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Original Article

Comparative Powder Microscopic and HPTLC Studies on Stem barks of *Symplocos racemosa* Roxb. and *Symplocos crataegoides* Ham.

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ABSTRACT

Introduction: Comparative powder microscopic and HPTLC studies were carried out on stem barks of *Symplocos racemosa* Roxb. and *Symplocos crataegoides* Ham. to differentiate its identity in Ayurvedic formulations. **Method:** Powder microscopic and HPTLC studies of these barks were undertaken on a comparative basis and results are reported in this paper. The authentic samples are cleaned, powdered and passed through sieve No. 80. A few mg of powder was stained with saffranin, Toluidine blue and Iodine and photographed under different magnifications with the help of NICKON Labphot -2. HPTLC studies were followed by Sethi and Wagner *et al* method. **Results:** The colour, taste, cork cells, fibres, sclereids, starch grains, calcium oxalate crystals, number of spots and Rf values of HPTLC are found to be differentiating diagnostic characters in powdered form of *S. racemosa* and *S. crataegoides*. **Conclusion:** The findings of the present study is believed to be helpful in standardization of Ayurvedic formulation containing stem bark of *S. racemosa* and *S. crataegoides* as ingredients in powder form. The study sets the specific microscopic protocol and HPTLC finger print of the two drugs and may lead to global acceptance and reputation of the Ayurvedic system.

Key words: Authentication, *Curna*, *Dasamularistam*, *Lodhra*, *Lodhrasavam*, Pharmacognosy.

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INTRODUCTION

The stem barks of the plants *Symplocos racemosa* Roxb. and *Symplocos crataegoides* Ham. (Fam. Symplocaceae) is extensively used in Indian medicine under the names of *Lodhra/Patikalodhra*. Mainly it is used to cure uterine complaints, vaginal and menstrual disorders. In Sanskrit the name *Lodhra* means that it stops ocular discharges. The important preparations using the drugs are *Nyagrodhadi Kvatha curna*, *Nyagrodhadi curna*, *Lodhrasavam*, *Dasamularistam* etc.[1-7] Most of the books on Indian Materia Medica equate *Symplocos racemosa* as well as *S. crataegoides* Ham., as the botanical source of *Lodhrah* or *Rodhrah*. Two varieties of *Lodhra* are described in the texts viz. *Sabara lodhra* and *Patika lodhra*. *Sabara lodhra* is equated with *Symplocos racemosa* and *Patika lodhra* is equated with *Symplocos crataegoides*.[8] The alcoholic extract of stem bark of *Symplocos racemosa* contains phenolic glycoside (benzoylsalireposide, salireposide), symplocuronic acid, sympocemoside, salirepin, β-amyrin, oleanolic acid and β-sitosterol.[9] Symposide(I) a new flavan-glycoside showing anti-fibrinolytic activity, was isolated from the stem bark of *S. racemosa* and characterized as (-)-epiafzelechin-7-β-D-glucopyranoside.[10]

The following compounds isolated from the ethanolic extract of the stem bark of *Symplocos crataegoides* (Syn: *S. paniculata*) 4-(8-hydroxyethyl) cyclohexan-1-oic acid(1); androst-5(6)-ene 17-one 3β-O-(β-D-glucopyranoside) (2); 9β,25-cyclo 3β-O-(β-D glucopyranosyl)-echynocystic acid (3); 9β,19-cyclo 24-methylcholan-5,22-diene 3β-O-(β-D-glucopyranosyl (1-6)α-D-rhamnopyranoside) (4); 30-Et 2α, 16α-dihydroxy 3β-O- (β-D-glucopyranosyl) hopan-24-oic acid (5); 32,33,34-trimethyl-bacteriohopan-16-ene 3-O-β-D-glucopyranoside (6); and flavones 3',4',5',6'-tetramethoxy 7-O-β-D-glucopyranosyl (1-3) β-D-glucopyranoside (7).[11]

Botanically *S. racemosa* and *S. crataegoides* are different species called by similar vernacular name *Lodhrah*. This leads to confusion in identifying the correct botanical source of the drug. For this purpose, Powder microscopic studies and HPTLC finger printing of these barks have been undertaken on a comparative basis.

MATERIALS AND METHODS

Collection and Identification

The stem bark of *Symplocos racemosa* Roxb. was purchased locally from market and authenticated by Prof. *P. jayaraman*, Director, Plant Anatomy Research Centre, Chennai. The authentic stem bark of *Symplocos crataegoides* Ham. was supplied by Dr. G. C. Joshi, Research Officer, Regional Research Institute of Himalayan Flora, CCRAS, Thapala, Uttarakhand. The photos of the medicinal plants and its barks are given in Figure 1. The specimen vouchers of the stem barks of *S. racemosa* and *S. crataegoides* were deposited in CSMRIASDD Museum (K219/SB22 and C222/BSB26).

Powder microscopic studies

The samples were cleaned, shade dried, powdered and passed through sieve No. 80. A few mg of powder was analyzed microscopically after clearing them in Chloral hydrate solution. A few mg of powder was

Table 1: Powder microscopic study of stem barks of *S. racemosa* and *S. crataegoides*

Parameter	*Symplocos racemosa* Roxb.	*Symplocos crataegoides* Ham.
Colour, odour, taste and texture	Yellowish brown; characteristic; slightly astringent; soft in texture.	Light brown; characteristic; initially slightly sour and after slightly bitter; soft in texture.
Cork cells	Polygonal comparatively larger in size 70 to 110 µm, slightly wavy anticlinal walls.	Polygonal smaller in size 50 to 70 µm straight anticlinal walls filled with brownish content.
Fibres	Long, narrow lumen, thick walled, lignified and blunt on one end, a few crystal fibres.	Libriform fibres with tertiary thickening are common, long, very narrow lumen, cylindrical, thick walled, lignified and tapering on both ends.
Sclereids	Brachysclereids, some sclereids rectangular, lignified with thick spiny outgrowth and sclerotic phellem cells or phelloids, rectangular and tabular in shape with simple pits in the lumen, 320 µm long and 130 µm wide.	Polyhedral, more or less iso diametric sclereids (Brachysclereids), thick walled, lignified 80 to 100 µm in size.
Starch grains	Numerous, simple as well as compound, having 2 to 6 components, Y shaped hilum in centre, measuring 4 to 13 µm in diameter.	Simple, round to oval, measuring 4 to 25 µm in diameter.
Crystals	Abundant Prismatic crystals of calcium oxalate.	Scarce Prismatic crystals of calcium oxalate.

Table 2: *Rf* values of chloroform and alcohol extracts

Types of lights with wavelength	Chloroform extracts [Mobile phase: Toluene: Ethyl acetate (8:2)]		Alcohol extracts [Mobile Phase: Toluene: Ethyl acetate: Formic acid (7 :3:0.1)]	
	S. racemosa	*S. crataegoides*	*S. racemosa*	*S. crataegoides*
UV-254 nm	0.77 Green	0.92 Green	0.32 Green	0.89 Green
	0.63 Green	0.17 Green		0.32 Green
	0.17 Green			
UV-366 nm	0.62 Blue	0.79 Red	0.92 Fluorescent Blue	0.92 Fluorescent Blue
	0.40 Blue	0.64 Fluorescent blue	0.83 Blue	0.83 Blue
	0.20 Blue	0.43 Red	0.77 Blue	0.77 Fluorescent Blue
	0.15 Fluorescent blue	0.37 Blue	0.31 Blue	0.31 Blue
	0.11 Fluorescent blue	0.25 Blue		
		0.20 Blue		
		0.11 Blue		
Visible Light (after derivatisation vanillin–sulphuric acid reagent)	0.63 Grey	0.78 Grey		0.90 Grey
	0.46 Grey	0.65 Violet		
	0.37 Grey	0.50 Grey		
		0.45 Grey		
		0.37 Violet		
		0.13 Grey		

stained with saffranin, Toluidine blue and Iodine as per the procedures[12] and photographed under different magnifications with the help of NICKON Labphot -2 microscopic unit.

Preparation of extracts for TLC/HPTLC studies

5 g of drug of each sample were shade dried and coarsely powdered and defatted with hexane. The plant materials were packed in a Soxhlet apparatus and extracted successively with chloroform and ethanol for 5 hrs separately. The extracts were filtered by using Whatmann No.1 filter paper. The extracts were concentrated on water bath and made up to 10 ml volumetric flask.

Method for developing TLC/HPTLC

Instrument: CAMAG (Switzerland), sample applicator: Camag Linomat-IV applicator with N_2 gas flow, photo documentation system: Digi store-2 documentation system with win cats and video scan software, scanner: Camag HPTLC scanner-3 (030618), win cats-IV, development chamber: Camag HPTLC 10×10, 10×20 twin trough linear development chamber, quantity applied: 10 µl for extracts and 4 µl for standards, stationary phase: Aluminium plate precoated with silica gel 60 F_{254} (E. Merck), plate thickness: 0.2 mm, scanning wavelength: 254 nm, laboratory condition: 20 ± 5°C and 53% relative humidity. The chloroform and alcohol extract

Figure 1: *S. racemosa and S. crataegoides*-Twig with flowers and stem barks.

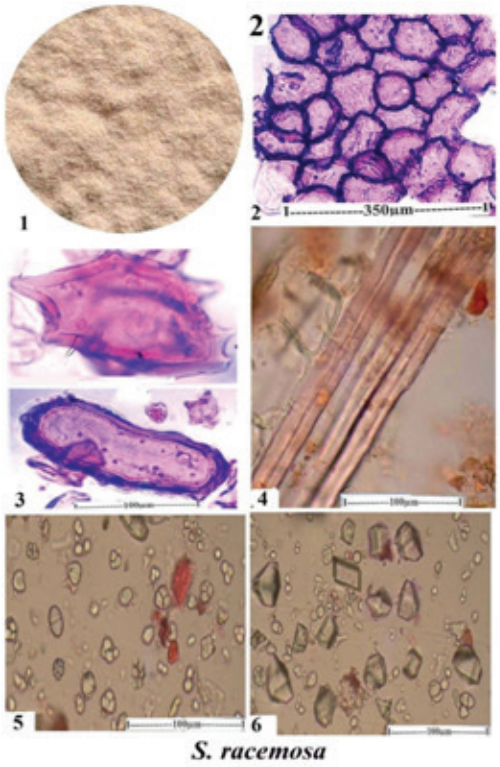

Figure 2: *Symplocos racemosa*.

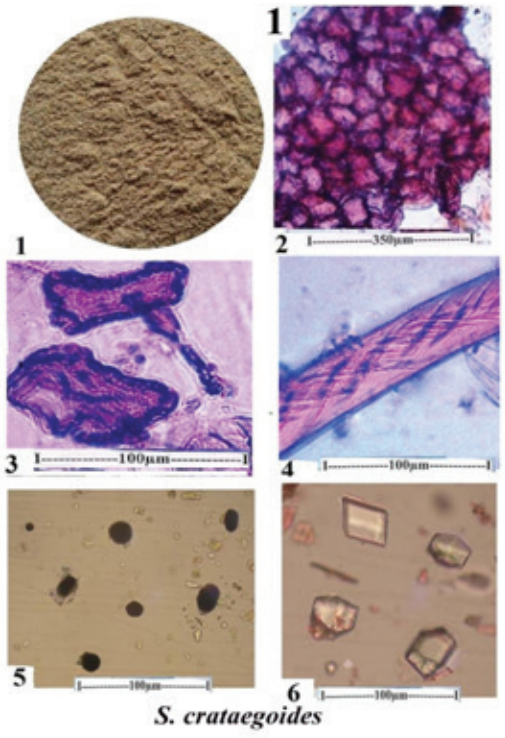

Figure 3: *Symplocos crataegoides*

Symplocos racemosa
1. Powder
2. Cork cells in surface view
3. Sclereids
4. Fibres
5. Starch grains (simple & compound)
6. Prismatic crystals of calcium oxalate (abundant).

Symplocos crataegoides
1. Powder
2. Cork cells in surface view
3. Sclereids
4. Libriform fibre (Tertiary thickening)
5. Starch grains (simple)
6. Prismatic crystals of calcium oxalate (scarce).

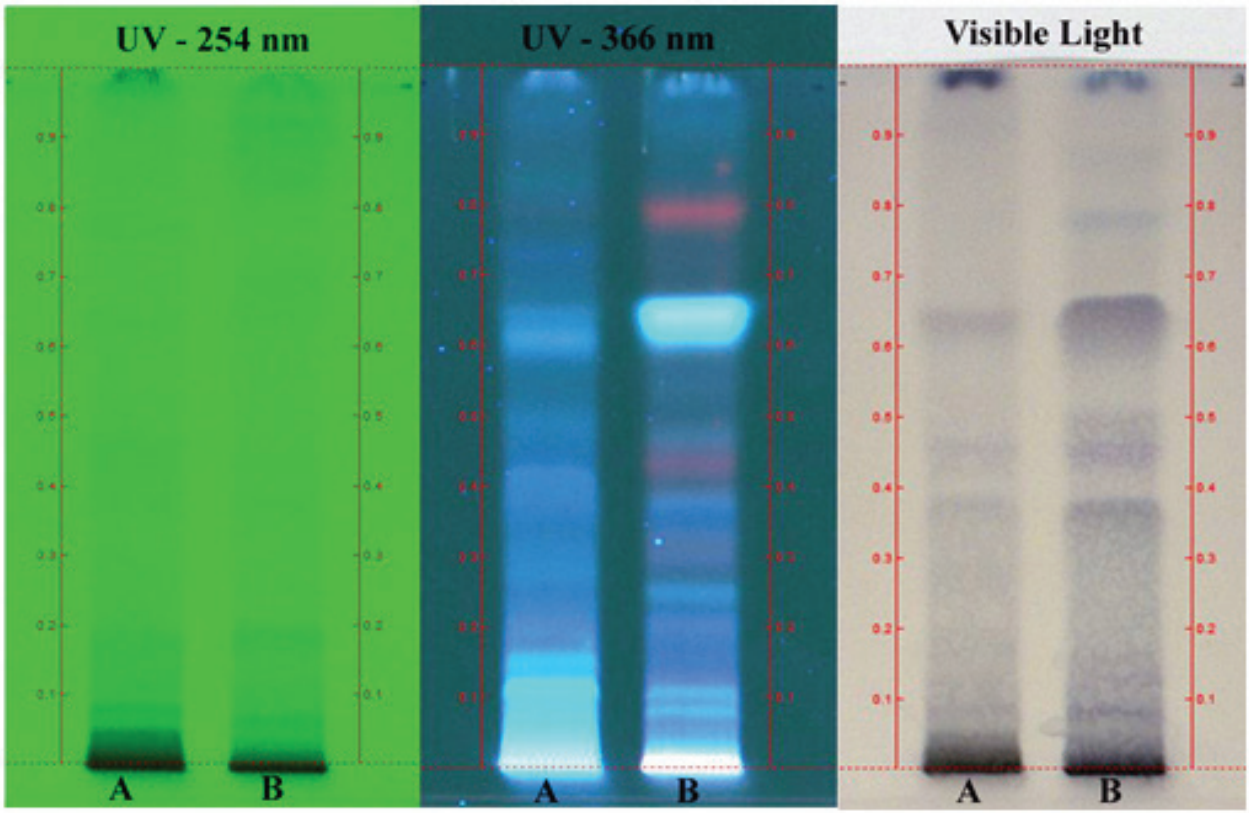

Figure 4: TLC profile of chloroform extracts of *S. racemosa* (A) and *S. crataegoides* (B).

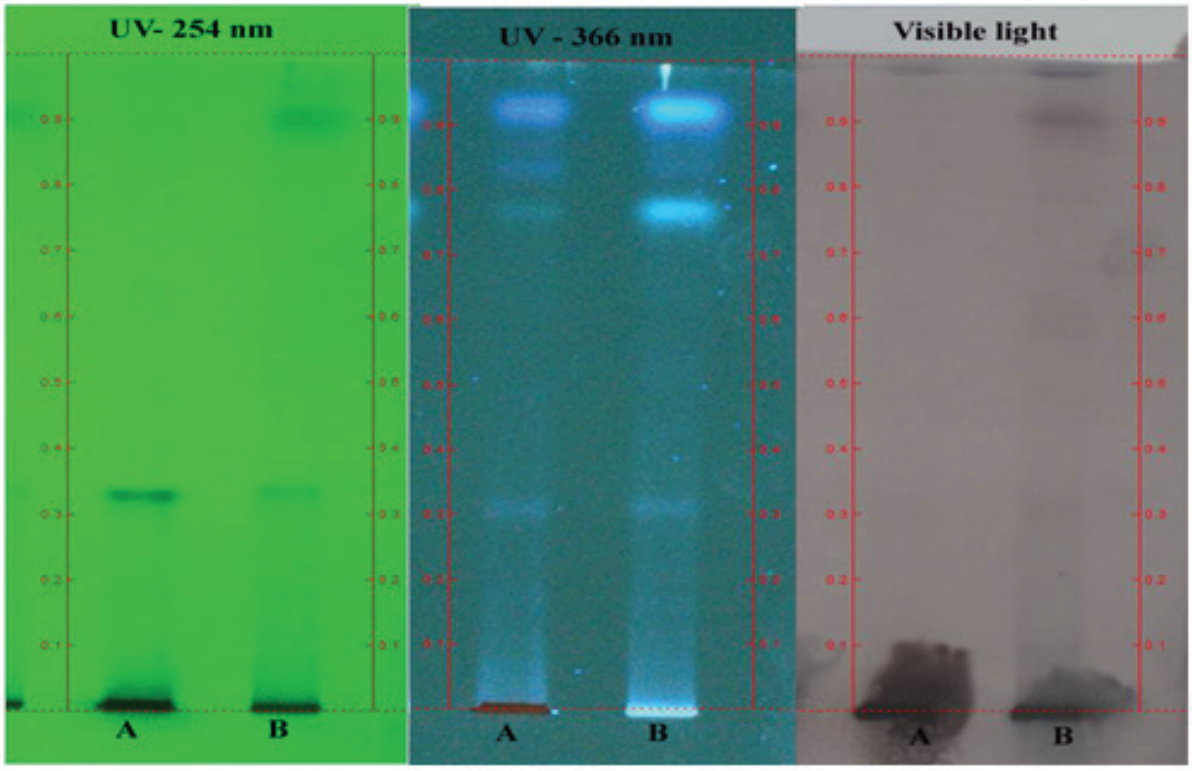

Figure 5: TLC profile of alcohol extracts of *S. racemosa* (A) and *S. crataegoides* (B).

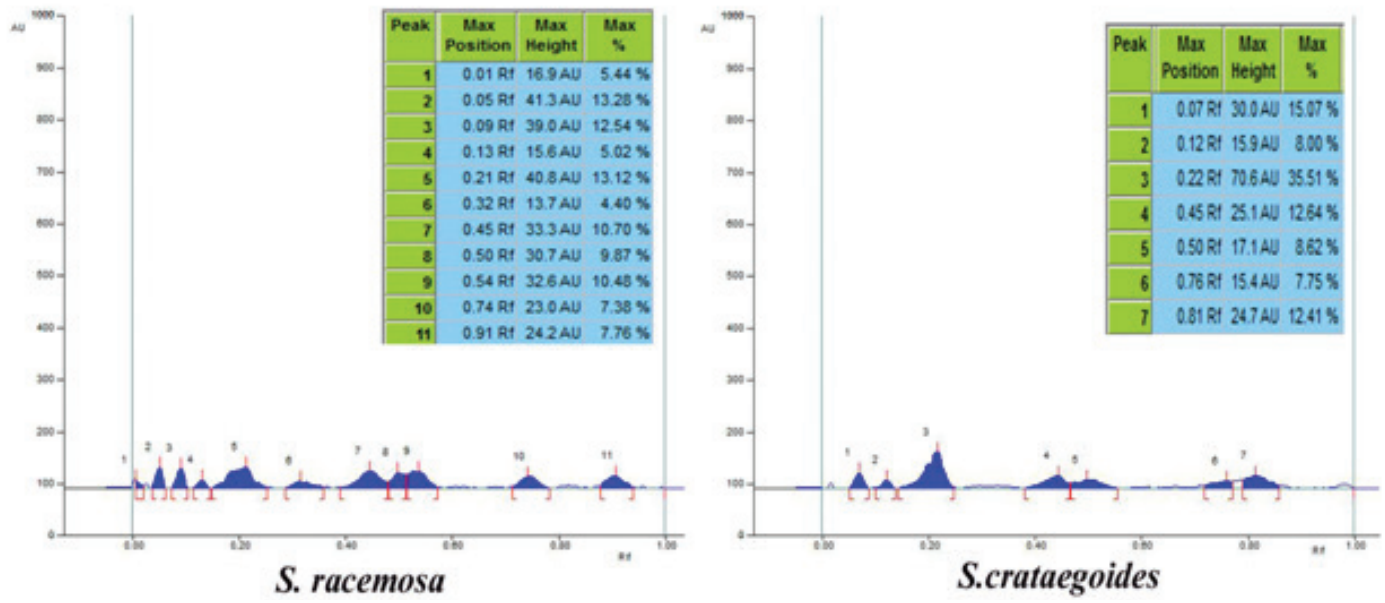

Figure 6: HPTLC profile of chloroform extracts of *S. racemosa* and *S. crataegoides*.

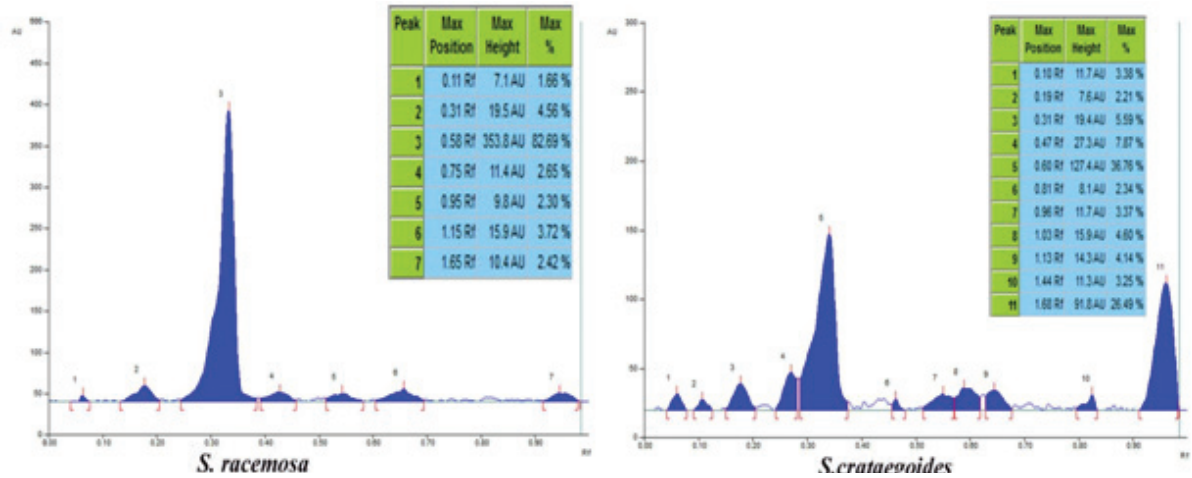

Figure 7: HPTLC profile of alcohol extracts of *S. racemosa* and *S. crataegoides*.

of both stem barks were chromatographed using toluene: ethyl acetate (8: 2), toluene: ethyl acetate: formic acid (7 : 3: 0.1) respectively.

The plate was developed upto a height of 8 cm, air dried, spots were observed under the UV light at 254 nm and 366 nm. The HPTLC finger print profiles were also recorded at 254 nm. Finally the plates were derivatized using vanillin sulphuric acid reagent heated at 105°C till colour spots appeared.[13,14]

RESULTS

Powdered stem bark of *Symplocos racemosa* and *Symplocos crataegoides* were studied for microscopic point of view and given in Table 1 and Figure 2 and 3.

Different compositions of the mobile phase for TLC and HPTLC analysis were tested in order to obtain high resolution and reproducible peaks. The TLC profile of chloroform and alcohol extract of *S. racemosa* and *S. crataegoides* is shown in Figure 4 and 5. The corresponding *Rf* values of various spots for chloroform and alcohol extract is given in Table 2.

HPTLC finger print profile of chloroform extract of *S. racemosa* and *S. crataegoides* showed 11 and 7 peaks respectively (Figure 6).

HPTLC finger print profile of alcohol extract of *S. racemosa* and *S. crataegoides* showed 7 and 11 peaks respectively (Figure 7).

DISCUSSION

Microscopic method of authentication is the first and fundamental step for standardization of herbal formulation. The findings of the present study is believed to be helpful in standardization of Ayurvedic formulation containing stem bark of *S. racemosa* and *S. crataegoides* as ingredients in powder form. HPTLC profile of chloroform and alcoholic extract provides a suitable method for monitoring the identity, purity and also standardization of the drug. The study sets the specific microscopic protocols of the two drugs and may lead to global acceptance and reputation of the Ayurvedic system.

CONCLUSION

The present study, analysed the powder microscopic characters of stem barks of *Symplocos racemosa* Roxb and *Symplocos crataegoides* Ham. and HPTLC fingerprint of chloroform and alcoholic extract of the same respectively. The results will be helpful in differentiating these barks in

powdered form or in authentication/identification of the crude drug/raw drug and in standardization of Ayurvedic formulation *Curna*.

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CONFLICT OF INTEREST

The author declare no conflict of interest.

ABBREVIATION USED

HPTLC: High Performance Thin Layer Chromatography; **Rf**: Retention factor; **TLC**: Thin Layer Chromatography; **UV**: Ultra violet.

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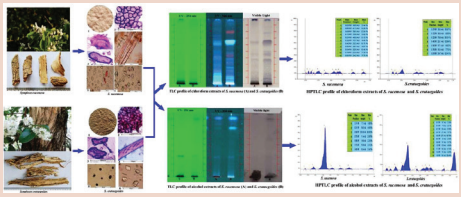
PICTORIAL ABSTRACT



SUMMARY

- Botanically *Symplocos crataegoides* and *Symplocos racemosa* are different species called by similar vernacular name Lodhrah.
- Comparative powder microscopic and HPTLC studies were undertaken to differentiate its identity.
- Findings of the study helpful in standardization of Ayurvedic formulation containing these plant drugs.
- This study sets the specific microscopic and HPTLC protocols of the two drugs and may lead to global acceptance and reputation of the Ayurvedic system.

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