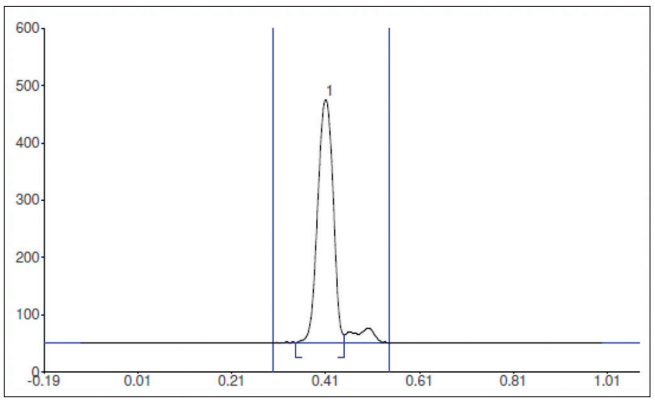

Figure 5: Track 4 - HPTLC peak of standard Rutin

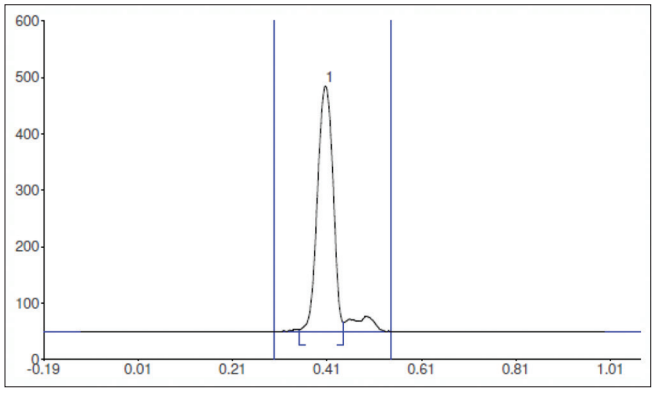

Figure 6: Track 7 - HPTLC peak of standard Rutin

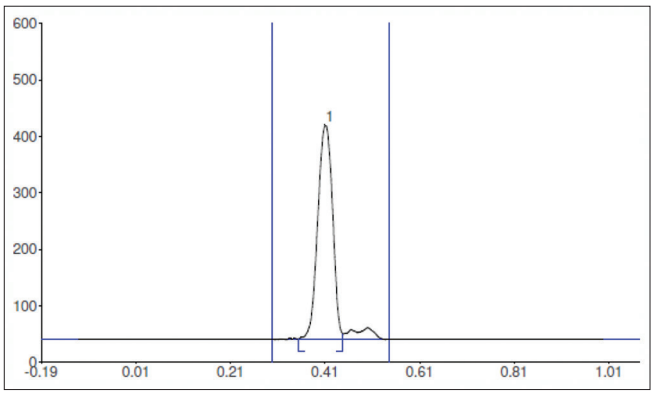

Figure 7: Track 8 - HPTLC peak of standard Rutin

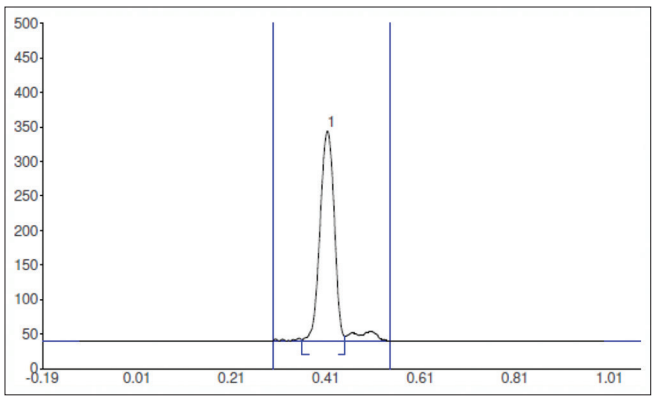

Figure 8: Track 9 - HPTLC peak of standard Rutin

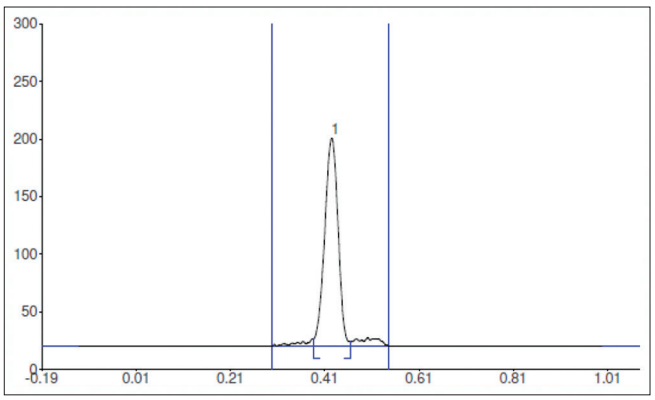

Figure 9: Track 10 - HPTLC peak of standard Rutin

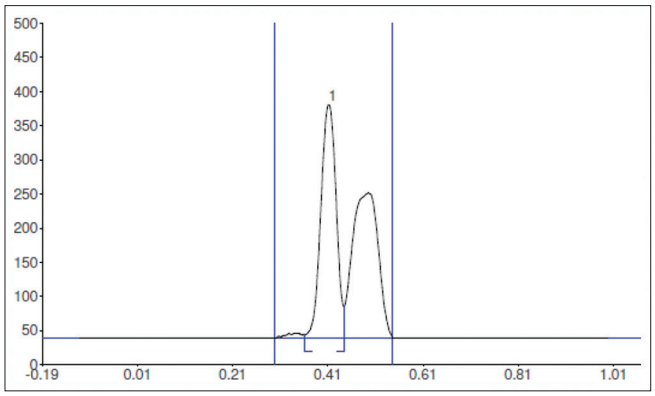

Figure 10: Track 5 - HPTLC peak of *Polyalthia longifolia*

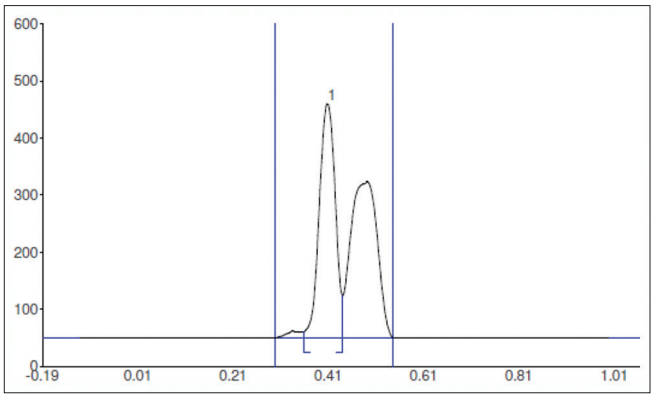

Figure 11: Track 6 - HPTLC peak of *Polyalthia longifolia*

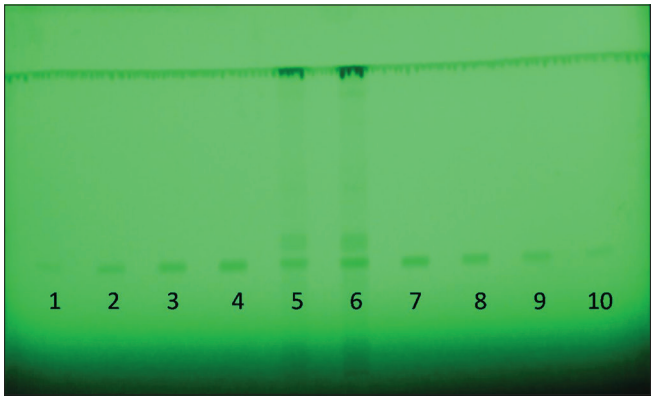

Figure 12: HPTLC image before (333 nm) derivatization

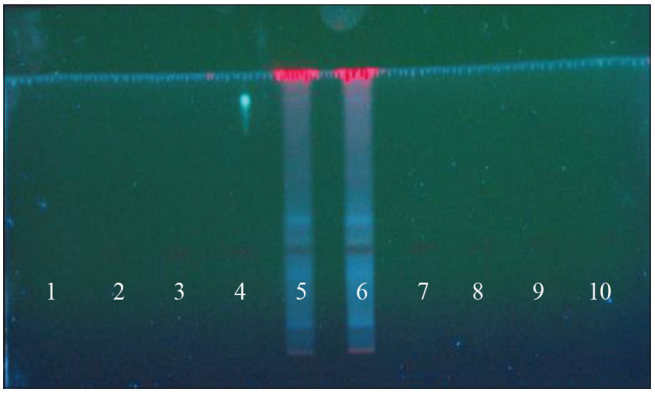

Figure 13: HPTLC image after (550 nm) derivatization

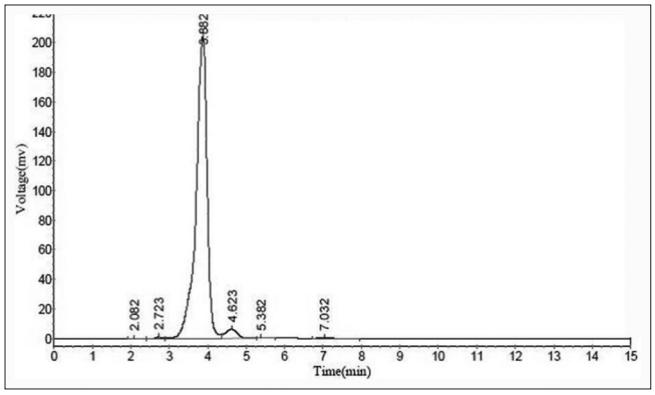

Figure 14: HPLC peak of standard Rutin

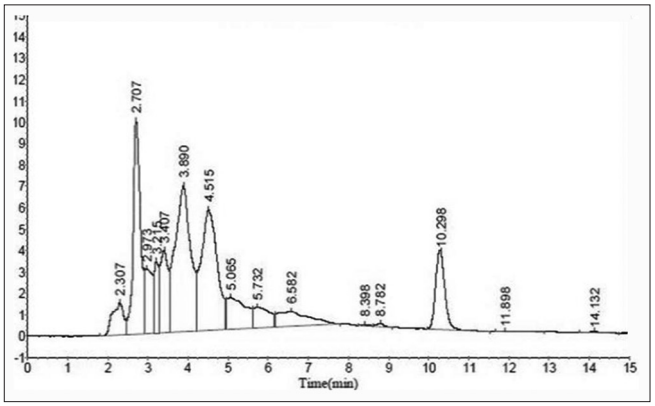

Figure 15: HPLC peak of *Polyalthia longifolia*

Table 4: HPLC analysis of *Polyalthia longifolia* and Rutin

Details of standard and sample	Observation parameter (ret time) area
Polyalthia longifolia ethanolic extract	(3.890) 183134.063
Rutin	(3.882) 4083121.25
Dilution ratio (standard:sample)	1:1
% of rutin	4.03% w/v

HPTLC=High performance thin layer chromatography

By HPLC estimation, the amount of rutin in ethanolic extract of PL leaves was found to be 4.03% w/v.

DISCUSSION AND CONCLUSION

Thus, rutin is an important active constituent isolated from many plant extracts. Further to our consideration, it is widely known for its immense pharmacological potential like anti-inflammatory, anticancer, antiarthritic etc. Our research article emphasis on the studies related to isolation of rutin in the cold ethanolic extract which was monitored by sophisticated chromatographic methods such as HPTLC and HPLC. Research on the extract gave us well resolved spots in HPTLC and good peak in HPLC. Hence, we urge the young

readers to step up in taking the task for exploring the extract of the local plant for isolating the other constituents and undertake studies on various pharmacological interventions by using different animal models.

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REFERENCES

1. Dhar U, Manjkhola S, Joshi M, Bhatt A. Current status and future strategy for development of medicinal plants sector in Uttaranchal, India. Curr Sci 2002;82:956-64.
2. DaSilva EJ, Hamilton A. Medicinal plants: A re-emerging health aid. Electron J Biotechnol 1999;2:1-4.
3. De Silva T. Industrial utilization of medicinal plants in developing countries. In: Bodekar G, Bhat KK, Burley J, Vantomme P, editors. Medicinal Plants for Forest Conservation and Healthcare. Nonwood Forest products series, No. 11. Rome, Italy: FAO; 1997. p. 38-48.
4. Hamilton A. Medicinal plants and conservation: Issues and approaches [serial on the Internet], 2003. p. 3. Available from: http://www.wwf.org.uk/filelibrary/pdf/medplantsandcons.pdf. [Last accessed on 2013 Nov 20].
5. Kamboj VP. Herbal medicine. Curr Sci 2000;78:35-9.
6. Warrier PK, Nambiar VP, Ramankutty C. In: Indian Medicinal Plants: A compendium of 500 species. Vol. 4. Chennai, India: Orient Longman Private Limited; 1994. p. 330-2.
7. (a) Anonymous. In: The Wealth of India: A dictionary of Indian Raw materials and Industrial parts. Vol. 2. Raw Materials Published by Council of Scientific and Industrial Research; 1950. p. 82. (b) Anonymous. In: The Wealth of India: A dictionary of Indian Raw materials and Industrial parts. Vol. 2. Raw Materials Published by Council of Scientific and Industrial Research; 1950. p. 186-8.
8. Sampath M, Vasanthi M. Isolation, structural elucidation of flavonoids from *Polyalthia longifolia* (Sonn.) Thawaites and evaluation of antibacterial, antioxidant and anticancer potential. Int J Pharm Pharm Sci 2013;5:336-41.
9. Sashidhara KV, Singh SP, Srivastava A, Puri A. Identification of the antioxidant principles of *Polyalthia longifolia var. pendulum* using TEAC assays. Nat Prod Res 2011;25:918-26.
10. Sharma RK, Mandal S, Rajani GP, Gupta N, Srivastava DP. Antiulcer and antiinflammatory activity of fresh leave extracts of *Polyalthia longifolia* in rats. Int J Drug Dev Res 2011;3:351-9.
11. Lee TH, Wang MJ, Chen PY, Wu TY, Wen WC, Tsai FY, *et al.* Constituents of *Polyalthia longifolia var. pendulum*. J Nat Prod 2009;72:1960-3.
12. Katkar KV, Suthar AC, Chauhan VS. The chemistry, pharmacologic and therapeutic applications of *Polyalthia longifolia*. Pharmacogn Rev 2010;4:62-8.
13. Sampath M, Vasanthi M. Isolation, Structural elucidation of flavonoids from *Polyalthia longifolia* Thawaites and evaluation of antibacterial, antioxidant and anticancer potential. Int J Pharm Pharm Sci 2013;1:3365-71.
14. Kokate CK. Practical Pharmacognosy. New Delhi, India: Vallabh Prakashan; 1989. p. 111-4.
15. Khandelwal KR. Practical Pharmacognosy-Techniques and Experiments. 10th ed. Pune: Nirali Prakashan; 2003. p. 149-56.
16. Harborne JB. Phytochemical methods: A guide to Modern Techniques of Plant Analysis. 3rd ed. New Delhi, India: Springer (India) Private limited Publication House; First Indian Reprint 2005. p. 1-295.
17. Nagore DH, Patil PS, Kuber VK. Comparison between high performance liquid chromatography and high performance thin layer chromatography for determination of diosgenin from the fenugreek seeds. Int J Green Pharm 2012;6:315-20.

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