High Performance Thin Layer Chromatography and High Performance Liquid Chromatography determination of Quercetin from *Polyalthia longifolia* leaves

Gaurav Mahesh Doshi[1, 2*] and Hemant Devidas Une[3]

[1]*Department of Pharmacology, Vivekanand Education Society's College of Pharmacy, Mumbai, Maharashtra, INDIA.*

[2]*Pacific Academy of Higher Education and Research University (PAHER), Udaipur, Rajasthan, INDIA.*

[3]*Department of Pharmacology, Y. B. Chavan College of Pharmacy, Rouzabaugh, Aurangabad, Maharashtra, INDIA.*

ABSTRACT

Introduction: *Polyalthia longifolia* Thwaites (PL) is an ornamentally important flowering tree with immense potential. Identification of phytochemical constituents from plant extracts is need of hour in developing countries like India. These elucidated constituents may play a pivotal role in providing remedies to plethora of diseases whether major or minor. Proper application of analytical technique is key to success in interpretation of these plant constituents. Flavanoids are widely noticeable in number of plant extracts. Flavonols class of compound like quercetin has been reported to possess good amount of ethno-pharmacological relevance from number of medicinally as well as local plants. **Objective:** In present research studies the aim was to find out by chromatographic techniques how much was percentage of quercetin present in the *Polyalthia longifolia* Thwaites leaves ethanolic extract. **Materials and Methods:** *Polyalthia longifolia* Thwaites leaves after ethanolic extraction and preliminary phytochemical studies were subjected to identification of quercetin by high performance thin layer chromatography (HPTLC) and high-pressure liquid chromatography (HPLC). **Results:** Preliminary phytochemical evaluation confirmed the presence of flavonoids. The R_f value of the extract was found to be conceding with the standard quercetin by using analytical techniques. Quercetin was found to be 84.26% w/w and 74.45% w/v by high performance thin layer chromatography (HPTLC) and high-pressure liquid chromatography (HPLC) respectively. **Conclusion:** The active constituent identified from *Polyalthia longifolia* Thwaites leaves extract was found to be identical to quercetin.

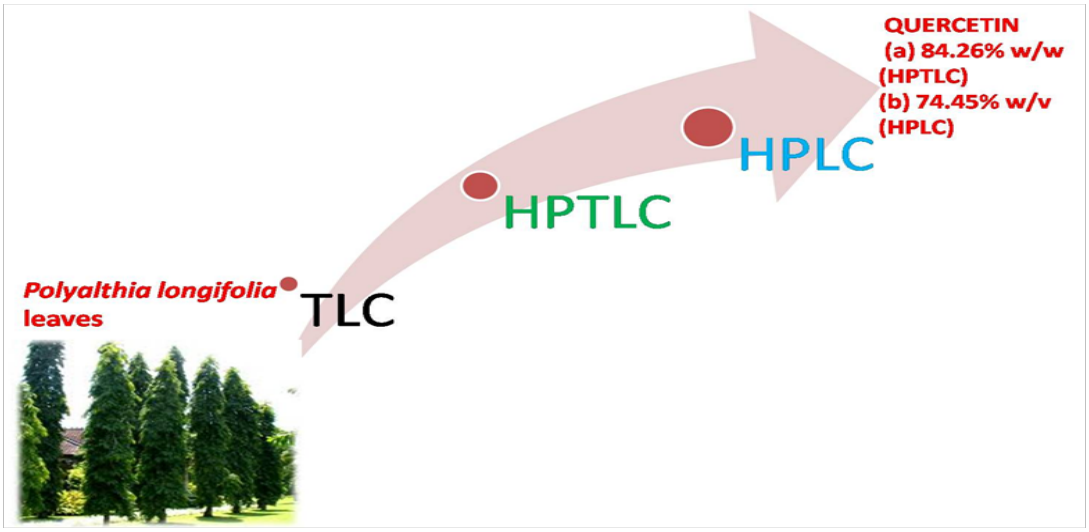
Key words: HPLC, HPTLC, *Polyalthia longifolia*, Quercetin, TLC.

INTRODUCTION

Plant constituents identification in modern era focuses on isolation and characterization of secondary constituents within a specific group of species with outcome that ideally some eluted constituents may be novel or have totally new structure. Hence, it is need of hour to clear interpret these isolated components in the selected plant extract. Both qualitative as well as quantitative measurements on the plant extracts provide determinations of the amount of the phytoconstituents present in the plants Flavonols exist in glycosidic combination and can be easily separated by paper chromatography.[1] *Polyalthia longifolia* Thwaites is a universal eagerly blossomed tree in India and widely known for number of pharmacological important activities along with various important reported constituents as stated by us in our previously published work of rutin identification from PL leaves.[2,3]

***Corresponding address:**
Mr. Gaurav M. Doshi
Department of Pharmacology, Vivekanand Education Society's College of Pharmacy, Mumbai, Maharashtra, India.
E-mail: gaurav.pharmacology@gmail.com

DOI: 10.5530/fra.2015.2.3



Graphical Abstract

Uses: *Polyalthia longifolia* (PL) is regarded as frequently blossomed tree in India and widely known for number of pharmacological important properties. The leaves of PL were shade-dried and extracted by ethanol followed by identification of quercetin by high performance thin layer chromatography and high-pressure liquid chromatography.

MATERIALS AND METHODS

Part A: Collection, authentication and extraction

The details have been reported by us in our previous studies of rutin identification.[2] PL ethanolic leaves extract were subjected to HPTLC and HPLC studies after carrying out preliminary phytochemical screening. Standard biomarker used for identification purpose was obtained from Yucca Enterprises, Mumbai, India and HPLC grade solvents used in experimentation were procured from Merck India Pvt. Ltd., Mumbai, India.

Part B: Analytical studies

Analytical studies 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 and High Pressure Liquid Chromatography (HPLC) for determining the percentage of the active constituents.

(a) Thin Layer Chromatography (TLC)

It consists of Mobile Phase of Toluene: Ethyl Acetate: Formic Acid (5: 4:0.2), Standard (Quercetin) and extract dissolved in (10 mg/ml) ethanol. The sample was filtered before spotting and chamber was saturated for 30 minutes.

(b) High Performance Thin Layer Chromatography (HPTLC)

The HPTLC was performed at the Anchrom Research Labs. Testing Pvt.Ltd, Mumbai, India. HPTLC plate's silica gel 60 F254 (20 x 10 cm) were used. The standard and sample

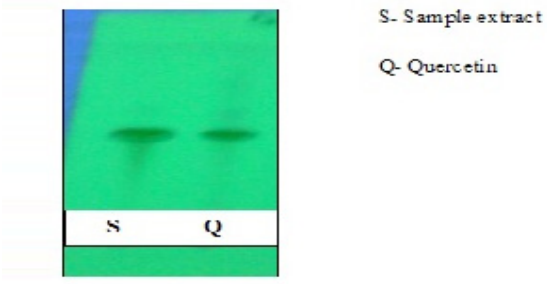


Figure 1: TLC image of quercetin in *Polyalthia longifolia* leaves

Table 1: HPTLC analysis of *Polyalthia longifolia* leaves and standard quercetin

Track No	Details	R_f	Area
1	Standard Quercetin (4 µg/l)	0.22	3652.8
2	Standard Quercetin (5 µg/l)	0.23	6449.0
3	Standard Quercetin (6 µg/l)	0.23	8103.6
4	Standard Quercetin (7 µg/l)	0.24	9865.2
5	Standard Quercetin (8 µg/l)	0.24	11438.8
6	Standard Quercetin (9 µg/l)	0.25	13094.8
11	*Polyalthia longifolia* ethanolic extract (5 µg/l)	0.25	12076.6
12	*Polyalthia longifolia* ethanolic extract (7 µg/l)	0.25	12503.1

Table 2: HPLC analysis of *Polyalthia longifolia* leaves and standard quercetin

Details of standard and sample	Observation Parameter (Ret Time) Area
PL extract	(6.762) 96940864
Standard quercetin	(6.700) 115630016
Dilution ratio (standard : sample)	1:1
% of quercetin	75.45 % w/v

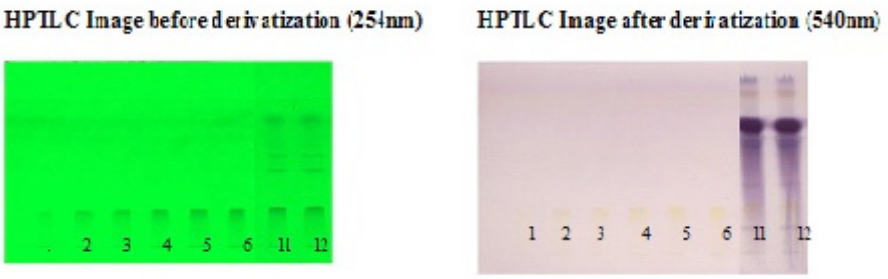


Figure 2: HPTLC images of quercetin in *Polyalthia longifolia* leaves

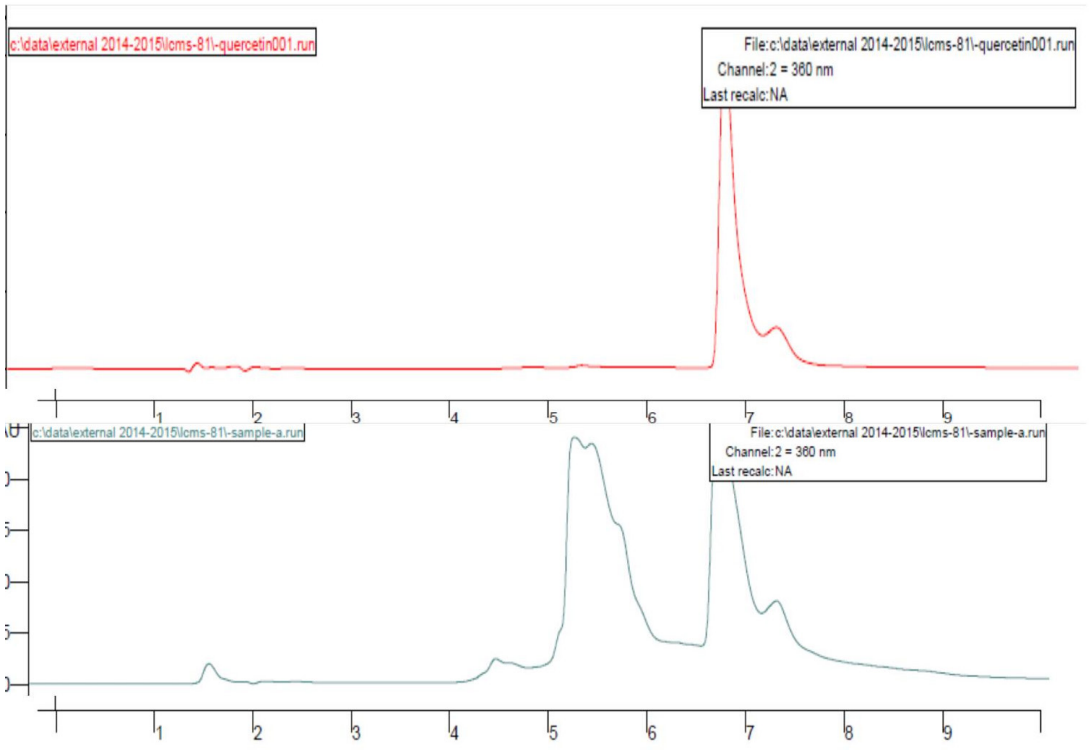


Figure 3: HPLC peak of standard quercetin and *Polyalthia longifolia* leaves

were prepared by dissolving 5 mg and 10 mg in 5 ml of methanol each. Spots of extract (5 and 7 µg/l) and standard (4, 5,6,7,8 and 9 µg/l) were applied on the plates.

The instrument used in studies is mentioned below with details as CAMAG Linomat 5 with application parameters, inert gas as spray gas, 100 nl/s and 0.2 µl was the dosage speed and predosage volume respectively. The sequence was comprising of syringe size (100 µl), there were 8 tracks and 12.00 mm and 8.00 mm was the application position

and band length respectively. The calibration parameters consists of mode of single level, calibration curve with area and peak heights.

$$\text{Percentage of Quercetin} = \frac{\text{sample area x standard dilution x purity}}{\text{standard area x sample dilution x 100}} \text{ x 100}$$

(c) High Pressure Liquid Chromatography (HPLC)

The Instrument details are Shimadzu LC-10 ATVP with Chromtech N 2000 data software. The other details are detector used was UV with 280 nm wavelength, Flowrate (1.5 ml/min), Injection volume (20 µl), Column dimensions (RP C-18, 250 x 4.6 mm, 5 µ). The procedure was comprising of mobile phase (Acetonitrile (95): (5) water), wavelength (360 nm), and flowrate (1 ml/min). 2 mg of standard and 5 mg of sample was weighed and dissolved in 2 ml and 5 ml of solvent respectively, from which 100 µl was taken and made up to 1 ml with solvent, from this stock solution 20 µl was injected in each constituent identification.

$$\text{Percentage of Quercetin} = \frac{\text{sample area x standard dilution x purity}}{\text{standard area x sample dilution x 100}} \text{ x 100}$$

RESULTS

(a) Extraction yield and preliminary phytochemical screening

The results obtained have been previously reported by us.[2]

(b) Thin layer chromatography

R_f for standard quercetin = 3.0/4.=0.62 and R_f for PL ethanolic extract = 3.1/4.9=0.63. By comparing, R_f value, the sample was found to be present positive for quercetin (Figure 1).

(c) High Performance Thin Layer Chromatography (HPTLC)

The ethanolic extract of PL leaves has shown well resolved spots on the HPTLC plate at Tracks 11 and Track 12 in comparison to standard quercetin at Tracks 1 to 6. The images were obtained under UV visible wavelength at 254 nm but well resolved spots were obtained only after derivatization at 540 nm. Quantization by HPTLC and by comparing the R_f value (maximum 0.25) confirms that the spots resolved were of quercetin identified by comparison with standard biomarker as dark brownish paste spots (Table 1 and Figure 2). By HPTLC studies, the amount of quercetin present in the PL extract was found to be 84.26% w/w (4.21 mg of quercetin present in 5 mg of PL leaves extract).

(d) High Performance Thin Layer Chromatography

The ethanolic extract of PL leaves has shown well resolved peak at 360 nm (retention time is 6.7 min) in comparison to quercetin at a flow rate of 1 ml/min using methanol and water (0.1% orthophosphoric acid) [pH 3.5] in the ratio of 60:40 (Table 2 and Figure 3). By HPLC estimation, the amount of quercetin in ethanolic extract of PL leaves was found to be 75.45% w/v.

DISCUSSION AND CONCLUSION

Phytochemical analysis is one of the major break through in the field of plant constituents along with structural interpretation. The stream covers simple techniques such as thin layer chromatography to hyphenated techniques such as mass and nuclear magnetic resonance spectroscopy. Flavonols aglycones are well established in plant extracts with omnipresence of common flavonols like kaempferol, quercetin and myricetin[4] Quercetin has been reported for various pharmacological activities with major ones in the field of inflammation, cancer and arthritis. Our current research studies of quercetin identification from PL leaves by HPTLC and HPLC confirms the presence of these constituent in major proportion. In addition, the quercetin and rutin identification could be correlated to our established immunomodulatory activity of the PL ethanolic extract. Future, lies on identifying the probable role of these component and its correlation to pharmacological potential.

ACKNOWLEDGEMENTS

We would like to acknowledge the College management and Principal, Dr. Supriya Shidhaye, who provided us all the facilities to do this work. We also acknowledge Radiant Research Services Pvt. Ltd. for their help in analytical services.

ABBREVIATIONS

HPTLC : High Performance Thin Layer Chromatography.
HPLC : High Pressure Liquid Chromatography HPLC
UV : Ultraviolet

Highlights of the paper

- Leaves of *Polyalthia longifolia* Thwaites (PL) are used widely in traditional offerings in India during festivals.
- In the current studies, the researchers have determined the percentage of the Quercetin present in PL by high performance thin layer chromatography (HPTLC) and high-pressure liquid chromatography (HPLC).
- Quercetin was found to be 84.26% w/w (HPTLC) and 74.45% w/v (HPLC) respectively.
- The R_f value of the extract was found to be conceding with the standard quercetin.

About Authors

Mr. Gaurav Doshi: *Presently working as Assistant Professor, Department of Pharmacology, Vivekanand Education Society's College of Pharmacy, Chembur (East), Mumbai- 400074. He has 27 nos. of journal paper; 54 citations to his credit. He is Ph.D Research Scholar of Department of Pharmaceutical Sciences, Pacific Academy of Higher Education and Research University, Udaipur, Rajasthan, India.*

Hemant Devidas Une: *Working as Associate Professor, Vice- Principal, Head of Department of Pharmacology, Y. B. Chavan College of Pharmacy, Rouzabagh, Aurangabad, Maharashtra, India. He has 18 nos. of journal paper; 85 citations to his credit.*

REFERENCES

1. Harborne JB. Phytochemical methods: A guide to Modern Techniques of Plant Analysis. 3rd ed. New Delhi, India: Springer (India) Private limited; 2005. p. 16-7.
2. Doshi GM, Chaskar PK, Zine SP, Une HD. Solicitation of HPLC and HPTLC for determination of rutin from *Polyalthia longifolia*. Pharmacogn. Res. 2014 May 16; (2): 234-9.
3. Doshi GM, Une HD. Screening of *Polyalthia longifolia* leaves as potential immunomodulatory target. Int. J. Pharmacol. 2015 Feb16; 11(2): 106-13.
4. Harborne JB, Mabry TJ, Mabry H. The Flavonoids: Advances in Research. Chapman and Hall, London; 1982.