A. Saraswathy et al. / Journal of Pharmacy Research 2011,4(6),1666-1669

Research Article
ISSN: 0974-6943

Available online through
www.jpronline.info

Pharmacognostic, physico-chemical and preliminary phytochemical studies on stem bark of *Caesalpinia coriaria* (Jacq.) Willd.

A. Saraswathy*, K. Amala, R. Shakila, N.S. Hema Kumar
Captain Srinivasa Murti Drug Research Institute for Ayurveda (CCRAS), Arumbakkam, Chennai-600 106, Tamilnadu

Received on: 11-02-2011; Revised on: 16-03-2011; Accepted on:21-04-2011

ABSTRACT

*Caesalpinia coriaria (*Jacq.) Willd., (Caesalpiniaceae) is rich in tannins and used in tanneries and also as dying material in textile industries. In indigenous system of medicine, its fruits are used for stomatitis and ulcer. The decoction of pods is used to stop bleeding piles. The stem bark is antiperiodic and is used in chronic fever. There is no previous reports on pharmacognosy of stem bark of this plant. Macro-micro-morphological, powder microscopy, fluorescence analysis, physico-chemical parameters and HPTLC finger print of the drug can be considered as pharmacopoeal standards to identify and authenticate the drug and also observed to characterise the crude drug.

Key words: Pharmacognosy, preliminary phytochemical, HPTLC finger print, Caesalpiniaceae

INTRODUCTION

Ingimaram of Siddha is botanically equated to *Caesalpinia coriaria* (Jacq.) Willd. Caesalpiniaceae; a small branching tree without prickles, stems unarmed, pinnae 7-8 pairs, leaflets 25-30, very narrow, 25 in long, flowers small, in short dense panicles, pale yellow or sometimes white, pods are twisted, fleshy, smooth and pale to blackish brown in colour.2-3 in. long, 3/4 in. broad and 1/8 in. thick. They are cultivated for its pods, which are a valuable tannin materials. Powder of pods astringent, antiperiodic, tonic[1,4,5,14]. As there is no detailed pharmacognostical studies on the stem bark of this plant. Therefore, the present paper attempts to reveal the pharmacognostical and physicochemical parameters, preliminary phytochemical screening and fluorescence analysis of the stem bark for identification of the drug in dry form.

Plate1 A & B. Flowering and fruiting twig of *C. coriaria*

MATERIALS AND METHODS

Fresh plant materials were collected from villages around Chennai when the plants were flowering and was identified with the help of Flora of Presidency of Madras[5]. Dried specimen (No. K/202, SB-8) was deposited in the crude drug museum of CSMRIASDD, Chennai. Free hand sections were taken, clearing and staining were done by the methods described by Johansen[7]. Drawings were made with the help of camera lucida.

Powder of the dried stem bark of *C. coriaria* was used for chemical analysis. Physico-chemical studies like total ash, acid insoluble ash, water soluble ash, alcohol and water solubility, loss on drying at 105ºC, and successive extractive values using Soxhlet extraction method were carried out as per the WHO guidelines[2]. Preliminary phytochemical tests were done as per the standard methods[6, 8, 9]. The fluorescence behaviour of the powdered drug in the day light and ultra violet light were carried out by moistening the powder in different alkaline and acidic solutions and viewing under the light of different wavelengths in a UV-chamber[3,12].

Hexane, benzene, chloroform, ethyl acetate and ethanol are of AR grade of SRL products. Aluminium plates precoated with silica gel $60F_{254}$ (E.Merck) were used for the HPTLC study. Linomat IV (Camag, Switzerland) applicator was used for the application of extracts on the TLC plates. CAMAG TLC Scanner 030618 with CATS V 4.06 software was used for the qualitative screening of the developed plates. CAMAG visualizer was used for the documentation of developed plates under UV 254 nm and UV 366 nm and under visible light after derivatization[10,11,13].

The coarsely powdered drug (4 g) was extracted successively with hexane, benzene, chloroform, ethyl acetate and ethanol using Soxhlet apparatus. The extracts were applied as 6 mm bands at 6 mm intervals leaving 10 mm space in X and Y axes. Two sets of TLC plates, one applied with hexane, benzene, chloroform extracts and ß-sitosterol using the mobile phase of Toluene : Ethyl acetate (10 : 10, v/v) and other applied with ethyl acetate, ethanol extracts and gallic acid using the mobile phase of Toluene : Ethyl acetate : Formic acid (5:2.5:0.5, v/v) were developed up to the height of 8 cm from the point of application. The developed plates were air dried and viewed under UV chamber at 254 nm and 366 nm and the images were recorded. The plates were scanner for finger print profile study at UV 254 nm using deuterium lamp. The plates were then derivatized with vanillin-sulphuric acid reagent and on subsequent heating at 105°C till the appearance of the colour. The image of the derivatized plate was recorded and scanned in visible wavelength 520 nm for finger print profile.

RESULT AND DISCUSSION

Macroscopic characters

Cut pieces of the stem barks are about 3-4 cm thick, externally rough, exfoliating bark like dark brown in colour, woody, fibrous, surface of the transversely cut stem bark shows dark brown colour, externally coffee brown and internally yellowish brown in colour, slightly bitter in taste and no characteristic odour.

Microscopic characters

Diagrammatic TS of the stem bark shows a broad zone of cork, differentiated into larger and smaller cells, forming a rhytidome, followed by cortex and phloem with 4 to 5 layers of continuous band of stone cell layers (Fig. 1).

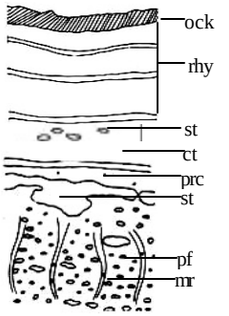

Figure-1. Diagrammatic TS of *C. coriaria* stem bark. ct, cortex; mr, medullary rays ock, outer cork; pf, phloem fibres; prc, prismatic crystals of calcium oxalate; st, stone cells; rhy, rhytidome.

Detailed transverse section of stem bark shows a wide zone of cork made up of alternative layers of

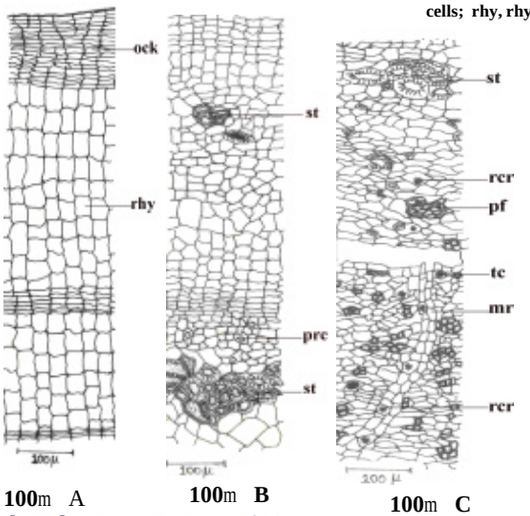

Figure 2. A. Detailed TS of Stem bark shows outer cork region, B. Inner cork and cortex region with stone cell layer, C. Phloem region with medullary rays. ct, cortex; mr, medullary rays; ock, outer cork; pf, phloem fibres; prc, prismatic crystals of calcium oxalate; rcr, rosette crystals of calcium oxalate; rhy, rhytidome; st, stone cells; tc, tannin cell.

***Corresponding author.**
A. Saraswathy
Captain Srinivasa Murti Drug Research Institute for Ayurveda (CCRAS), Arumbakkam, Chennai-600 106, Tamilnadu
Tel.: + 91-
E-mail:*csmdria@gmail.com*

larger and smaller cells forming a rhytidome occupying almost one third of the area of entire bark, outer a few external layers are exfoliating, the cells are thin walled rectangular, tangentially elongated cells filled with brown content. Inner most layer of cork cells contain a few lignified stone cells, found scattered in groups with or without brown content followed by cortex consisting of 3 to 4 layers of continuous band of thick lignified stone cell region with various size and shapes, a few with prismatic crystals of calcium oxalate and starch grains. Phloem very wide consisting of groups of stone cells and usual elements of sieve tubes, companion cells, phloem parenchyma, phloem fibres traversed by phloem rays. Phloem fibres arranged in tangential bands alternating with phloem parenchyma, consisting of thin walled cells, containing rosette crystals of calcium oxalate and starch grains simple or compound having 2 or 3 in groups measuring upto 12 µ in length. Tanniniferous cells also occur in the phloem region. In outer and middle phloem regions phloem tissues get crushed and form tangential bands of ceratenchyma. Phloem rays many, 2-3 seriate, thin-walled radially elongated cells, a few cells with rosette crystals of calcium oxalate crystals measuring upto 25 µ in size.

Powder microscopy

Microscopic examination of the powder shows simple and compound starch grains two or three components rounded to oval in shape measuring upto 12 µ in length, fragments of thin walled lignified septate fibres, uni to multiseriate medullary rays cells with rosette crystals of calcium oxalate, prismatic crystals of calcium oxalate measuring upto 30 µ in size, rosette crystals of calcium oxalate crystals upto 25 µ in size, various size, shapes and thickness of stone cells and sclereids with pits and small lumen upto 250 µ in length, cork cells in surface view and tannin cells (Fig. 3).

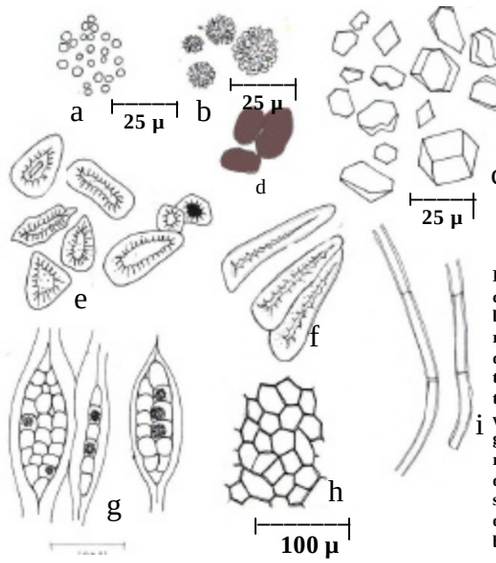

Figure 3. Powder Microscopy of *C. coriaria* stem bark. a. starch grains; b. rosette crystals of calcium oxalate; c. prismatic crystals of calcium oxalate; d. tannin cells; e. stone cells with crystals; f. sclereids; g. medullary ray cells with rosette crystals of calcium oxalate; h. cork cells in surface view; i, fragments of thin walled septate fibres.

Explanation for the abbreviation

Ct- cortex,Ock- Outer cork,Mr- medullary rays,Pcr- prisamatic crystal of calcium oxalate,Pf - phloem fibres ,Rcr -rosette crystals of calcium oxalate,Rhy-rhytidome,St- stone cell,Tc-tannin cell

Salient microscopic characters

1. Prismatic crystals of calcium oxalate crystals found in the cortex region.
2. Rosette crystals of calcium oxalate crystals found in the phloem and medullary ray region.
3. Elongated sclereids, stone cells with prominent pits and small lumen in various size, shapes and thickness.
4. Simple and compound starch grains are found in the cortex and phloem region.
5. Uni to Multiseriate medullary rays cells with rosette crystals of calcium oxalate.
6. Thin walled lignified septate fibres.

Table-1 Physico-chemical parameters of the stembark of *C. coriaria*

Sl.No	Parameters	Results (n=3) ±SD
1.	% Loss on drying at 105ºC	5.76 ±0.29
2.	% Total ash	7.55± 0.36
3.	% Water soluble ash	0.6±0.05
4.	% Acid in-soluble ash	1.29±0.11
5.	% Extractive values:	
	a *n*-Hexane	51.50±0.57
	b Benzene	1.23±0.13
	c. Chloroform	0.67±0.20
	d. Ethyl acetate	3.55 ±0.24
	e. Ethanol	3.53±0.18
6.	% Solubility at room temp.	
	a. Ethanol	19.09±1.08
	b. Water	10.36±0.95
7.	Alkalinity of water soluble ash (cc of 0.1 N HCl/g)	0.4 ±0.05

SD- Standard Deviation

Table-2 Preliminary phytochemical tests for extracts of stem bark of *C. coriaria*

S.No	Test	*n*-Hexane Extract	Chloroform Extract	Ethyl Acetate Extract	Alcohol Extract
1.	Alkaloid	-	-	-	-
2.	Quinone	-	-	-	-
3.	Coumarin	-	-	-	-
4.	Flavone	-	-	+	+
5.	Steroid	+	+	+	+
6.	Phenol	-	-	+	+
7.	Tannin	-	-	+	+
8.	Glycoside/Sugar	-	-	+	+
9.	Iridoid	-	-	-	-
10.	Terpenoid	+	+	+	+

Table-3. Fluorescence analysis of the stem bark powder of *C. coriaria*

S.No	Powder	Day light	UV at 254 nm	UV at 366 nm
1.	Powder as such	Dirty green	Brownish green	Dirty green
2.	Powder + Water	Light green	Dark green	Bluish green
3.	Powder + 1N NaOH (aqueous)	Dark orange	Dark green	Bluish green
4.	Powder + 1N NaOH (alcoholic)	Brown	Dark green	Pink
5.	Powder + 1N HCl	Pale green	Dark green	Bluish green
6.	Powder + 50% H_2SO_4	Brown	Dark green	Light green
7.	Powder + *n*-Hexane	Green	Brown	Reddish pink
8.	Powder + Chloroform	Brownish green	Brown	Reddish pink
9.	Powder + Ethyl acetate	Yellowish green	Dark brown	Reddish pink
10.	Powder + Ethanol	Brown	Dark green	Yellowish brown

Physico-chemical parameters and fluorescence analysis

Physico-chemical data of the stem bark of *C. coriaria* are tabulated in Table-1. Quantitative standards revealed that the ash content was 7.55% and low amount of acid insoluble siliceous matter was detected in the stem bark. The water soluble extractive value of the stem bark indicates the presence of inorganic content. The alcohol soluble extractive value indicates the content of polar constituents like sugars, phenols, tannins, alkaloids, steroids, quinones, glycosides and flavonoids from the stem bark. Phytochemical screening of *C. coriaria* stem bark shows the presence of flavone, phenol, tannin, glycoside, steroids and terpenoid in ethyl acetate and alcohol extract, whereas steroids and terpenoide are found in *n*-hexane and chloroform extracts, the results are given in Table-2. The observations of fluorescence analysis are given in Table-3.

TLC/HPTLC studies

Under UV 254 nm, the *n*-hexane extract showed four major spots at R_f 0.16, 0.35, 0.47 and 0.58 and three minor spots at 0.28, 0.32, 0.35 and 0.42 (Figure 4). The benzene extract showed four major spots at R_f 0.17, 0.46, 0.57, 0.68 and 0.76; six minor spots at R_f 0.07, 0.12, 0.21, 0.26, 0.31 and 0.36 (Figure 5). The chloroform extract showed one major spot at R_f 0.25; two minor spots at R_f 0.03 and 0.16 (Figure 6). Under UV 366 nm, the hexane extract showed four spots at R_f 0.24, 0.30, 0.55 and 0.40 (major)(Figure 7). The benzene extract showed two major spot at R_f 0.49 and 0.61; five minor spots at R_f 0.09, 0.25, 0.41, 0.53 and 0.74(Figure 8). The chloroform extract showed one spot at R_f 0.74(Figure 9).

HPTLC finger print profile of *C. coriaria* stem bark at UV 254 nm, 366nm and after derivatisation under visible light

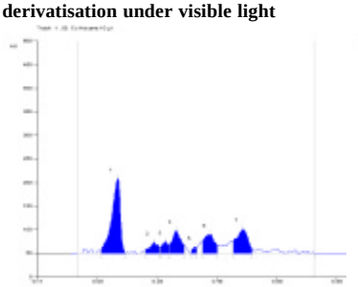

Figure 4. Hexane extract

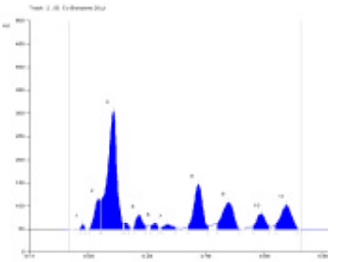

Figure 5. Benzene extract

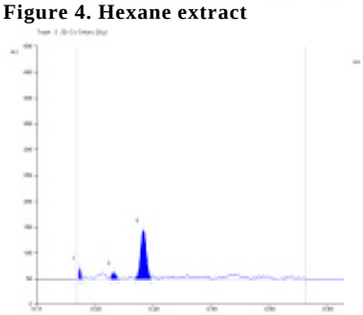

Figure 6.Chloroform extract

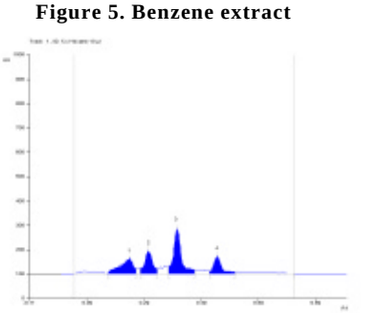

Figure 7. Hexane extract

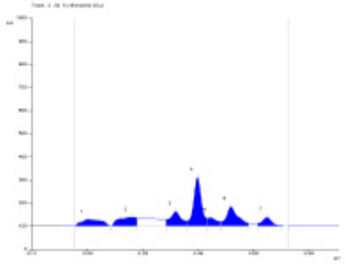

Figure 8. Benzene extract

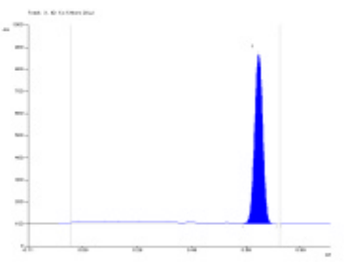

Figure 9.Chloroform extract

HPTLC finger print profile of *C. coriaria* stem bark under visible light after derivatization with vanillin-sulphuric acid

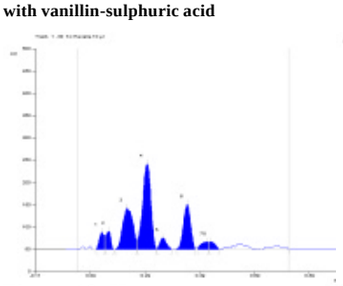

Figure 10. Hexane extract

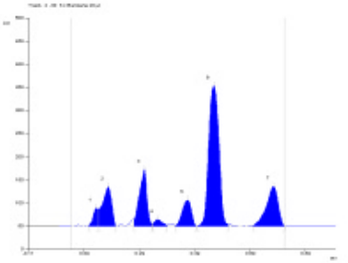

Figure 11. Benzene extract

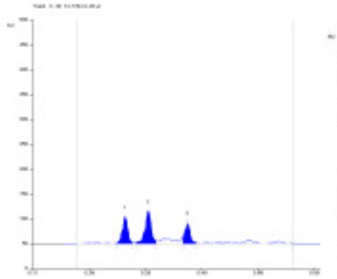

Figure 12.Chloroform extract

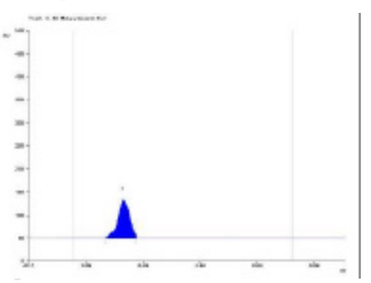

Figure 13. ß-Sitosterol

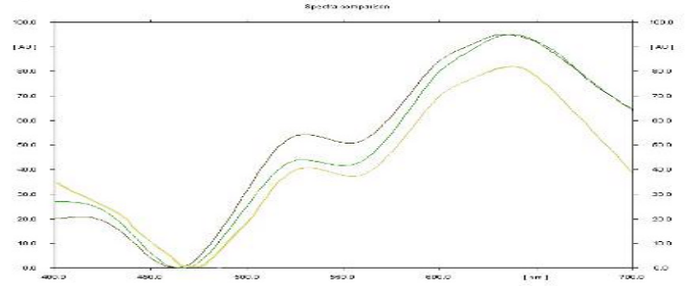

Figure 14. UV supper imposable spectra of ß-sitosterol with other extracts

Photo documentation of 1.Hexane, 2.Benzene, 3.Chloroform extracts of *C. coriaria* stem bark and 4. ß-Sitosterol

Figure 15. Under UV 254 nm

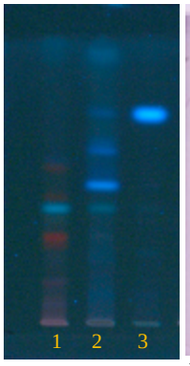

Figure 16. Under UV 366 nm

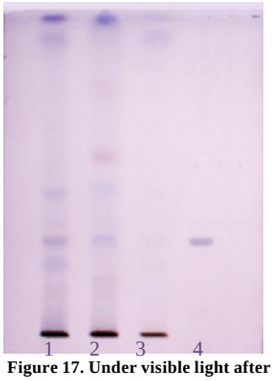

Figure 17. Under visible light after derivatization with vanillin-sulphuric acid

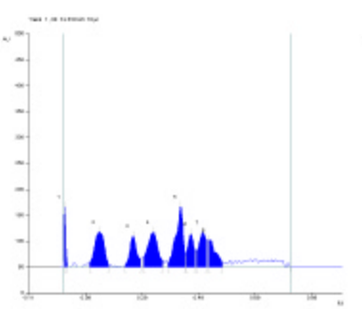

Figure 18. Ethyl acetate extract

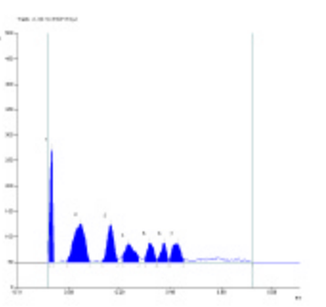

Figure 19. Ethanol extract

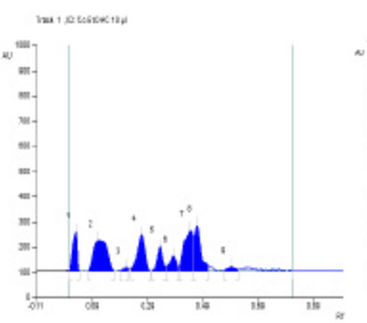

Figure 20. Ethyl acetate extract

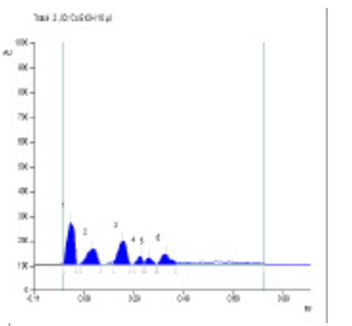

Figure 21. Ethanol extract

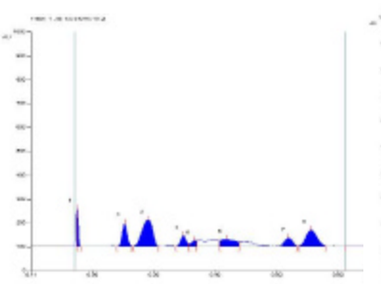

Figure 22. Ethyl acetate extract

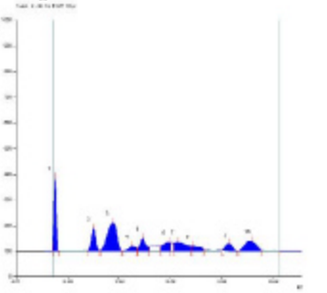

Figure 23. Ethanol extract

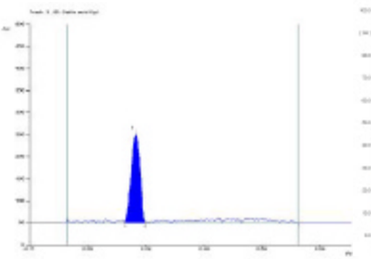

Figure 24. Gallic acid

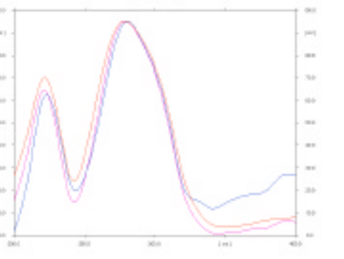

Figure 25. UV supperimposable spectra of gallic acid with other extracts

In the derivatized plate, hexane extract showed three major spots at R_f 0.22, 0.30 and 0.44; five minor spots at 0.13, 0.16, 0.22,0.35, 0.52 and 0.53(Figure 7). The benzene extract showed one major spot at R_f 0.56; six minor spots at R_f 0.13, 0.18, 0.31, 0.35, 0.46 and 0.77(Figure 8). The chloroform extract showed three spots at R_f 0.22, 0.30, 0.44(Figure 9). The spot at R_f 0.30 corresponds to ß-sitosterol (Figure 10). The UV superimposable spectra of spots at R_f 0.30 is shown in the Fig.11.TLC photodocumentation of *n*-hexane, benzene, chloroform extracts and ß-sitosterol are shown in Fig. 12-14.

Under UV 254 nm, the ethyl acetate extract showed five major spots at R_f 0.14, 0.26, 0.33, 0.43 and 0.50; two minor spots at R_f 0.46 and 0.53 (all green) (Figure 15); the ethanol extract showed four major spots at R_f 0.14, 0.25, 0.32 and 0.52; two minor spots at R_f 0.41, 0.47 (Figure 16). Under UV 366 nm, the ethyl acetate extract showed five major spots at R_f 0.11, 0.27, 0.34, 0.45 and 0.47; three minor spots at R_f 0.21, 0.39 and 0.59; the ethanol extract showed three major spots at 0.12, 0.25 and 0.42; two minor spots at R_f 0.32 and 0.35 (Figure 18). In the derivatized plate, ethyl acetate extract showed two major spots at R_f 0.28 (brown) and 0.81 (purple); four minor spots at 0.13 (purple), 0.37 (purple) (Figure 19), 0.41 (yellow), 0.74 (purple). The ethanol extract showed two major spots at R_f 0.28 (brown) and 0.81 (purple); four minor spots at 0.13 (purple), 0.37 (purple) and 0.74 (purple) (Figure 20).

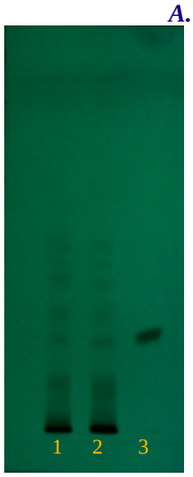

Figure 26. Under UV 254 nm

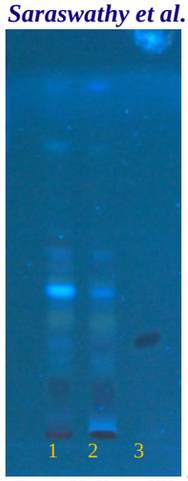

Figure 27. Under UV 366 nm

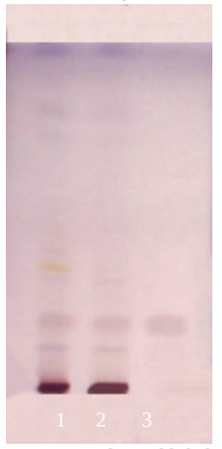

Figure 28. Under visible light after derivatization with vanillin-sulphuric acid

The spot at R_f 0.25 corresponds to gallic acid (Figure 21), which is present in ethyl acetate and ethanol extracts. The superimposable UV spectra of R_f 0.25 gallic acid with that of extracts confirmed its present in the stem bark (Figure 22). TLC photodocumentation of ethyl acetate, ethanol extracts and gallic acid are shown in Fig. 23-25.

CONCLUSION

Morphology as well as microscopical parameters of the dried stem bark of *C. coriaria* were studied and described along with physico-chemical, preliminary phytochemical, fluorescence analysis and HPTLC studies for authentification and its quality control in fragmentary condition as well as in whole form. This study will contribute to the existing knowledge over the standardization aspects of the raw drug *C. coriaria*.

ACKNOWLEDGEMENT

Authors are thankful to the Director, Central Council for Research in Ayurveda and Siddha, New Delhi for facilities.

REFERENCES

1. Anonymous, The Wealth of India, A Dictionary of Indian Raw materials and Industrial products Vol.2C. Publication and Information Directorate, Council of Scientific and Industrial Research, New Delhi, 1988, pp. 2.
2. Anonymous, Quality Control Methods for Medicinal Plant Materials, World Health Organisation, Geneva, 1998, pp.62-64.
3. Brain KR, Turner TD. The Practical Evaluation of Phytopharmaceuticals. Wright Scientehnica, Bristol, 1975, pp.78-80.
4. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants, Drug Research Laboratory, Jammu Kashmir, Sri Nagar, 1956.
5. Gamble JS, Flora of Presidency of Madras. Botanical Survey of India, Calcutta, 1967, Vol-I, pp.278-279.
6. Harborne JB., Phytochemical Methods. Jackman H. (Ed.), London, 1973, pp.70.
7. Johansen OA.. Plant Microtechniques. Mc.Grow Hill, New York, 1940, pp.182-203.
8. Lala PK. Lab Manuals of Pharmacognosy (5th Edition). CSI Publishers and Distributors, Calcutta, 1993.
9. Overton KH. Isolation, Purification and Preliminary Observation in Elucidation of Structures by Physical and Chemical Methods, Bently KW. (Ed.), *Inter Science Pub*., New York, 1963, pp.34.
10. Sethi PD. High Performance Thin Layer Chromatography (1st Edition). CBS Publishers and Distributors, New Delhi, 1996,Vol-X.
11. Stahl I. Thin Layer Chromatography, A Laboratory Hand Book (Student Edition). Springer-Verlag, Berlin. 1969,pp. 52-86; 127-128, 900.
12. Trease GE, Evans WC. Pharmacognosy (13th Edition). Bailliere Tuidall, London, 1989, pp. 799-803.
13. Wagner H., Bladt S. Plant Drug Analysis, A Thin Layer Chromatography Atlas (2nd Edition). Springer-Verlag, Germany.
14. Yoganarasimhan SN. Medicinal Plants of India. Regional Research Institute (Ay.), Bangalore, India, 2000,Vol-II. pp- 91

Source of support: Nil, Conflict of interest: None Declared