DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR THE DETERMINATION OF β -BOSWELLIC ACID FROM BOSWELLIA SERRATA ROXB. (EXUDATE)

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ABSTRACT

A simple, rapid, selective and quantitative HPTLC method has been developed for determination of β-Boswellic acid in different samples of Boswellia serrata Roxb. exudate. The n-hexane extract of Boswellia serrata Roxb.(exudate) samples were applied on TLC Aluminium plate pre coated with Silica gel60 GF254 and developed using Toluene : Ethyl acetate: Formic acid (5:4.5:0.5) v/v as a mobile phase. The plate was sprayed (derivatized) with Anisaldehyde- Sulphuric Acid reagent followed by heating at 110°C for 10 minutes and detection and quantification were carried out densitometrically using an UV detector at wavelength of 530 nm. Content of marker compound in the samples were found similar.

Keywords: β-Boswellic acid, Boswellia serrata Roxb., Kundru exudate, Shallaki, HPTLC

INTRODUCTION

Boswellia serrata Roxb. Ex Colebr. Syn. Boswellia serrata var. glabra (Roxb) Bennett, Boswellia glabra Roxb. (Family- Burseraceae) is well known as Kundru or Shallaki and distributed in dry forests from Punjab to West Bengal and in Peninsular India. Common at the foot of Western Himalaya, in Rajasthan, Gujrat, Maharashtra, Madhya Pradesh, Bihār Orissa Andhra Pradesh and further south in the Peninsula. Reported to be threatened in North Eastern Region of India. It is a medium size to large sized, deciduous tree, upto 18 mm in height, an evergreen and spiny tree. Leaves are alternate, imparipinnate and crowded towards the end of branches; leaflets 17-31, opposite, sessile, ovate or ovate –lanceolate, crenate and pubescent.

Flowers are small and white, in axillary racemes or penicles. Drupes the fruits are about 1.2 cm long and trigonous, splitting into 3 valves and subtended by the woody disk. Seeds are compressed and pendulous. Flowering is during March-April and fruiting in winter season. The oleo-resin exudes during winter and gets deposited on the various parts of tree trunk. The oleo-resin is secreted in the schizogenous duct in the bark which are scattered just below the bast fibres.

The oleo-resin exudes as colourless semi-fluid liquid which gradually becomes whitish to golden yellow and solidifies slowly with time. Sometimes, it is reddish brown, greenish yellow or dull yellow to orange in colour. Oleo-gum resin exudates and bark are used in medicine. The bark is sweet, acrid, cooling and tonic. It is good for asthma, dysentery, ulcers, haemorrhoids and skin diseases. Gum-resin exudates obtained from the plant is sweet, bitter, astringent and commercially known as Indian oil balm or Indian frankincense or Sallai guggul.

It is used as antipruritic, expectorant, diuretic, eccholic, antiseptic, antisyneretic, diaphoretic, stomatchic and uterine tonic. It is a remedy for diarrhea, dysentery, ringworm, boils, fevers (antipruritic) skin and blood diseases, cardiovascular diseases, mouth sores, vaginal discharges, hair loss, jaundice, hemorrhoids, syphilitic diseases, irritable menses and to stimulate the liver. Modern medicine and pharmacology point to Boswellia serrata's use as a anti arthritic, anti-inflammatory, anti-hyperlipidemic (control blood lipids), antithrombotic (anti- coronary plaque), analgesic (pain-reliever) and hepatoprotective (protects the liver).

The gum resin contains a mixture of triterpene acids known as Boswellic acid (α,β,γ boswellic acid) acetyl-β boswellic acid, 11-keto-β boswellic acid, acetyl-11-keto- β boswellic acid and their derivatives. Volatile oil contains α-thujene, α-phellandrene, β-phellandrene, α-terpineol, d-limonene, myrcene, α-terpene, p-cymene. A diterpene alcohol serratol and four tetracyclic triterpene acids 3-α-acetoxytricolor, -8,24-dien-21-oic acid, 3-ketotricolor-8, 24-dien-21-oic acid, 3-α-hydroxytricolor-8,24-dien-oic acid, 3-β-hydroxytricolor-8,24-dien-21-oic acid.

It is also found to contain arabinose, rhamnose, glucose, galactose, fructose, idose, galacturonric acid and β sitosterol isolated from gum. Essential oil from gum gave phenol-ocresol, m-cresol, p-cresol, thymol, and carvacrol and carboxylic acid- α campholonic acid, 2,2,4-trimethylcyclopent-3-en-1-yl acetic acid and campholytic acid.

The research has implicated a beneficial role for the resin in the treatment osteoartharitis, soft tissue rheumatism, low back pain, gout and rheumatoid arthritis is a creeping crippling disease causing great physical suffering, it is possible to alleviate physical pain, increase movement (mobility) and prevent further tissue injury through proper treatment. Treatment with Boswellia serrata, on the other hand Boswellic acid significantly reduced the infiltration of leucocytes into the knee joint in turn significantly reducing inflammation causing immune white blood-cell response.

So that Boswellic acid is an active constituents and used as a marker. Literature survey reveals that the TLC and HPTLC methods are reported but no method as yet is reported for the determination of β-Boswellic acid in Boswellia serrata Roxb. exudates.

With increasing demand for herbal products in medicines and cosmetics there is an urgent need for standardization. So the aim of
the work is to develop a simple, rapid, selective and cost effective HPTLC method for the determination of β-Boswellic acid in *Boswellia serrata* exudate.

**MATERIAL AND METHOD**

**Plant material**

The Kundru exudate was procured from the Local Market, Ghaziabad. It was identified and authenticated by the Botanists of Pharmacopoeial Laboratory for Indian Medicine, Ghaziabad. One genuine sample also taken from the Museum of Pharmacopoeial Laboratory for Indian Medicine, Ghaziabad.

**Equipment**

A Camag (Switzerland) HPTLC system equipped with a sample applicator Linomat V, Twin trough glass Chamber (20x10 cm²) with SS lid, TLC Scanner III, Reprostar III and Wincats an integrated Software 4.02 (Switzerland), Rotavapour.

**Chemical & reagents**

Analytical grade; Alcohol, Toluene, ethyl acetate, Formic acid, Chloroform, Methanol, Anisaldehyde, Sulphuric acid and n-Hexane were used; obtained from S.D. Fine Chem. Ltd. (Mumbai, India). TLC Aluminium pre coated plate with Silica gel 60 GF₂₅₄ (20x10 cm²; 0.2 mm thick) used were obtained from E. Merck Ltd. (Mumbai, India). Reference standard- β-Boswellic acid procured from Natural Remedies Pvt. Ltd, Bangalore, India.

**Sample & standard preparation**

1g of coarsely powdered drug samples were extracted with 10 ml n-Hexane for 24 hours by cold extraction method. The extracts were filtered by Whatmann filter paper and make up to 10 ml in a volumetric flask.

**Standard preparation**

5mg of standard β-Boswellic acid dissolved in 3ml of n-Hexane and made up to 5ml in standard volumetric flask.

**H.P.T.L.C (High Performance Thin Layer Chromatography)**

TLC Aluminium pre coated plate with Silica gel60 GF₂₅₄ (20x10 cm²; 0.2 mm thick) was used with Toluene : Ethyl acetate: Formic acid (5:4.5:0.5) V/V as mobile phase. n-Hexane extract of samples and β-Boswellic acid standard solution applied on plate by using Linomat V applicator. Camag Twin Trough Glass Chamber (20x10 cm²) with SS lid was used for development of TLC plate.

The Twin Trough Glass Chamber was saturated with mobile phase for 30 minutes. TLC plate was developed to 8 cm distance above the position of the sample application. The plate was removed from the chamber and air dried at room temperature.

This plate was sprayed (derivatized) with Anisaldehyde- Sulphuric Acid reagent followed by heating at 110°C for 10 minutes and HPTLC finger print profile was snapped by Camag Reprostar III, before derivatization under UV Light 254 nm, 366 nm and after derivatization (Fig.1). The plate was derivatized with Anisaldehyde-Sulphuric Acid reagent followed by heating at 110°C for 10 minutes and scanned immediately using Camag TLC Scanner III at wavelength 530nm.

Wincats an integrated Software 4.02 was used for the detection as well as for the evaluation of data. It was observed that β-Boswellic acid appeared at Rᵣ 0.84 (dark violet colour). The peaks, graph and spectra obtained were given in Fig.2 and 3 and Rs values, colour of bands (Table No.1), quantity of β-Boswellic acid, linearity, standard deviation & regression coefficient found via graph (Table No. 2) and calculated quantity of β-Boswellic acid & % recovery were given in Table No. 3.

**RESULTS AND DISCUSSION**

Of the various mobile phases tried, the mobile phase containing Toluene : Ethyl acetate: Formic acid (5:4.5:0.5) V/V and the active principle β-Boswellic acid resolved as a dark violet colour band at Rᵣ 0.84 very efficiently from the other components in n-hexane extract of *Boswellia serrara* Roxb. (exudates) (Fig.1).

Sharp peaks of β-Boswellic acid (Standard and samples) were obtained when the plate was scanned at wavelength 530nm (Fig.2). Quantity of β-Boswellic acid found in samples were obtained automatically (Table No. 2) via graph (Fig.3) and % β-Boswellic acid found in samples and % recovery were calculated (Table No.3).

Quantity of β-Boswellic acid found in Local Market Sample, Ghaziabad (U.P.) is 1.5678mg in 1g drug sample (0.15678% w/w) and quantity of β-Boswellic acid found in Museum Sample of PLIM, Ghaziabad is 1.7100 mg in 1g drug sample (0.17100% w/w). The % recovery of β-Boswellic acid in Local Market Sample, Ghaziabad (U.P.) is 98.10% w/w and 97.05% w/w in Museum Sample of PLIM, Ghaziabad (U.P.). The mean % recovery was 97.58%.

The accuracy and reproducibility of the method was established by means of recovery experiment. The mean recovery was close to 100% which indicates the accuracy of the method.

The robustness of the method was studied, during method development, by determining the effect of small variation, of mobile phase composition (±2%), chamber saturation period, development distance, derivatization time, and scanning time (10% variation of each). No significant change of Rᵣ or response to β-Boswellic acid was observed, indicating the robustness of the method.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Sample from</th>
<th>Local market sample, ghaziabad</th>
<th>Museum sample of plim, ghaziabad</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Quantity of</td>
<td>1.5678mg</td>
<td>1.7100 mg</td>
</tr>
<tr>
<td>2</td>
<td>% β-Boswellic acid</td>
<td>0.15678% w/w</td>
<td>0.17100% w/w</td>
</tr>
<tr>
<td>3</td>
<td>% Recovery</td>
<td>98.10% w/w</td>
<td>97.05% w/w</td>
</tr>
</tbody>
</table>
Table 1

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Detection/visualization</th>
<th>Kundru exudate (Track No. T1, T2, T3 and T4)</th>
<th>Standard-β-Boswellic acid (Track No. S1, S2 and S3)</th>
<th>Rf values</th>
<th>Colour of band</th>
<th>Rf values</th>
<th>Colour of band</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Under UV 254 nm</td>
<td>0.52 dark grey</td>
<td>0.56 dark grey</td>
<td>0.56</td>
<td>dark grey</td>
<td>0.69</td>
<td>dark grey</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.69 dark grey</td>
<td>0.76 dark grey</td>
<td></td>
<td></td>
<td>0.89</td>
<td>dark grey</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.76 dark grey</td>
<td>-</td>
<td>No significant ban</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Under UV 366 nm</td>
<td>0.52 blue</td>
<td>0.69 blue</td>
<td>0.69</td>
<td>bright sky blue</td>
<td>-</td>
<td>No significant ban</td>
</tr>
<tr>
<td>3.</td>
<td>After derivatization</td>
<td>0.13 light violet</td>
<td>0.27 light violet</td>
<td>0.27</td>
<td>dark violet</td>
<td>0.34</td>
<td>dark violet</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.34 dark violet</td>
<td>0.40 violet</td>
<td>0.40</td>
<td>violet</td>
<td>0.52</td>
<td>violet</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.52 violet</td>
<td>0.62 dark violet</td>
<td>0.62</td>
<td>dark violet</td>
<td>0.69</td>
<td>dark violet</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.69 dark violet</td>
<td>0.76 dark brown</td>
<td>0.76</td>
<td>dark brown</td>
<td>0.81</td>
<td>dark brown</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.81 dark brown</td>
<td>0.89 dark brown</td>
<td>0.89</td>
<td>dark brown</td>
<td></td>
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</table>

Table 2

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Track No.</th>
<th>Volume applied on plate</th>
<th>Quantity applied on plate</th>
<th>Quantity of β-Boswellic acid via graph</th>
<th>Linearity &amp; Regression Coefficient and Standard deviation via graph</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>T1</td>
<td>9μl</td>
<td>900μg</td>
<td>1.424μg</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>T2</td>
<td>9μl</td>
<td>900μg</td>
<td>1.562μg</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>S1</td>
<td>10μl</td>
<td>10μg</td>
<td>10.000μg</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>S2</td>
<td>5μl</td>
<td>5μg</td>
<td>5.000μg</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>S3</td>
<td>1μl</td>
<td>1μg</td>
<td>1.000μg</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>T3</td>
<td>(9+1)μl</td>
<td>900μg+1μg</td>
<td>2.397μg -1μg = 1.397μg</td>
<td>Y = -717.263 + 54.644 * X + -0.027 * X^2 ( r = 0.99999 ) sdv = 0.00%</td>
</tr>
<tr>
<td>7.</td>
<td>T4</td>
<td>(9+1)μl</td>
<td>900μg+1μg</td>
<td>2.516μg -1μg = 1.516μg</td>
<td></td>
</tr>
</tbody>
</table>

T1- n-Hexane extract of Local Market sample, Ghaziabad
T2- n-Hexane extract of Museum Sample of PLIM, Ghaziabad
S1- β-Boswellic acid standard solution (1mg/ml), S2- β-Boswellic acid standard solution (1mg/ml)
S3- β-Boswellic acid standard solution (1mg/ml), T3- n-Hexane extract (spiked with std. solution) of Local Market Sample, Ghaziabad
T4- n-Hexane extract (spiked with std. solution) of Museum Sample of PLIM, Ghaziabad
Fig. 1: T.L.C. Finger print of *Boswellia serrara* Roxb. (Exudate)

Peak of Boswellic Acid (@530nm)

Fig. 2: Peaks of *Boswellia serrara* Roxb. (Exudate) in all Tracks

Peaks of *Boswellia serrata* Extract (@530nm)
CONCLUSION
The proposed HPTLC method is simple, rapid, accurate, reproducible, selective and economic and can be used for routine quality control analysis of *Boswellia serrara* Roxb. (exudate) powder and quantitative determination of β-Boswellic acid in exudate powder.

REFERENCES
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