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Quantification of Dioctyl phthalate from *Ehretia laevis* Roxb by HPTLC

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ABSTRACT

The present study was designed to quantify dioctyl phthalate (DOP), a bioactive molecule, present in Ehretia laevis from hexane extract, prepared under different conditions. A simple High Performance Thin Layer Chromatographic (HPTLC) method has been developed for the quantification of dioctyl phthalate. The quantification of analytes has been carried out using a mobile phase hexane: ethyl acetate (7:3) on pre-coated aluminium plates (Silica gel Merck 60 F_{254}). Densitometric determination was carried out .The spots were located at 254 nm. The amount of dioctyl phthalate present in the extracts has been estimated by comparing the peak area using the standard sample. Linearity of the standard was found in the concentration range of 3 to 5 µg/spot. The correlation coefficient value was 0.960. The proposed HPTLC method was found to be simple, faster and reliable for quantification of a bioactive molecule, dioctyl phthalate.

Keywords: Boraginaceae, *Ehretia laevis*, Dioctyl Phthalate and HPTLC.

INTRODUCTION

Indian traditional medicinal practice is based on the various systems including Ayurveda, Siddha and Unani.The evaluation of drug is mostly based on phytochemical, pharmacological and allied approaches including various instrumental techniques like chromatography, microscopy and others.

As mentioned in Ayurveda, *E. laevis* Roxb, belonging to family Boraginaceae, is an important medicinal plant. The plants of genus have significant medicinal importance and find uses in traditional medicine as a remedy for the treatment of diarrhea, cough, cachexia, syphilis,

toothache, stomach and venereal diseases and as an antidote to vegetable poison [1]. All parts of the *E. laevis* plant are used for different medicinal purposes. Decoction of the fresh root is used in the treatment of syphilis and that of the stem bark for the treatment of diphtheria. The paste of tender leaves is used externally to cure eczema, and the powder of flowers with milk is employed as an aphrodisiac [2]. It has many uses such as ornaments, pot herbs, dye for wood, stone, medicines, wines, cosmetics etc. [3]. The inner bark of the tree and the insipid fruit of *E. laevis* are eaten in times of scarcity. The leaves are used as cattle fodder [1]**.**

Dioctyl phthalate was identified as one of the active principles, displaying chronic toxicity against *Aedes aegypti* and *Culex quinquefasciatus* types of larvae [4].

Therefore an attempt has been made to develop HPTLC method, which is simple and reliable for analysis of dioctyl phthalate in crude extract.

EXPERIMENTAL SECTION

Reagents and Chemicals

All solvents used were of AR-grade and obtained from Merck, Mumbai (India).

Plant Material

The leaves of *Ehretia laevis* were collected from Pune, Maharashtra, India during the month of July. It was identified and authenticated at Botanical Survey of India, Pune, Maharashtra, India. Its voucher number is BSI / WC / Tech / 2006 /185.

Preparation of Extracts

Under different conditions, hexane extract were prepared, using Soxhlet extractor, at room temperature and under reflux condition. The air shade dried powdered material (100g) was extracted with Soxhlet extractor for 72 hrs using n-hexane (40–60).The solvent was recovered under reduced pressure. The hexane extract (**A,** 9%w/w) obtained as yellow thick viscous oil and it was preserved in a refrigerator [5, 6]. It demonstrated entomological activity. Bioguided broad fractionation of hexane extract (**A**) was carried out to get two major fractions as, Hexane: Pet ether1:1 **(A1,** 1.2 %**)** and Hexane **(A2,** 2.3%**)**.

In the second method, powdered material (10 g) was extracted using hexane (50 ml) by soaking it for 24 hours at room temperature. The solvent was recovered under reduced pressure to obtain crude mass (**B,** 2.5%).

In the third method, powdered material (10 g) was refluxed for 18 hours using hexane (50 ml). The solvent was recovered under reduced pressure to obtain crude mass (**C,** 5 %).

Chromatographic Conditions

Stationary phase:	Pre-coated silica gel plates Merck 60 F_{254} (10 µ, 10 cm, 0.2 mm thickness).
Mobile phase:	Hexane: Ethyl Acetate (7:3)
Lamp:	Deuterium
Wavelength:	254 nm
Application mode:	CAMAG Automatic TLC Sampler III
Development mode:	CAMAG Twin Trough Chamber
Scanner:	CAMAG TLC Scanner 3 and CATS software

Experimental conditions:

Temperature 25± 2 ^{0}C,
Relative humidity 40%

Preparation of Standard

A solution of dioctyl phthalate (1 mg / ml) was prepared by dissolving 2.5 mg of sample in chloroform solvent. The working standard solution was 0.3 mg/ml.

Calibration curve for Standard

Dioctyl phthalate, a bioactive molecule was isolated by column chromatography of Soxhlet hexane extract (**A**).The standard solution of dioctyl phthalate (3 µg to 5 µg per respective spot) was applied in triplicate on TLC plate. Quantitative evaluation of the plate was performed in absorption / reflection mode at 254 nm using a slit width of 6.0 □0.30 mm, scanning speed 20 mm/s with a computerized CAMAG, TLC scanner-3 integrated with CATS – III software. The plate was developed and scanned as per the chromatographic conditions and the peak areas were recorded.

HPTLC Quantification in Test Samples

2 mg of various extracts, as prepared above, were weighed accurately and dissolved in chloroform to make up the volume of 1 ml. The standard stock solution was spotted on to TLC plates and run in different solvent systems. 10 µL of extracts per spot of these solutions were applied along with standard solution on to a precoated silica gel plates (in triplicates). The mobile phase consisting of Hexane: Ethyl acetate in the ratio of 7:3 v/v was found optimum. Densitometric scanning was done at 254 nm as all extracts showed maximum response at that wavelength. In order to reduce the neckless effect, HPTLC chamber was saturated for 20 min using saturation pads. The mobile phase was run up to a distance of 8 cm; which takes approximately 20 min for complete development of the HPTLC plate.

The content of dioctyl phthalate of various extracts was determined by comparing the area of the chromatogram with the calibration curve of working standard. The Rf value of the standard dioctyl phthalate (0.8) was compared with the Rf value of the extracts. The average content of dioctyl phthalate in different extracts was expressed as mg / g of extract.

RESULTS AND DISCUSSION

Spectral characteristics of the peak of standard and that of the extracts were compared for identification of dioctyl phthalate. Calibration curve of dioctyl phthalate was obtained by plotting peak areas verses concentration applied. It was found to be linear in the range of 3 µg to 5 µg per spot. Equation of the calibration curve is y = 1822.5x +174.67. The correlation coefficient was found to be 0.960 and thus exhibits good linearity between concentration and area.

Table 1

Sr. No.	Extracts	Concentration of Dioctyl Phthalate (mg/g of extract)
1	A	95.03
2	A1	137.158
3	A 2	83.188
4	B	65.55
5	C	123.862

CONCLUSION

The developed HPTLC method is simple, precise, specific and accurate for quantitative estimation of dioctyl phthalate present in *E.laevis*. The chromatographic method is validated according to ICH guidelines. Statistical tests indicate that the proposed method reduces the duration of analysis and appears to be equally suitable for the routine analysis in pharmaceutical formulation in quality control laboratories, where time factor is important.

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4-8