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QUANTIFICATION OF STEROIDS AND TRITERPENOIDS FROM INDIAN MEDICINAL PLANTS BY HPLC

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ABSTRACT

The study aimed at identification and quantification of phytoconstituents present in the biologically active roots of Pavetta tomentosa, leaves of Commiphora caudata and rhizomes of Maranta arundinacea. A reversed-phase High Performance Liquid Chromatography (HPLC) method with a single solvent system has been used for quantification of different steroids and triterpenoids. The established HPLC method can be successfully used for efficient quantitative analysis of betulin in P. tomentosa roots, β-sitosterol in rhizomes of M. arundinacea and E-guggulsterone in leaves of C. caudata.

Keywords: Betulin, *β*-sitosterol, *E*-guggulsterone, *Pavetta tomentosa, Maranta arundinacea, Commiphora caudata,* HPLC

INTRODUCTION

Pavetta tomentosa Linn. (Fam. Rubiaceae) is a shrub distributed throughout the deciduous forests of India. The flowers are reported to be consumed fresh as food and the infusion is used as a cosmetic. The roots are said to possess purgative, diuretic and tonic properties, jaundice, headache, urinary diseases [1]. The root bark has been reported to possess D-mannitol [2].

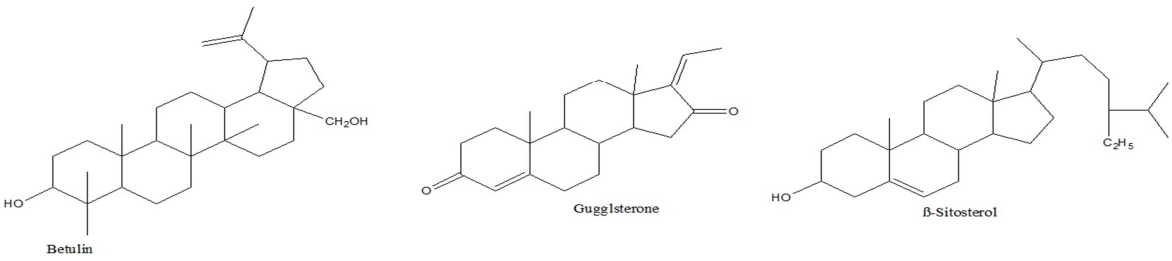

Fig. 1 Structure of betulin, *E*-gugglsterone and β sitosterol

Maranta arundinacea (Fam: Marantaceae) is a herb mainly cultivated in tropical regions India. Rhizomes are considered to be acrid, rubefacient and used in wound healing. Leaves are used by local people for packaging of meat and fish. [3]

Commiphora *caudata* (Fam. Burseraceae) commonly known as Hill Mango is a common tree found in South India. Leaves and fruits are used to improve digestion and increase appetite. Resin is carminative while bark is used against snake bite. [4]

Literature survey revealed that not much study has been conducted on constituents of this plant which is been used in various purposes since ancient times. Preliminary phytochemical investigation on the above plants showed presence of steroids and triterpenoids. Therefore purpose of the study was to identify the active phytoconstituent present in the plants which can be used as marker for these plants. Further quantification of the identified phytoconstituents was carried out using HPLC with a single solvent system for steroid or triterpenoid markers. The study involved the identification by Thin Layer Chromatography and quantification of the same by High Performance Liquid Chromatography (HPLC).The method can be used for identification, quantification as well as standardization of plant material or extract or herbal formulations containing the quantified markers.

MATERIAL AND METHODS

Plant material

P. tomentosa roots were collected from Mulshi, Pune, India; Rhizomes of *M. arundinacea* were collected from Mangalore, India & Leaves of *C. caudata* were collected from Chittoor district, Andhra Pradesh, India. Authentication was done and a voucher specimen (ICT/ MNPRL/ PT/ 01), (ICT/ MNPRL/ MA/ 02) & (ICT/ MNPRL/ CC/ 01) were deposited in Medicinal Natural Products Research Laboratory, ICT, Mumbai. All the plant materials were dried at 60°C and were powdered.

Chemicals

All solvents used were of analytical grade. HPLC grade acetonitrile and methanol were purchased from Merck India. Water used was double distilled. Solvents were filtered through a 0.45 µm filter (Millipore Bedford, MA, USA) and degassed in an ultrasonic bath (Remi Instruments, Mumbai, India) before use. Betulin standard (Purity > 90%) and was isolated in-house and confirmed using spectral studies. *β*-sitosterol and *E*-guggulsterone were purchased from Total Herb Solution Pvt. Ltd, Mumbai & Sigma-Aldrich Ltd respectively.

Instrumentation

HPLC analysis was performed with a Jasco (Hachioji, Tokyo, Japan) system consisting of an intelligent pump (PU-4180), a high-pressure mixer, a manual sample injection valve equipped with a 20 µl loop, and a PDA detector (MD-4010). HPLC column of 250×4.6 mm internal diameter, 5 µm particle size, HiQsil C18 HS, 4.6mm I.D. x 250mm (Make-KYA Technologies Corporation, Made in Japan) was used, with 86:14 mixtures of acetonitrile: water as isocratic mobile phase at a flow rate of 1 ml/min. The injection volume was 20 µl. HPLC was performed at ambient temperature and data were analysed on a computer equipped with ChromNav software.

Sample preparation

2g each of powdered drug (*P.tomentosa* root, *C.caudata* leaves & *M. arundinacea* rhizomes) was accurately weighed and extracted with 75ml of methanol using soxhlet apparatus for 3h. The extract was transferred to a 100-ml volumetric flask, and volume was made up using methanol.

Standard Preparation

Standard stock solution (1 mg/ml) was prepared using methanol. From the stock solution, standard solutions of six concentrations (range 10, 20, 40, 60 & 80 µg/ml) were prepared by appropriate dilution.

TLC analysis

Thin layer chromatography was performed as a preliminary study to qualitatively determine the constituents present in the plants. Analysis was done on pre-coated silica gel G60 F_{254} (E. Merck) using Petroleum ether: Ethyl acetate in ratio 8:2, Toluene : Acetone in ratio 9 : 1 and Petroleum ether : Ethyl acetate : Methanol in ratio 6 : 2 : 0.5 were used as mobile phase for betulin, *β*-sitosterol & *E*-

guggulsterone. Visualization was done by derivatizing using Anisaldehyde sulphuric acid reagent for betulin & *β*-sitosterol while for *E*-guggulsterone it was under UV 254 nm.

HPLC Analysis

The percentage content of betulin, *β*-sitosterol & *E*-guggulsterone was determined by HPLC method. Since there was no interference with that of the marker compound (Fig 1), this method can be used for separation, quantification and identification of steroids as well as triterpenoids.

RESULT AND DISCUSSION

The main objective of this method was to identify and quantify the phytoconstituents present in the above plants. The powder was extracted using methanol as solvent and used as sample solution for TLC. The sample solution showed the presence of alkaloids, tannins, steroids and triterpenoids. On further investigation using standard solutions, it was found that betulin is present in the roots of *P.tomentosa*, *E*-gugglsterone in leaves of *C. caudata* and *β*-sitosterol in rhizomes of *M.arundinacea*. Different combinations of methanol, acetonitrile and water were tried in variable ratios and finally Acetonitrile: Water in the ratio 86: 14 was found to be appropriate for all the three standards with an R_f of 5.5 min for *β*-sitosterol , 6 min for E- guggulsterone and 20 min for betulin.

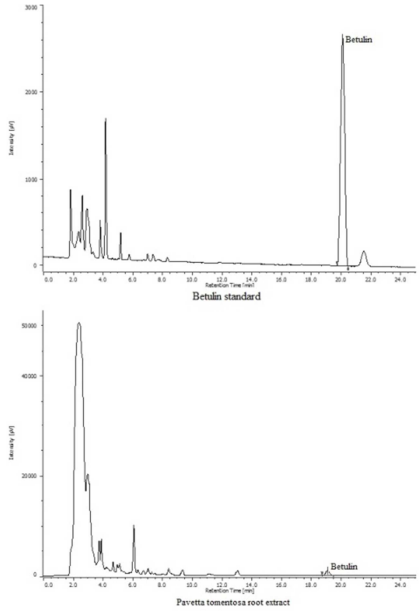

Fig. 2 HPLC chromatogram of standard betulin and *Pavetta tomentosa* root extract

The mobile phase showed no interference with other constituents hence was found to be suitable for estimation of steroids and triterpenoids.Since the compounds were having different λ_{max} i.e 210 nm for *β*-sitosterol & betulin while 242 nm for E-guggulsterone, HPLC analysis was done using PDA detector so that the same system can be used to quantify the steroid or triterpenoid compounds having different absorption.The percentage content of betulin was 0.028%, *E*-guggulsterone 0.059% while *β*-sitosterol ranges from 0.0016 %.

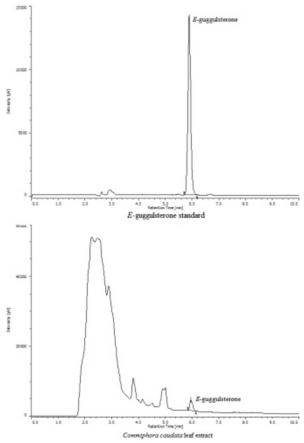

Fig. 3 HPLC chromatogram of standard *E*-gugglsterone and *Commiphora caudata* leaf extract

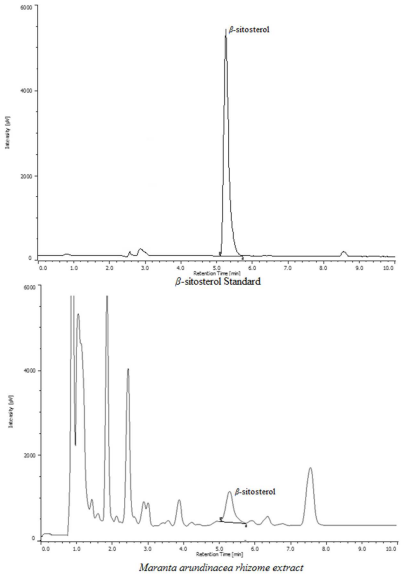

Fig. 4 HPLC chromatogram of standard *β*-sitosterol and *Maranta arundinacea* rhizome extract

CONCLUSION

A rapid isocratic HPLC method was used for estimation and quantification of betulin, *β*-sitosterol & *E*-guggulsterone in the above-mentioned plants. The developed HPLC method was found to be simple and specific; therefore, it can be further used for identification and standardization of plant material containing betulin, *β*-sitosterol & *E*-guggulsterone.

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Conflict of Interest
The authors declare no conflict of interest.

REFERENCES

1) The Wealth of India. Council of Scientific and Industrial Research, New Delhi (India), Volume VII (N-Pe), **1966**;282-283.
2) Banerjee, A.K. and Ghosh C: Isolation of D-mannitol from *Pavetta tomentosa*. Science and Culture **1956**; 22:114.

3) The Wealth of India. Council of Scientific and Industrial Research, New Delhi (India), Volume VI (L-M), **1962**;302-303.
4) Reviews on Indian Medicinal Plants. Medicinal Plants Unit Indian Council of Medical Research, New Delhi, Volume 7 (Cl-Co), **2008**;436.