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QUANTIFICATION OF PHENOLIC COMPOUNDS IN HERBAL RAW MATERIALS USING HPTLC

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ABSTRACT

The research work envisages quantification of phenolic compounds using High Performance Thin Layer Chromatography in medicinally important plants Ficus lacor fruits, Caesalpinia bonduc bark, Althaea officinalis seed. A single solvent system Toluene: ethyl acetate: formic acid was used for analyzing the constituents. The analysis revealed the presence of luteolin in Ficus lacor fruit, gallic acid in Caesalpinia bonduc bark and scopoletin in Althaea officinalis seed.

Keywords: phenolic compounds, *Ficus lacor*, *Caesalpinia bonduc*, *Althaea officinalis*

INTRODUCTION

Phenolic compounds are important class of phytochemicals present in almost all the herbal raw materials. These include mainly tannins that are used in tanning leather, flavonoids are used as anti-oxidant, coumarins as anti-coagulant as well as these all compounds are used in preparation of dyes. Thus it is necessary to study these compounds through various analytical studies. According to literature survey, the medicinal plants *Ficus lacor* fruits, *Caesalpinia bonduc* bark, *Althaea officinalis* seed undertaken for research in the paper have rarely been studied for the presence of phenolic compounds. This study thus aims in using same HPTLC system for quantification of phenolic compounds in these plants.

Ficus lacor Buch. (Moraceae) is a large spreading tree with sub-globose fruits. The fruits are white when ripe or flushed with red and dotted. It is found almost throughout India and is commonly cultivated as an ornamental tree. The leaves of the tree are used as fodder, the decoction of bark is used as a gargle and as a wash for ulcers. The fruits are edible[1]. Fruits are reported to be used in treatment of diabetes, syncope, etc[2]. Decoction of buds has been reported to be used for ulcer and leucorrhoea. The seeds were used earlier to cure gastric problems, dysentery, boils, etc[3]. *Caesalpinia bonduc* (Caesalpiniaceae) is a shruby climber found

throughout the plains of India. The bark is known to be used as rubefacient and as a local application for sores[4].

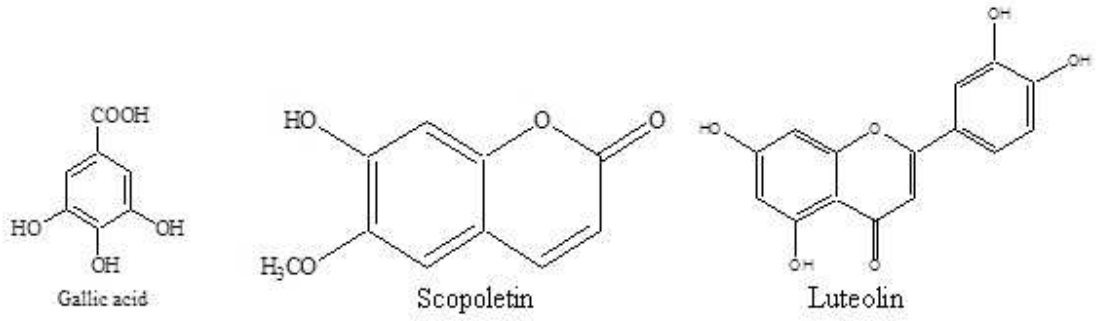

Fig. 1 Structure of scopoletin, gallic acid and luteolin

Althaea officinalis (Malvaceae) seed is a perennial herb found mainly in north Himalaya in Kashmir. The whole plant is official in several Pharmacopoeias. The herb is useful in medicine as demulcent and emollient due to presence of mucilage, starch and pectin[5].

Literature survey revealed that not much work has been done on the identification as well as quantification of the phenolic compounds present in seeds of *A. officinalis*, fruits of *F. lacor* and bark of *C. bonduc*. Therefore the present work aimed at identification and quantification of major phenolic compounds present in these raw materials. The established HPTLC method, thus, can be used in identification, quantification and standardization of raw materials as well as extracts of different species containing polyphenols.

MATERIALS AND METHODS

General Experimental Procedures

All chemicals and reagents used were of analytical grade. Toluene, ethyl acetate, formic acid and methanol used were purchased from S.D. Fine Chemicals of laboratory grade. Standard luteolin and gallic acid (purity > 98%) was purchased from Total Herbs Solution Pvt. Ltd., Mumbai, India. Standard scopoletin was purchased from Sigma Aldritch, Mumbai, India. Separation experiments were performed on silica-based HPTLC F_{254} plates that were products of Merck (Darmstadt, Germany). Standard and sample solutions were applied by Desaga Applicator (Desaga, Germany) and quantified using proQuant software (*Biostep* Desaga, Germany).

Plant material

A. officinalis seeds, *F. lacor* fruits was collected from Mumbai, India and *Caesalpinia bonduc* was collected from Nashik, Maharashtra. A voucher specimen of the raw material (ICT/MNPRL/AO/02), (ICT/MNPRL/FL/01) and (ICT/MNPRL/CB/01) respectively was deposited in Medicinal Natural Product Research Laboratory, Institute of Chemical Technology, Mumbai.

Standard preparation

Standard stock solution (1 mg/ml) each of scopoletin, luteolin and gallic acid was prepared using methanol.

Sample preparation

5 g each of powdered drug (*A. officinalis* seed, *F. lacor* fruit and *C. bonduc* stem) was extracted separately with 50 ml methanol in Soxhlet apparatus for 3 h. The extract obtained transferred to 100 ml volumetric flask and volume made up with methanol.

HPTLC chromatographic analysis

Before sample application, the silica plates (20 cm × 20 cm or 10 cm× 10 cm) were activated at 110°C for 15 min. Standard solution (1mg/ml in methanol) was used for the preparation of a five-point calibration curve corresponding to an amount of 0.4-2 µg/spot for scopoletin, 0.4-2 µg/spot for luteolin and 0.4-2 µg/spot for gallic acid. All the extracts obtained from the experimental runs were spotted in triplicate. Standard and sample solutions were applied in the form of a band using a 10 µl syringe (Hamilton, Bonaduz, Switzerland) by Desaga Applicator. The linear ascending development was carried out in a twin trough chamber prior saturated with 10 mL mobile phase (Toluene: ethyl acetate: formic acid, 1: 9: 0.1 v/v) for 15min at room temperature (25 ˚C and 40% relative humidity). After development, plates were dried, and the components were visualized under UV 254 nm. Identification of phenolic compounds in the extracts were performed on the basis of R_f and chromatographic behaviour with those of an authentic standard. The band was scanned and quantified densitometrically at 254 nm (λ_{max} of polyphenols). For quantification, the densitometry scanning was performed in the absorbance mode, slit width 8.00 mm× 0.20 mm, scanning speed 20 mm/s. Quantification was performed using linear regression equations of respective compounds. Precision of retention factor (R_f) values was based on 10 subsequent measurements. The HPTLC run of standard and sample track is shown in Fig. 2.

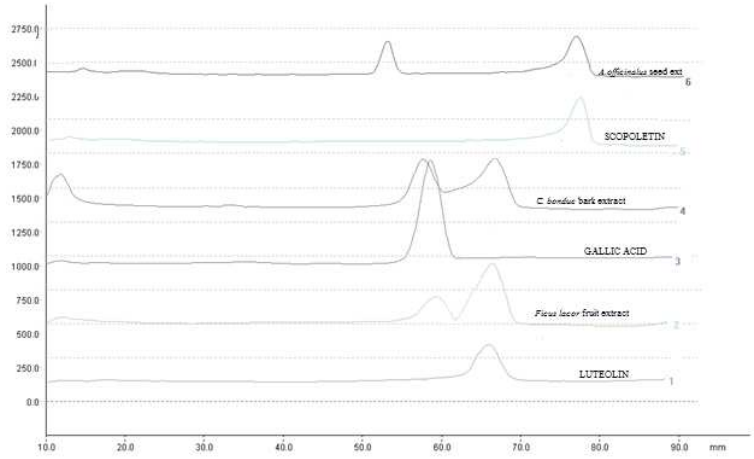

Fig. 2 HPTLC densitogram of standard scopoletin, gallic acid, luteolin, *A. officinalis* extract (2-3), *C. bonduc* extract and *F. lacor* extract (4-5)

RESULTS AND DISCUSSION

While developing HPTLC method, different parameters like solvent system composition, wavelength, band separation and symmetry were taken into consideration to obtain good resolution. HPTLC method was developed for estimation of phenolic compounds in the plant samples. Different trials were carried out using acetone, chloroform, ethyl acetate, methanol, toluene and *n*-hexane. The solvent system comprising of *n*-hexane: ethyl acetate in different ratios resulted in no separation. Addition of methanol separated the bands but the reproducibility was not observed. When chloroform and methanol in different proportions were taken, the resolution was found to be poor. Finally toluene and ethyl acetate was gave better separation of bands with comparatively good peak symmetry. Also no interference was observed with the other constituents in the extract except tailing which was removed by adding formic acid. Thus the final system comprised of toluene: ethyl acetate: formic acid, 1: 9: 0.1.

The results indicated that the method may be suitable for the estimation of phenolic compounds in different species. The percentage content in *A. officinalis* seed, *C. bonduc* bark and *F. lacor* fruits was found to be 0.002%, 0.076% and 0.008% respectively.

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Conflict of Interest
The authors declare no conflict of interest.

REFERENCES

1) The Wealth of India: Raw materials, vol 4 (Council of Scientific & Industrial Research, New Delhi, **1956**; p. 36.
2) Ayurvedic Pharmacopoeia of India, part I, vol IV, Government of India, Ministry of Health & Family Welfare, Department of Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homeopathy (AYUSH), New Delhi, **2008**; p. 136-137
3) Rai SK, Subedi S, Mishra S: Utilization Pattern of medicinal plants in Thumpakhar, Village Development Committee, Sindhupalchok, Nepal. Botanica Orientalis, **2004**; 4**:** 75-78.
4) The Wealth of India: Raw materials, vol 3, Council of Scientific & Industrial Research, New Delhi, **1992**; p. 6-8
5) The Wealth of India: Raw materials, vol 1, Council of Scientific & Industrial Research, New Delhi, **1985**; p.207-208