



## PHYSICO-CHEMICAL STANDARDISATION AND DEVELOPMENT OF HPTLC METHOD FOR THE DETERMINATION OF $\beta$ -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  $\beta$ -Boswellic acid in *Boswellia serrata* Roxb. exudates and its formulation. The n-hexane extract of *Boswellia serrata* Roxb. (exudate) samples were applied on TLC Aluminium plate pre coated with Silica gel60 GF<sub>254</sub> 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:**  $\beta$ -Boswellic acid, *Boswellia serrata* Roxb., Kundru exudate, Shallaki, HPTLC, Quantification, BACFO, Ayurvedic Formulations, Family-Burseraceae.

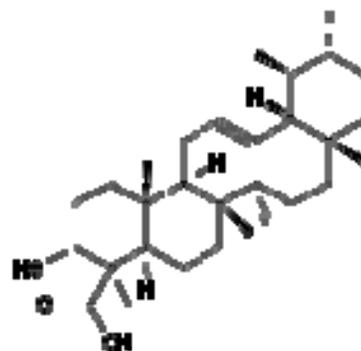
### INTRODUCTION

*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, Maharastra, Madhya Pradesh, Bihar 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 m in height, an evergreen and spiny tree. The oleo-resin exudates out 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 oilbanum or Indian frankincense or Sallai guggul. It is used as antipyretic, expectorant, diuretic, ebolic, antiseptic, antidiarrhetic, diaphoretic, stomachic and urethrorrhea, orchioopathy, bronchitis, asthma, cough, skin diseases, ulcers, tumours, cystic breast, chronic laryngitis, jaundice and arthritis. It shows anti-inflammatory and anti-arthritis activity have been mainly attributed to a component in the resin containing  $\beta$ -Boswellic acid and is used for rheumatic patients.<sup>1-9</sup>

In addition to use for arthritis this gummy resin is also mentioned in traditional Ayurvedic texts as a remedy for diarrhea, dysentery, ringworm, boils, fevers (antipyretic) skin and blood diseases, cardiovascular diseases, mouth sores, vaginal discharges, hair loss, jaundice, hemorrhoids, syphilitic diseases, irregular menses and to stimulate the liver. Modern medicine and pharmacology point to *Boswellia serrata*'s use as an anti arthritic, anti-inflammatory, anti-hyperlipidemic (control blood lipids), antiatherosclerotic (anti-coronary plaque), analgesic (pain-reliever) and hepoprotective (protects the liver).<sup>10-14</sup>

The gum resin contains a mixture of triterpene acids known as Boswellic acid ( $\alpha, \beta$  boswellic acid) acetyl-  $\beta$  boswellic acid, 11-keto-  $\beta$ -boswellic acid, acetyl-11-keto-  $\beta$ -boswellic acid and their derivatives.<sup>10</sup> Volatile oil contains  $\alpha$ -thujene,  $\alpha$ -phellandrene,  $\beta$ -phellandrene,  $\alpha$ -terpineol, d-limonene, myrcene,  $\alpha$ -terpene, *p*-cymene<sup>11</sup>; a diterpene alcohol serratol and four tetracyclic triterpene acids 3-  $\alpha$ - acetoxytirucall -8,24-dien-21-oic acid, 3-ketotirucall-8, 24-dien-21-oic acid, 3-  $\alpha$ - hydroxytirucall -8,24-dien-

oic acid, 3-  $\beta$ - hydroxytirucall -8,24-dien-21-oic acid.<sup>1-14</sup>. It is also found to contain arabinose, rhamnose, glucose, galactose, fructose, idose, galacturonic acid and  $\beta$  sitosterol isolated from gum<sup>12-16</sup>. Essential oil from gum gave phenol-o-cresol, m-cresol, p-cresol, thymol, and carvacrol and carboxylic acid-  $\alpha$ -campholenic acid, 2,2,4-trimethylcyclopent-3-en-1-yl acetic acid and campholytic acid<sup>2</sup>.



Structure of  $\beta$ -boswellic acid

The research has implicated a beneficial role for the resin in the treatment osteoarthritis, 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<sup>16</sup> "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  $\beta$ -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  $\beta$ -Boswellic acid in *Boswellia serrata* exudate.

### MATERIAL AND METHOD

#### Material

(i) Kundru exudate was procured from the Local Market, Ghaziabad.

(ii) An herbal product **Shallaki (*Boswellia serrata*) Capsules**, B. No. BCP-289 containing *Boswellia serrata* exudate was procured from the Local Market, Ghaziabad.

#### Label claimed

Each capsules contains: *Boswellia serrata* (Shallaki)-250mg,

Excipients: Gum Acacia, Purified Talc, Potato starch

No artificial

**Organoleptic Characters:** Colour – White; Smell - Slightly pungent; Touch powdery; Taste – slightly acid.

#### Determination of physico-chemical constants

The following Physico-chemical constants has been analysed and result given in Table No. 1

#### H.P.T.L.C.- (High Performance Thin Layer Chromatography) equipment

A Cammag (Switzerland) HPTLC system equipped with a sample applicator Linomat V, Twin trough glass Chamber (20x10 cm<sup>2</sup>) with SS lid, TLC Scanner III, Reprostar III and Wincats an integrated Software 4.02 (Switzerland), Rotavapour.

#### Chemical & reagents

Analytical grade; Toluene, ethyl acetate, Formic acid, Chloroform, Methanol, Anisaldehyde, Alcohol, 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 GF<sub>254</sub> (20x10 cm<sup>2</sup>; 0.2 mm thick) used were obtained from E. Merck Ltd. (Mumbai, India). Reference standard-  $\beta$ -Boswellic acid procured from Natural Remedies Pvt. Ltd., Bangalore, India.

#### Sample & standard preparation

**Sample 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  $\beta$ -Boswellic acid dissolved in 3ml of n-hexane and made up to 5ml in standard volumetric flask.

#### Chromatography

TLC Aluminium pre coated plate with Silica gel60 GF<sub>254</sub> (20x10 cm<sup>2</sup>; 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  $\beta$ -Boswellic acid standard solution applied on plate by using Linomat V applicator. Cammag Twin Trough Glass Chamber (20x10 cm<sup>2</sup>) 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 Cammag Reprostar III, before derivatization under UV 254 nm, 366 nm and after derivatization (Fig.1). The derivatized plate was 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.

#### Linearity of detector response and assay

In order to establish linearity, standard solution of  $\beta$ -Boswellic acid (1mg/ml) applied on TLC Aluminium pre coated plate with Silica gel60 GF<sub>254</sub> (20X10 cm<sup>2</sup>; 0.2 mm thick), 6 $\mu$ l, 4 $\mu$ l, 2 $\mu$ l on Track No. S1, S2 & S3 respectively and for assay, 9 $\mu$ l of n-hexane extract of both samples applied on Track No. T1 & T2. TLC plates was developed to 8 cm distance above the position of the sample application and removed from the chamber and air dried at room temperature.

This HPTLC finger print profile was snapped by Cammag 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  $\beta$ -Boswellic acid appeared at R<sub>f</sub> 0.62 (dark violet colour). The peaks, graph and spectra obtained were given in Fig. 2 and 3 and R<sub>f</sub> values, colour of bands (Table No. 2), quantity of  $\beta$ -Boswellic acid, linearity, standard deviation & regression coefficient found via graph (Table No. 3) and calculated quantity of  $\beta$ -Boswellic acid was given in Table No. 4.

**Table 2: TLC details of n-hexane extract of *Boswellia serrata* Roxb (exudate)**

Sr. No.	Detection/ visualization	BACFO capsule , Kundru exudate (Track No. T1 and T2)		Standard- Boswellic acid (Track No. S1, S2 and S3)	
		R <sub>f</sub> values	Colour of band	R <sub>f</sub> values	Colour of band
1.	Under UV 254 nm	0.52	dark grey	-	No significant band
		0.56	dark grey		
		0.69	dark grey		
		0.76	dark grey		
		0.89	dark grey		
2.	Under UV 366 nm	0.52	blue	-	No significant band
		0.69	blue		
		0.89	bright sky blue		
3.	After derivatization	0.13	light violet	0.62	dark violet
		0.20	light violet		
		0.27	light violet		
		0.34	dark violet		
		0.40	violet		
		0.52	violet		
		0.62	dark violet		
		0.69	dark violet		
		0.76	dark brown		
		0.81	dark brown		
0.89	dark brown				

**Table 1: Physico-chemical standardization of *Boswellia serrata* Roxb (Exudate)**

S. No.	Name of physico-chemical constants	BACFO capsule Local market, Ghaziabad	Kundru exudate Local market, Ghaziabad
1.	Moisture content	2.77% w/w	3.12% w/w
2.	pH (of 5% aq. Solution)	6.79	6.72
3.	Total ash	2.09% w/w	2.78% w/w
4.	Acid in-soluble ash	0.29% w/w	1.01% w/w
5.	Water soluble ash	0.23% w/w	0.67% w/w
6.	Water soluble extractives	27.51% w/w	28.91% w/w
7.	Ethanol soluble extractives	46.44% w/w	48.12% w/w
8.	Chloroform soluble extractives	8.02% w/w	7.63% w/w
9.	Hexane soluble extractives	34.67% w/w	33.21% w/w
10.	Volatile Oil	12.20%w/v	11.87%w/v

**Table 3: Quantity applied on plate and values found via graph**

Sr. No.	Track No.	Volume applied on plate	Quantity applied on plate	Quantity of $\beta$ -Boswellic acid via graph	Linearity & regression coefficient and standard deviation via graph
1.	T1	9 $\mu$ l	1800 $\mu$ g	2.117 $\mu$ g	$Y = 4717.263 + 54.644 * X + -0.027 * X^2$ $r = 0.99999 \quad s_{dv} = 0.00\%$
2.	T2	9 $\mu$ l	1800 $\mu$ g	2.718 $\mu$ g	
3.	S1	6 $\mu$ l	6 $\mu$ g	6.000 $\mu$ g	
4.	S2	4 $\mu$ l	4 $\mu$ g	4.000 $\mu$ g	
5.	S3	2 $\mu$ l	2 $\mu$ g	2.000 $\mu$ g	

T1- n-Hexane extract of BACFO Capsule, Local Market sample, Ghaziabad  
 T2- n-Hexane extract of Kundru Exudate, Local Market sample, Ghaziabad  
 S1-  $\beta$ -Boswellic acid standard solution  
 S2-  $\beta$ -Boswellic acid standard solution  
 S3-  $\beta$ -Boswellic acid standard solution

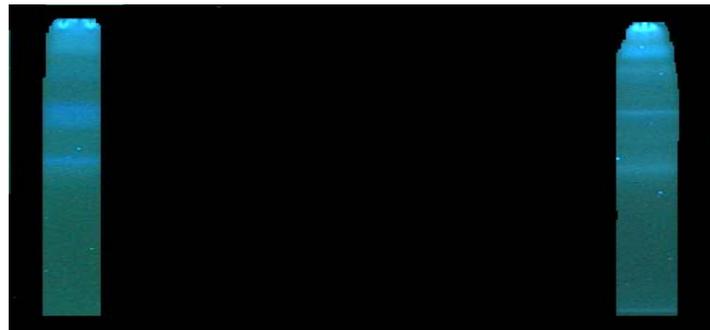
**Table 4: Summary of results**

Sr. No. ↓	Sample from →	BACFO capsule	Local market sample, Ghaziabad
1.	Quantity of $\beta$ -Boswellic acid in 1g	1.1761mg	1.5100mg
2.	% $\beta$ -Boswellic acid	0.11761 % w/w	0.15100 % w/w



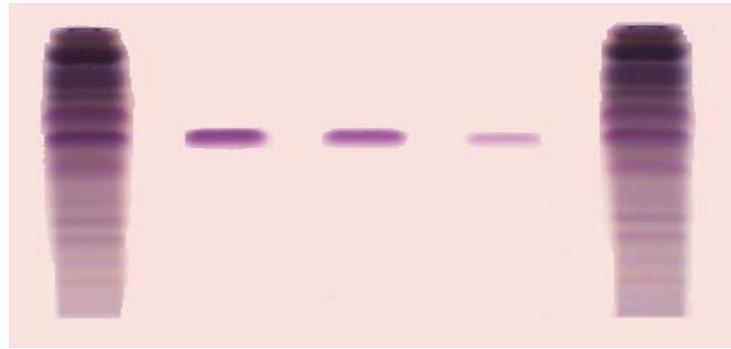
T1                      S1      S2      S3                      T2

UV-254 nm



T1                      S1      S2      S3                      T2

UV-366 nm



T1 S1 S2 S3 T2  
After Derivatization

Fig. 1: H.P.T.L.C. Finger print of BACFO Capsule

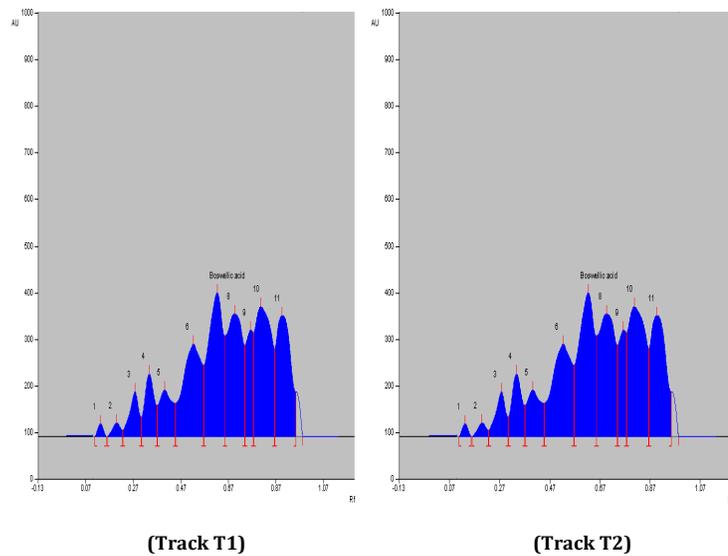
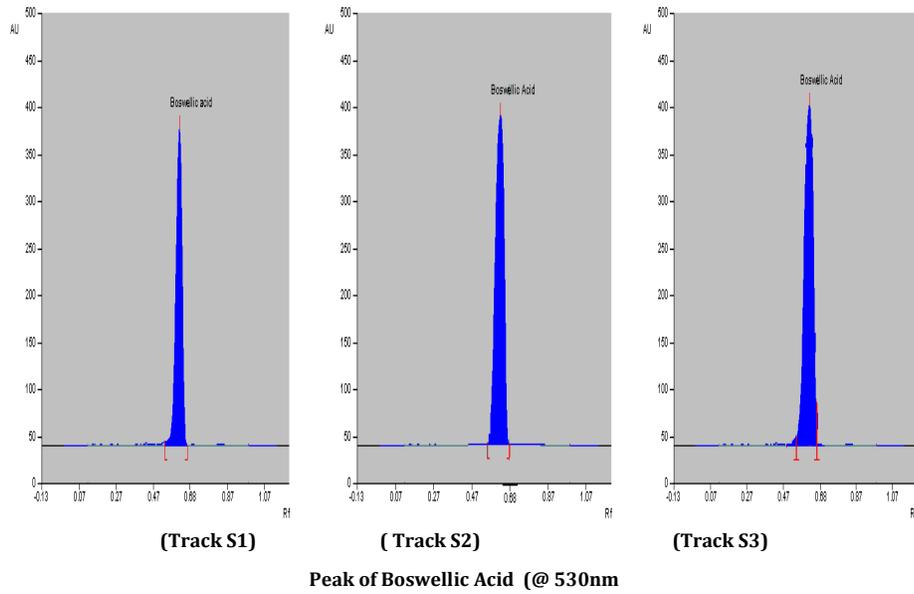
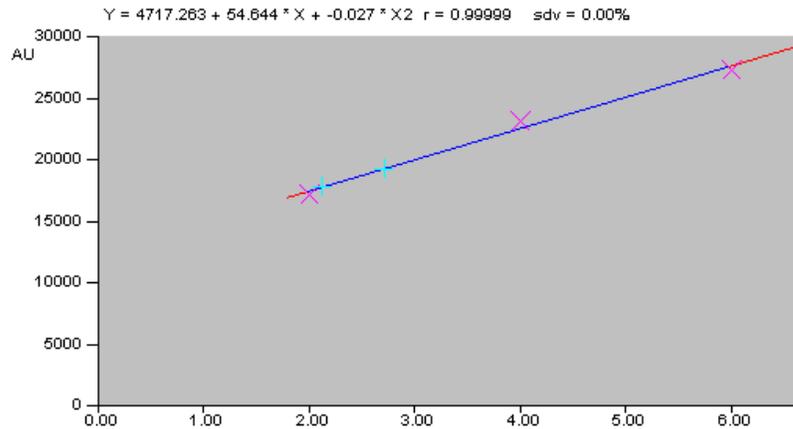
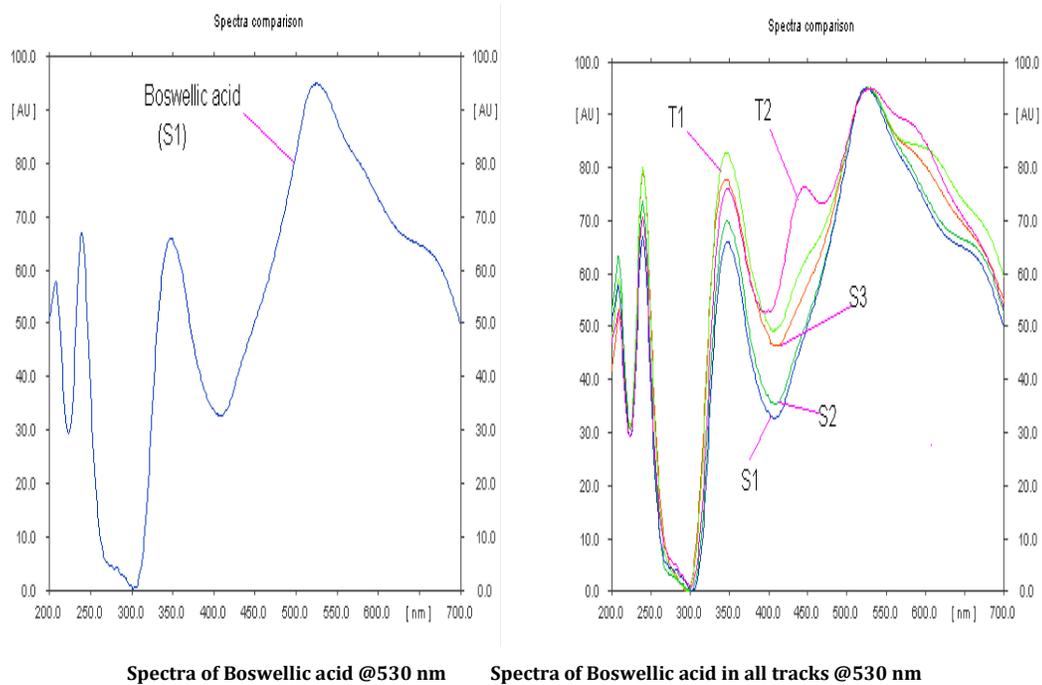


Fig. 2: Peaks of BACFO (SHALLAKI) and *Boswellia serrata* in all Tracks



Area  
Graph area vs AU



Spectra of Boswellic acid @530 nm

Spectra of Boswellic acid in all tracks @530 nm

Fig. 3: Graph and spectra of *Boswellia serrata* Roxb. (Exudate)

## 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  $\beta$ -Boswellic acid resolved as a dark violet colour band at  $R_f$  0.62 very efficiently from the other components in n-hexane extract of *Boswellia serrata* Linn. (exudates) and BACFO Capsule (Fig. 1). Sharp peaks of  $\beta$ -Boswellic acid (Standard and samples) were obtained when the plate was scanned at wavelength 530nm (Fig. 2). Quantity of  $\beta$ -Boswellic acid found in samples were obtained automatically (Table No. 3) via graph (Fig. 3) and %  $\beta$ -Boswellic acid found in samples (Table No. 4).

Quantity of  $\beta$ -Boswellic acid found in BACFO Capsule is 1.1761mg in 1g drug sample (0.11761 % w/w) and quantity of  $\beta$ -Boswellic acid found in Local Market Sample, Ghaziabad (U.P.) is 1.5100mg in 1g drug sample (0.15100 % w/w).

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

## CONCLUSION

The proposed HPTLC method is simple, rapid, accurate, reproducible, selective and economic and can be used for routine quality control analysis of *Boswellia serrata* Linn. (exudate) powder and quantitative determination of  $\beta$ -Boswellic acid in compound formulations.

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