Asian Journal of Chemistry Vol. 22, No. 10 (2010), 8117-8120

Quantification of β-Sitosterol from Three *Jatropha* Species by High Performance Thin Layer Chromatography

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Chromatographic techniques can be used to document phytochemical fingerprint and quantitate chemical markers to identify morphological and geographical variation in the raw herbal material. The three *Jatropha* species *viz.*, *J. nana, J. gossypifolia* and *J. glandulifera* (family-Euphorbiaceae) are used in traditional systems mainly for the treatment of diarrhoea, dysentery, liver disorders and inflammation. While *Jatropha nana* reported for opthalmia. β-Sitosterol is a phytosterols which is ubiquitous throughout the plant kingdom and reported to possess wide range of biological activities. The petroleum ether extracts were dissolved in methanol and the quantification of analytes has been carried out using mobile phase toluene:methanol (9:1 v/v) on precoated aluminium silica gel plates (Merck) and densitometric determinations was carried out after derivatization with anisaldehyde sulphuric acid reagent. The plates were scanned at 525 nm by absorption/reflection mode. Calibration curves were prepared and the amount of β-sitosterol in the extracts has been estimated by comparing the respective peak areas of the standard. The linearity of standard was found in the concentration of range of 100-500 ηg. The correlation coefficient value was 0.9978. High performance thin layer chromatography (HPTLC) method has been developed and validated for the analysis of β-sitosterol in leaves rout- and seed oil 8 *Jatropha* species. This was found to be faster, reliable and sensitive for quantification of β-sitosterol.

Key Words: *J. nana*, *J. gossypifolia* and *J. glandulifera*, β-Sitosterol and HPTLC.

INTRODUCTION

In traditional system of medicine leaves and roots of *J. gossypifolia* (Jgo) and *J. glandulifera* (Jgl) are mainly used for the treatment of diarrhoea, liver disorders and inflammation[1-4]. β-Sitosterol is a main phyto-sterol found in numerous plants including rice, wheat, corn, nut, peanut *etc*. It is structurally related to cholesterol[5]. β-Sitosterol has recorded an amazing health benefits in immune dysfunctions, antipyretic[6], antioxidant[7], hepatoprotective[8], hypercholesterolemia[9], inflammatory disorders and rheumatoid arthritis[10], colon cancer[11], benign prostatic hypertrophy[12,13] and breast cancer[14]. There is no report available for presence of β-sitosterol in *Jatropha* species. Preliminary phytochemical screening, TLC fingerprinting and co-TLC studies (with β-sitosterol) of *Jatropha* seed oil, leaves and roots revealed the presence of

β-sitosterol and an identical spot as that of standard β-sitosterol was observed. Further it was confirmed by R_f values comparison and multi wavelength scanning.

Previously, β-sitosterol has been quantified by liquid chromatography and tandem mass spectrometry using atmospheric pressure photoionization (APPI-LC-MS-MS)[15]. While there are other reports using liquid chromatography (LC) with evaporative light scattering detection (ELSD)[16] online liquid chromatography-gas chromatography (LC-GC)[17] and gas chromatography[18].

As there is no quantification data available about the levels of β-sitosterol in these *Jatropha* species. Considering its wide therapeutic applications, as an alternative quantification techniques, as one of the marker constituent to ensure identity and quality of this plant a sensitive, specific and reproducible HPTLC method was developed for the quantification of β-sitosterol in the three *Jatropha* species.

EXPERIMENTAL

The seed, leaves and roots of *J. nana*, *J. gossypifolia* and *J. glandulifera* were collected from surrounding areas of Pune and Solapur, Maharashtra, India in May-June 2007. The voucher specimens were deposited in Agharkar Herbarium of Maharashtra Association (AHMA), Pune. The collected plant materials were dried under shade and coarsely powdered. The seed oil is extracted with petroleum ether (60-80 ºC) by soxhlet extraction method. The leaves and root samples were extracted with petroleum ether (60-80 ºC) at room temperature by cold maceration method. All the chemicals used in the experiments were of analytical grade procured from Merck. Reference standard β-sitosterol was purchased from Sigma Chemicals, USA.

Chromatographic conditions: Stationary phase: Pre-coated silica gel plates Merck 60 F_{254} (10 × 10, 0.2 mm thickness). Experimental conditions: temperature 25 ± 2 ºC. Relative humidity-40 %. Mobile phase-toluene:methanol (9:1 v/v). Spotting device: Linomat III Automatic sample spotter, CAMAG (Switzerland). Development mode: CAMAG twin trough chamber, CAMAG, Densitometer: TLC Scanner III, CATS software, CAMAG.

Sample preparation: Dried and well matured seeds, leaves and root powders were exhaustively extracted with petroleum ether at room temperature by cold maceration method. The seed oil is extracted by soxhlet extraction method. The extracts were filtered and concentrated under reduced temperature and pressure. The yields were 40.00, 2.30, 1.30, 29.00, 1.40, 0.92, 27.00, 1.40 and 1.10 % for *J. nana*, *J. gossypifolia* and *J. glandulifera* seed, leaves and roots, respectively.

Preparation of standard solution: The stock solution of β-sitosterol (1 mg/mL) was prepared in methanol. The stock solution was quantitatively transferred into a 10 mL volumetric flask to give solution of appropriate concentration range of β-sitosterol.

Calibration curve of the standard: Standard solution of β-sitosterol (100-500 ng/mL) was applied in triplicate on precoated silica gel 60 F_{254} HPTLC plates (E. Merck), of uniform thickness of 0.2 mm. The plates were developed in a solvent

system of toluene:methanol (9:1 v/v) in CAMAG twin trough chamber up to a distance of 80 mm previously equilibrated with mobile phase for 0.5 h. After development, the plate was dried in air and sprayed by using anisaldehyde sulphuric acid reagent solution and subsequently heated at 120 ºC for derivatization. These plates were scanned at 525 nm absorbance/reflection mode by CAMAG Scanner III and CATS software was used to analyze the plates. The peak areas were recorded. Res-pective calibration curves were prepared by plotting peak area *verses* concentration of β-sitosterol.

HPTLC quantification of the extracts: The completely dried petroleum ether extracts were accurately weighed and stock solutions of 10 mg/mL were prepared. Theses stock solutions were further diluted with methanol to get solutions of 1 mg/mL 10 µL/spot of these solutions were applied on to a precoated silica gel 60 F_{254} HPTLC plates in triplicates. The plates were developed by ascending mode to a distance of 8 cm and scanned as per said conditions. The β-sitosterol content of various extracts were determined by comparing the area of chromatogram with the calibration curve of working standards. The R_f value of standard β-sitosterol (0.52) was compared with the R_f value of the extracts. The average content of the β-sitosterol in different species of *Jatropha* extracts were expressed in mg/g of the extract.

RESULTS AND DISCUSSION

The different mobile phases were tested and the desired resolution was achieved by toluene:methanol 9:1 v/v. The β-sitosterol was not showing any absorption before derivatization, therefore the spots were visualized after spraying with anisaldehyde sulphuric acid reagent. The maximum absorption of the standard peak β-sitosterol was compared with the spots of the extracts. Calibration of β-sitosterol was obtained by plotting peak areas *verses* concentration applied. It was found to be linear range of 100-500 ηg per spot. Equation of calibration curve is $Y = 0.5621x + 8.685$. Thus, exhibits good linearity between concentrations and area. The amount of β-sitosterol in petroleum ether extract of seed oil, leaves and roots were *J. nana* 932.2, 181.9 and 221.4 mg/g. While in *J. gossypifolia* 464.6, 65.8 and 19.0 mg/g and in *J. glandulifera* it is 274.1, 53.6 and 258.6 mg/g, respectively (Fig. 1).

Conclusion

In this study it has been demonstrated that HPTLC fingerprint can be employed to identify the source of the plant powder to the specific part of the plant that is constituent in the powder. The quantification of β-sitosterol in powder of different plant parts has been established. The proposed HPTLC method was found to be simple, rapid, accurate and reproducible for the estimation of β-sitosterol in seed oli, leaves and roots of *Jatropha* species. The correlation coefficient value was 0.9978. This method was found to be faster, simple and reliable for analysis of β-sitosterol. The plant part show specific variations in their content of this phytochemical marker. Thus, HPTLC reported in this study provides a chromatographic fingerprint suitable for confirming the identity and purity of three *Jatropha* species.

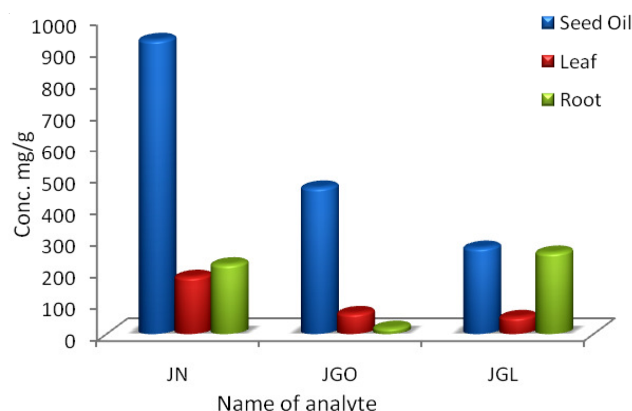

Fig. 1. Quantitative estimation of β-sitosterol in three *Jatropha* species by HPTLC

ACKNOWLEDGEMENTS

The authors are thankful to Director, Agharkar Research Institute, Head, Botany Group and Mrs. Ashwini Misar for help during experiment.

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(*Received*: 5 April 2010; *Accepted*: 2 August 2010) AJC-8944