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

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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 60F254 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 classics1. 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 disease2,3. 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 healthcare4. 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 studies5,6. 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 polyherbal 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 Klebsiella pneumonia and a gram positive Staphilococcus aureus8,9. Varuna (Crataeva nurvala Buch. -Ham.) is also a good diuretic, litholytic and antimicrobial. Experimental model proves its urolitholytic 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 LD50 of 50% ethanolic extract of stem bark was found to be more than 1000 mg/kg i.p. in adult rats10,11.
Organoleptic characters of prepared drug material

<table>
<thead>
<tr>
<th>Description</th>
<th>Fine powder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>Dark brown</td>
</tr>
<tr>
<td>Odour</td>
<td>No characteristic odour</td>
</tr>
<tr>
<td>Taste</td>
<td>Bitter</td>
</tr>
</tbody>
</table>

Physiochemical parameters of the drug

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss on drying</td>
<td>12.5%</td>
</tr>
<tr>
<td>Ash content</td>
<td>27.5%</td>
</tr>
<tr>
<td>Acid insoluble ash</td>
<td>2.3%</td>
</tr>
<tr>
<td>Water soluble extractive</td>
<td>60.3%</td>
</tr>
<tr>
<td>Alcohol soluble extractive</td>
<td>35.6%</td>
</tr>
<tr>
<td>pH</td>
<td>8</td>
</tr>
</tbody>
</table>

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. 

Quantification of Quercitin 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 F254 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 F254 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.
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.

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 (Rf 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 (Rf 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. 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-chemical, 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 in Crataeva nurvala and Bryophyllum pinnatum has been developed and validated. The data could be used as a QC standard.

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REFERENCES