Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2016; 8(7); 1187-1190

ISSN: 0975-4873

Research Article

HPTLC Method for Quantitative Determination of Quercetin in a Polyherbal Compound for Urolithiasis

Barik Laxmi Dhar*, Ratha Kshirod Kumar, Das Manosi, Hazra Jayram

National Research Institute of Ayurvedic Drug Development, Kolkata 4-CN Block, Sector V, Bidhannagar, Kolkata-700091.

Available Online: 12th July, 2016

ABSTRACT

A sensitive and reliable high performance thin layer chromatographic method has been developed for quantification of quercetin in a polyherbal compound for urolithiasis. Methanolic extract of *Crataeva nurvala* and *Bryophyllum pinnatum* was chromatographed on silica gel $^{60}F_{254}$ Aluminum plates plates with Chloroform: Methanol: Formic acid in the ratio (7.5:1.5:1; v/v/v) as mobile phase. The Quercitin quantification was 1.82% (w/w) and 3.2 % (w/w) for *Crataeva nurvala* Buch. -Ham and *Bryophyllum pinnatum* Lam. respectively.

Keywords: HPTLC, quercetin, *Crataeva nurvala*, *Bryophyllum pinnatum*,Urolithiasis

INTRODUCTION

Mutrashmari is a formidable disease resembling with *'Yama'* the god of Death. It is classified under *Astamahagada* (eight fatal conditions) in Ayurvedic classics[1]. On the basis of similarity in the clinical findings it is compared to Urolithiasis. It is a consequence of complex physiochemical process which involves sequence of events in the formation of urinary stone. The prevalence of the disease is 10% in men and 5% in women in their lifetime with a secondary recurrence of 50% in both genders. Hereditary, few metabolic disorders, and some diet factors play important role in the genesis of the disease[2,3]. The conventional treatment available for the disease is quite expensive and beyond the reach of common men, also these drugs are failed to prevent the recurrence of stone formation. Consequently, quest for establishment of an effective and cheaper antiurolithic drug is the need of the hour. India is endowed with a rich diversity of medicinal plants and traditional medicinal knowledge. The Indian system of medicines (AYUSH) prevail in the country is also enriched with the herbal, mineral, metallic, and animal drug resources for various ailments. Many remote Indian pockets also have traditional folk healer, having proficiency to use these medicinal drugs for their primary healthcare[4].Many numbers of ethno-medicinal plants have reported form India for anti-urolithic activity and few of them are only validated through preclinical and proper clinical studies[5-7]. Pharmaceutical standardization of various single and compound of Ayurveda, Siddha and Unani (ASU) drug is also published in the form of monographs (Ayurvedic Pharmacopeia of India). In the present paper, a poly-herbal anti-urolithic compound is explored to endorse a step towards the standardization of Ayurvedic formulation. Parna beej (*Bryophyllum pinnatum* Lam.) & Varun (*Crataeva nurvala* Buch. -Ham.), both medicinal plants are the ingredients in equal ratio of the poly-herbal compound and are used to treat various urinary tract disorders like Ashmari (urolithiasis), Mutrakrichra (dysuria) by the traditional health practitioners of north eastern part of India since long. These herbs contain a wide range of active chemical compounds, including alkaloids, triterpenes, glycosides, flavonoids, steroids, bufadienolides, lipids and organic acids. Experimental study and Clinical study proves that these two drugs in equal quantity have potency to cure urinary calculus. *Bryophyllum pinnatum* exhibits anti-nociceptive, analgesic, anti-inflammatory, antiulcer, neuro-sedative, muscle relaxant, diuretic, litholytic, and hypoglycemic properties. It also shows antimicrobial activity in vitro against *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebisella pneumonia* and a gram positive *Staphylococcus aureus*[8,9]. Varuna (*Crataeva nurvala* Buch. -Ham.) is also a good diuretic, litholytic and antimicrobial. Experimental model proves its uorlitholytic effect against 0.75% of ethylene glycol and remarkably diminished the crystal deposition in the Kidney. Chloroform extract of stem bark of *C. nurvala* is reported to be effective against both gram positive (B. cereus) and gram negative (*E. coli*) mediated urinary tract infection. Alcoholic extract of Crataeva nurvala (250 and 500 mg/kg for 10 days) proves its protective activity against cisplatin (5 mg/kg) induced nephrotoxicity. Ethanolic extract of root bark of *Crataeva nurvala* (150 and 300 mg/kg) shows wound healing and collagenation potential in-vivo. The LD_{50} of 50% ethanolic extract of stem bark was found to be more than 1000 mg/kg i.p. in adult rats[10,11].

MATERIALS AND METHODS

Whole plant parts of Parnabeej *(Bryophyllum pinnatum* Lam.) and stem bark of Varuna (*Crataeva nurvala* Buch. -

*Author for Correspondence: ldbarik1963@yahoo.co.in

Organoleptic characters of prepared drug material	
Description	*Fine powder*
Colour	*Dark brown*
Odour	*No characteristic odour*
Taste	*Bitter*

Physiochemical parameters of the drug	
Loss on drying	12.5%
Ash content	27.5%
Acid insoluble ash	2.3%
Water soluble extractive	60.3%
Alcohol soluble extractive	35.6%
pH	8

Ham.) were procured from the local herb supplier of Kolkata and were authenticated by the Institute as per usual norms. Both the drug were cleaned, shed dried and cut in to small pieces and kept in a separate vessel. Decoction of the above drugs was prepared separately as per the methods mentioned in Ayurvedic classics. Prepared decoction was filtered and dried under rotary vacuum driers for complete removal of moistures. Then, it was weighed and kept in a tight closed glass vessel for analytical study. The drugs were further analyzed for physiochemical parameters as per the API norms. Quantification of Quercitin using standard HPTLC analysis was done[12].

Quantification of Quercetin by HPTLC study of Varun (Crataeva nurvala) Parnabeej (Bryophyllum pinnatum) extracts

Method development

A CAMAG HPTLC system (Switzerland) comprising CAMAG Linomat 5 applicator, CAMAG TLC scanner 3, CAMAG Wincats software, version 1.44, Hamilton syringe (100µl), CAMAG Reprostar 3, CAMAG TLC plate heater, CAMAG UV Cabinet were used for the study. Silica gel $^{60}F_{254}$ Aluminum plates (Merck) was used as stationary phase. Hexane: Ethyl acetate (8:2; v/v) was used as mobile phase. Methanol was used as solvent.

Preparation of standard solution

Accurately weighed 2 mg of Quercetin was dissolved in 10 ml of methanol in a volumetric flask to obtain 0.2mg / ml Quercetin. This solution was used as calibration.

Preparation of Sample solution

50mg fine dust of individual plant part was gently refluxed in 60ml methanol for 2hrs and filtered through whatman filter paper (No. 41 pore size: 20-25µm). The residue was refluxed again with 40ml methanol for 2hr. and filtered it as before. The combined filtrates were evaporated to make it a final volume of 10ml. Now 1ml of this solution was diluted to 10 ml with methanol and this was used for estimation of Quercetin in the plant.

HPTLC method and Chromatographic conditions

The chromatographic estimation was performed using the following conditions. Stationary phase was precoated silica gel $^{60}F_{254}$ aluminium sheets (20x10cm) and the mobile phase used was Chloroform: Methanol: Formic acid in the ratio (7.5:1.5:1; v/v/v). The chamber saturation

Observed at 254 nm

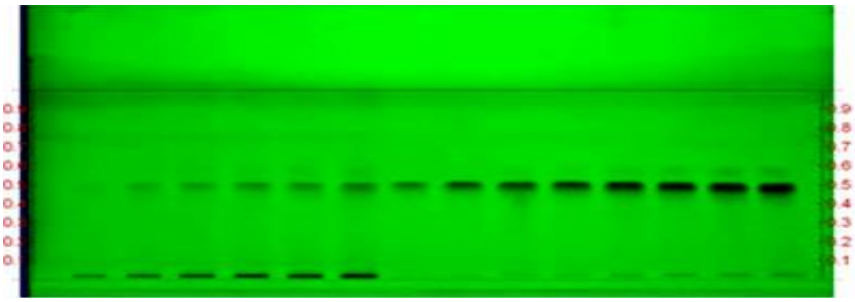

Figure 1: Photography of HPTLC.

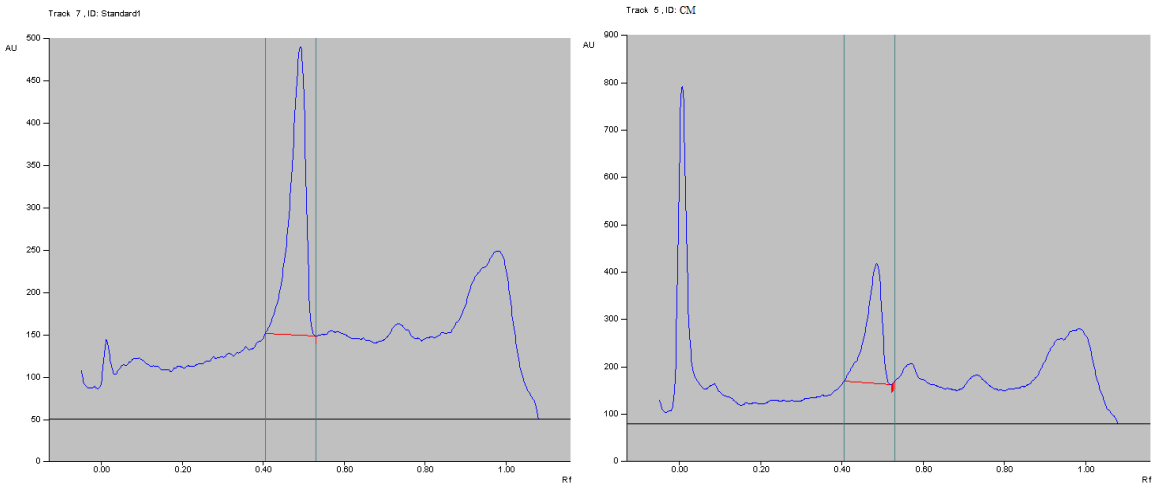

Figure 2&3: HPTLC graph shows presence of standard quercetin in *crataevanurvala*.

Observed at 254 nm

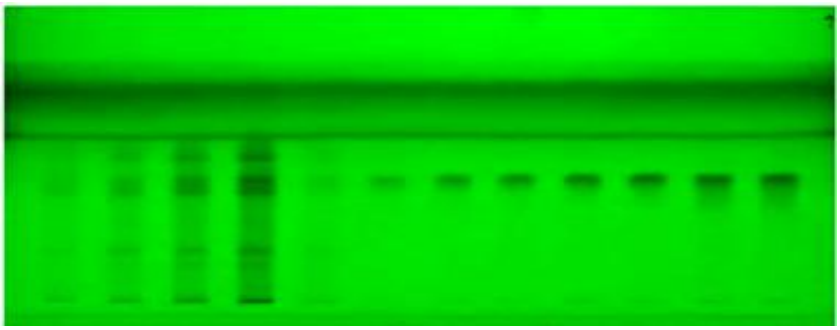

Figure 4: Photography of HPTLC.

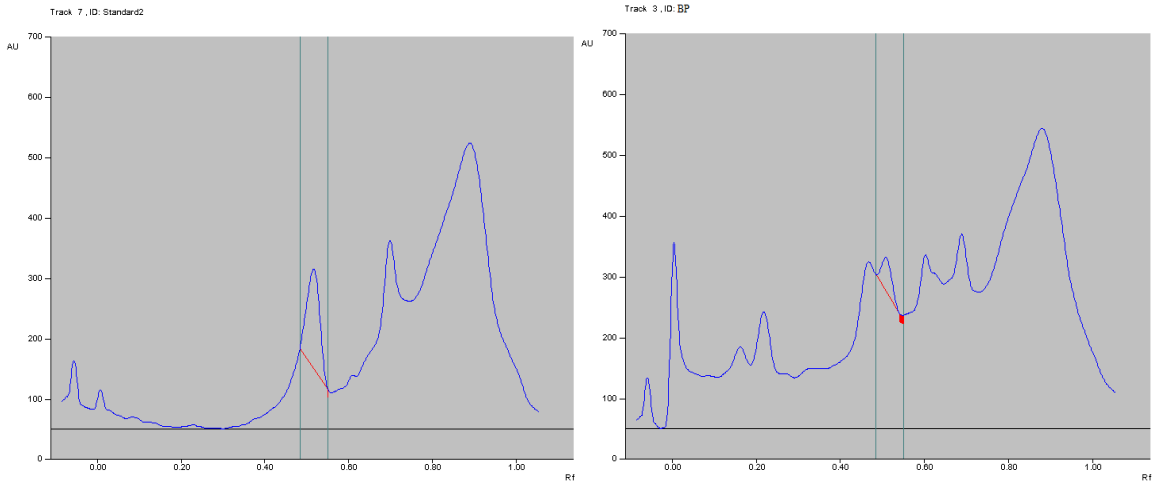

Figure 5&6: HPTLC graph shows presence of standard quercetin in *Bryophyllum pinnatum*.

time employed was 20 mins and the developing distance was 8 cm. scanning wavelength of 278 nm with a slit dimension of (6.0x 0.45) mm. micro and scanning speed of 20mm/s were employed[13].

RESULTS

Quantification of Quercetin by HPTLC study of Varun (Crataeva nurvala) extract

HPTLC studies revealed well resolved peaks of extracts containing quercetin. The spots of the entire chromatogram were visualized under UV 254nm (Fig. 1) and the percentage of quercetin (R_f 0.51) in *Crataeva nurvala* extracts was found to be 1.82% (w/w).

Quantification of Quercetin by HPTLC study of Parnabeej (Bryophyllum pinnatum) extract

HPTLC studies revealed well resolved peaks of extracts containing quercetin. The spots of the entire chromatogram were visualized under UV 254nm (Fig. 4) and the percentage of quercetin (R_f 0.51) in *Bryophyllum pinnatum* extracts was found to be 3.2 % (w/w).

DISCUSSION

HPTLC is a valuable quality assessment tool for the identification and quantification of chemical constituents present in plant drugs. The retention factor (Rf) values obtained from it can be used to identify compounds due to their uniqueness for each compound. The TLC procedure was optimized with a view to quantify the herbal extract. The mobile phase used was Chloroform: Methanol: Formic acid in the ratio (7.5:1.5:1; v/v/v) with Rf = 0.51 for quercetin (Fig.1&4). Well-defined spots were obtained when the chamber was saturated with mobile phase for 20 min at room temperature. In the present study, the Rf values of individual compounds appearing as spots vertically have been noted (the less polar compounds moving higher up the plates resulting in higher Rf values), which may thus be used as a quality control profile for this drug. The TLC plate was visualized under UV light at 254 nm, without derivatization. A photograph of a TLC plate after chromatography of quercetin standard and a methanol extract of the *C. nurvala* and *B. pinnatum* shown in Figure 1&4. The identity of the quercetin bands in sample chromatograms was confirmed by the chromatogram obtained from the sample with that obtained from the reference standard solution (Fig. 3 & 4; Fig. 5&6) and by comparing retention factors of quercetin from sample and standard solutions. The peak corresponding to quercetin from the sample solution had same retention factor as that from the quercetin standard (Rf= 0.51) (Fig. 3 & 4; Fig. 5&6). A preparative TLC method reported in the literature was developed for isolation of quercetin[14,15]. The limits of detection (LOD) and quantification (LOQ) were 1.82% (w/w) and3.2 % (w/w) for *Crataeva nurvala* and *Bryophyllum pinnatum* respectively.

CONCLUSION

Thus the organoleptic, microscopic characters, physico-chemcial, fluorescence study, preliminary phytochemical

screening and HPTLC fingerprint analysis can be used as a diagnostic tool for the correct identification of the formulation. The adulterants if any in these plant materials can be easily identified by using these results. Here, a rapid, simple, accurate and specific HPTLC method for quantitative estimation of quercetin present *Crataeva nurvala* and *Bryophyllum pinnatum* has been developed and validated. The data could be used as a QC standard.

ACKNOWLEDGEMENT

The authors acknowledge the Director General, CCRAS for providing necessary facilities and support to carry out the work.

REFERENCES

1. Singhal GD and Colleagues. (2007). Susruta-Samhita Ancient India Surgry.2nd edn.pp-522-28.
2. Trinchieri, A. (2008). Epidemiology of urolithiasis: an update. Clinical Cases in Mineral and Bone Metabolism, 5(2), 101–106.
3. B, S., P, H., & Rao, P. N. (2013). a Case Study on Mutrashmari (Urolithiasis) Using Yavaksharadi Yoga. International Journal of Research in Ayurveda and Pharmacy, 4(1), 138–140. http://doi.org/10.7897/2277-4343.04145.
4. Kshirod Kumar, R., & Mishra, S. S. (2012). Journal of Pharmaceutical and Scientific Innovation.Use of Agnikuanri (Ammania baccifera L.) In ringworm by some of the villagers of Birmaharajpur block (Sonepur district), 1(4),37–38.
5. Mikawlrawng, K., & Kumar, S. (2014). Current scenario of urolithiasis and the use of medicinal plants as antiurolithiatic agents in Manipur (North East India): A Review, *2*(1), 1–12.
6. Baheti, D. G., & Kadam, S. S. (2013). Antiurolithiatic activity of some traditional medicinal plants against calcium oxalate induced urolithiasis in rats. *International Journal of Pharmaceutical, Chemical and Biological Sciences*, *3*(4), 1276–1285.
7. Tiwari, A., Soni, V., Londhe, V., Bhandarkar, A., Bandawane, D., & Nipate, S. (2012). An overview on potent indigenous herbs for urinary tract infirmity: Urolithiasis. *Asian Journal of Pharmaceutical and Clinical Research*, *5*(Suppl.1), 7–12.
8. Nagaratna, A., & Hegde, P. L. (2015). A comprehensive review on Parnabeeja [Bryophyllum pinnatum (lam.) Oken], *3*(5), 166–171.
9. Kamboj, A., & Saluja, A. K. (2013). Development of validated HPTLC method for quantification of stigmasterol from leaf and stem of Bryophyllum pinnatum. *Arabian Journal of Chemistry*. http://doi.org/10.1016/j.arabjc.2013.10.006
10. Bhattacharjee, A., Shashidhara, S. C., & Aswathanarayana. (2012). Phytochemical and ethno-pharmacological profile of Crataeva nurvala Buch-Hum (Varuna): A review. *Asian Pacific Journal of Tropical Biomedicine*, *2*(2 SUPPL.). http://doi.org/10.1016/S2221-1691(12)60379-7.
11. Haque, M. E., Islam, M. N., Gupta, D. Das, Hossain, M., Shekhar, H. U., & Shibib, B. A. (2008). Triterpenoids from the Stem Bark of *Crataeva nurvala*. *Dhaka University Journal of Pharmaceutical Sciences*, *7*(1), 3–6.
12. Anonymous. (2001). The Ayurvedic Pharmacopoeia of India, Part I, Vol.I,1st edn, Government of India, Ministry of health and family welfare, Department of ISM & H, New Delhi (India).
13. Rungsung, W., Dutta, S., Kumar Ratha, K., Mondal, D. N., & Hazra, J. (2015). Pharmacognostical and Phytochemical Study on the Stem of Ephedra gerardiana. *Journal of International Research in Medical and Pharmaceutical Sciences*, *2*(3), 80–85.
14. Anonymous. (2005). Quality Standards of Indian Medicinal Plants, Vol. 3, Published by Indian council of Medicinal Research, New Delhi, page no. 34.
15. Sachin U. Rakesh, P. R. Patil, V. R. Salunkhe, P. N. Dhabale, K. B. Burade. (2009). International Journal of ChemTech Research.1(4),931-936.