Journal of Pharmacognosy and Phytochemistry 2015; 4(4): 93-96

Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2015; 4(4): 93-96
Received: 11-09-2015
Accepted: 13-10-2015

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Comparative phytochemical study of stem bark versus small branches of *Anthocephalus cadamba* using high performance thin layer chromatography

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Abstract
Objective: To compare the phyto-constituents present in the stem bark and small branches of *Anthocephalus cadamba* on the basis of high performance thin layer chromatography in order to evaluate whether the small branches of this plant may be substituted with the stem Bark.
Methods: CAMAG HPTLC system equipped with semi-automatic applicator Linomat-IV and win CATS 1.4.2 software was used. *n*-Hexane, ethyl acetate and ethanol extracts of the stem bark and small braches were developed in suitable mobile phase using standard procedures and visualized in UV 254 and 366 nm and in white light after derivatization with in anisaldehyde-sulphuric acid reagent.
Results: The HPTLC fingerprinting of the *n*-hexane, ethyl acetate and ethanol extracts of stem bark and small branches showed almost similar phytochemical profile.
Conclusion: HPTLC fingerprint profile of stem bark and small branches are found almost similar, therefore small branches may be used in place of stem bark and vice-versa after comparison and confirmation of same for pharmacological activities. The method can also be used for identification of different *Anthocephalus cadamba* species and adulterants.

Keywords: *Anthocephalus cadamba*, Stem bark, Small branches, HPTLC fingerprinting

1. Introduction
The Indian system of medicine depends on plants and plant derived products for treating ailments. Different anatomical parts of plant such as leaves, stem, stem bark, root, rhizome, flower, stamen, fruit, seed, gall etc. are used to formulate various formulations used in Indian system of medicines like Ayurveda. Dealers and manufacturers of Ayurveda, Siddha and Unani drugs face the difficulty in getting the regular supply of plant raw materials, particularly of roots, rhizome and bark of big trees. It is difficult to get huge amount of stem bark from the big tree because removal of the stem bark from the trunk of the tree makes the plant weak and susceptible to damage by insects and natural elements. The usages of stem barks of the trunk are therefore forbidden with an aim to conserve and protect the medicinal plants from extinction and make them available for future generation.
Anthocephalus cadamba (Family-Rubiaceae) commonly called Kadamba is a medicinal plant widely used in Ayurveda. As per the Ayurvedic literature, stem bark of this plant is used in daha (burning sensation), yonidosha (disorders of female genitals), vrana (ulcer), raktapitta (haemorrhagic diseases) visakita–dansaja vrana (poisonous insect bite) [1]. The bark is also reported to be used as tonic, febrifuge, astringent [2-5], analgesic, antidiuretic [2], abortifacient [6] and in the treatment of syphilis [7] and stomatitis [2]. Phytochemical screening of the stem bark of the plant revealed the presence of alkaloids, flavanoids, steroids, terpenoids, fats and reducing sugars [8-10]. The stem bark mainly contains cadambine [11] 3-*O*-{*α*-L-rhamnopyranosyl}-quinovic acid -28-O-{β-D-glucopyranosyl}ester, 3-*O*-{*β*-D-glycopyranosyl}-quinovicacid-28-*O*-{*β*-D-glycopyranosyl}ester [12], 3*α*-dihydrocadambine [11, 13], 3*β*-dihydrocadambine, 3*β*-isodihydrocadambine [13], isodihydrocadambine [14], cadambagenic acid [13, 15], quinovic acid [14], saponin A, saponin B [16, 17], saponin C, saponin D [18], oleanolic acid [13], 4, 22-chloestadien-3-one, 3*β*-ergost-5-en-3-ol [19] while *β*-Sitosterol [15], sitostenone, *γ*-sitosterol, stigmasterol [19], deoxycordifoline, kelampaysides A, kelampaysides B, 8- epi-kingiside, loganic acid, loganin, loganol, 5*α*-carboxystrictosidine, sweroside, 3′-*O*-caffeoylsweroside, vallesiachotamine, isovallesiachotamine, strictosidine lactam [20] and in Indian system of medicine. Present study is carried out in *A. cadamba* to evaluate the possibilities

of using small branches in place of stem bark which will help sustainable utilization.

2. Material and Methods

2.1. Plant Material

The stem bark and small branches of *A. cadamba* were collected from Gwalior, identified and authenticated by the Shree N.K. Pandey, Research Officer (Botany), NRIASHRD, Gwalior.

2.2. Instrumentation

A CAMAG HPTLC system (Muttenz, Switzerland) equipped with a semiautomatic TLC applicator Linomat IV, twin trough plate development chamber, Win CATS software version 1.4.2. and Hamilton (Reno, Nevada, USA) Syringe (100 µL).

2.3 Material and Reagents

All chemicals, reagents and solvents used during the experimentation were of analytical grade and HPTLC plates were purchased from E. Merck Pvt. Ltd. (Mumbai, India).

2.4. HPTLC Profiles

HPTLC studies were carried out following the method of Sethi [22], Stahl [23] and Wagner *et al.* [24].

2.4.1. Sample Preparation

The stem bark and small branches were powdered coarsely. One gram powdered samples of each of stem bark and small branches were accurately weighed and exhaustively extracted by *n*-hexane, ethyl acetate and ethanol (each 100 ml) separately using soxhlet apparatus. The extracts were filtered and concentrated under reduced pressure and made up to10 ml in standard flasks separately.

2.4.2. Developing Solvent System

The mobile phase used for developing the *n*-hexane, ethyl acetate and ethanol extracts of stem bark and small branches was toluene: Ethyl acetate 8:2 (v/v).

2.4.3. Sample Application

The samples were spotted in the form of bands of width 10 mm with a 100 µl Hamilton syringe on aluminium TLC plates pre-coated with Silica gel $60F_{254}$ of 0.2 mm thickness with the help of TLC semi-automatic applicator Linomat IV attached to CAMAG HPTLC system, which was programmed through Win CATS software version 1.4.2. 10 µL of each extracts of stem bark and small branches were applied in two tracks as 10 mm bands at a spraying rate of 10 seconds μL^{-1}. Track 1 was stem bark and track 2 was small branches for each of the extracts applied.

2.4.4. Development and Detection of Spots

Development of the plate up to a migration distance of 80 mm was performed at 27 ± 2 °C with mobile phase for each extracts in a CAMAG HPTLC chamber previously saturated for 30 min. After development the plate was dried at 60 °C in an oven for 5 min and visualized under wavelength 254 nm and 366 nm for ultra violet detection. The developed plate was then dipped in anisaldehyde-sulphuric acid reagent for derivatization and dried at 105 °C in hot air oven till the colour of the band appears and visualized under white light. Images were captured by keeping the plates in photodocumentation chamber and R_f values were recorded by Win CATS software.

3. Results and Discussion

In this study comparative HPTLC profile of *n*-hexane, ethyl acetate and ethanol extracts of stem bark and small branches of *A. cadamba* were recorded to reveal the chemical pattern of each extract.

The HPTLC profile of *n*-hexane extract of stem bark and small branches (Table 1 and Figure 1) showed one band at R_f 0.56 (black) and no band, respectively when visualized under UV at 254 nm. At UV 366 stem bark and small branches showed four and three bands respectively, out of which three bands at R_f 0.58 (red), 0.59 (florescent blue), 0.67 (red) were found similar indicating the presence of three similar compounds in hexane extract of stem bark and small branches. Visualization under white light after derivatization with anisaldehyde sulphuric acid reagent, stem bark and small branches both showed four bands, out of which two bands at R_f 0.40 (blue), 0.84 (blue) were found similar indicating the presence of two similar compounds in hexane extract of stem bark and small branches.

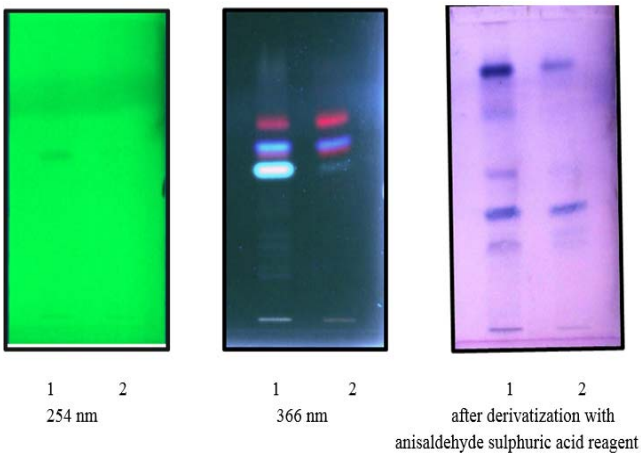

Fig 1: HPTLC profile of *n*-hexane extracts of stem bark and small branches of *A. cadamba.* (track 1: stem bark, track 2: small branches)

Table 1: R_f value of *n*-hexane extract of *A. cadamba*

Wave-length	*n*-Hexane extract	
	Stem bark	Small branches
254	0.56	No band
366	0.51, 0.58, 0.59, 0.67	0.58, 059, 0.67
Visible light after derivatization	0.29, 0.40, 0.52, 0.84	0.40, 0.84

The HPTLC finger print profile of ethyl acetate extract of stem bark and small branches (Table 2 and Figure 2) showed one band at R_f 0.78 (black) and no band, respectively when visualized under UV at 254 nm. At UV 366 stem bark and small branches showed eight and seven bands respectively, out of which six bands at R_f 0.23 (florescent blue), 0.30 (red), 0.59 (red), 0.74 (red), 0.81(blue), 0.90 (red) were found similar indicating the presence of six similar compounds in ethyl acetate extract of stem bark and small branches. Visualization under white light after derivatization with anisaldehyde sulphuric acid reagent, stem bark and small branches both

showed five and three bands respectively, out of which three bands at R_f 0.38, 0.53, 0.90 (all blue) were found similar indicating the presence of at least three similar compounds in ethyl acetate extract of stem bark and small branches.

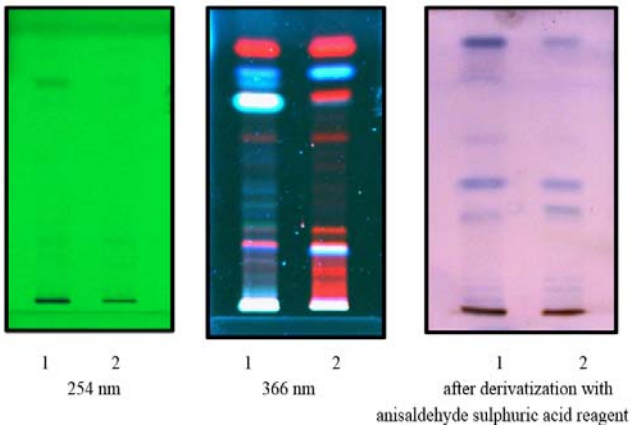

Fig 2: HPTLC profile of ethyl acetate extracts of stem bark and small branches of *A. cadamba.* (Track 1: stem bark, track 2: small branches)

Table 2: R_f value of ethyl acetate extract of *A. cadamba*

Wave-length	Ethyl acetate extract	
	Stem bark	**Small branches**
254	0.78	No band
366	0.23, 0.30, 0.41, 0.59, 0.74, 0.77, 0.81, 0.90	0.14,0.23, 0.30,0.59, 0.74,0.81, 0.90
Visible light after derivatization	0.17,0.38, 0.53, 0.69, 0.90	0.38, 0.53, 0.90

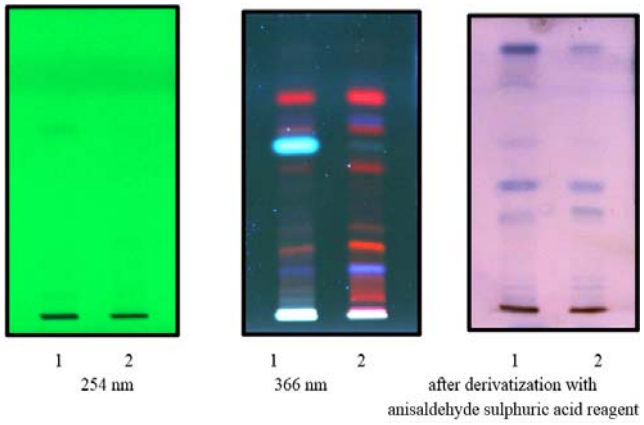

Fig 3: HPTLC profile of ethanol extracts of stem bark and small branches of *A. cadamba.* (track 1: stem bark, track 2: small branches)

The HPTLC finger print profile of ethanol extract of stem bark and small branches (Table 3 and Figure 3) showed no band in both stem bark and small branches when visualized under UV at 254 nm. At UV 366 stem bark and small branches showed five and eight bands respectively, out of which five bands at R_f 0.18 (blue), 0.25 (red), 0.60 (florescent blue), 0.65 (red), 0.76 (red) were found similar indicating the presence of five similar compounds in ethanol extract of stem bark and small branches. Visualization under white light after derivatization with anisaldehyde sulphuric acid reagent, stem bark and small branches both showed three with same R_f values at 0.35, 0.46, 0.98 (all blue) indicating the presence similar compounds in ethanol extract of stem bark and small branches.

Table 3: R_f value of ethanol extract of *A. cadamba*

Wave-length	Ethanol extract	
	Stem bark	**Small branches**
254	No band	No band
366	0.18, 0.25, 0.60, 0.65, 0.76	0.09, 0.19, 0.26, 0.33, 0.53, 0.65, 0.69, 0.76
Visible light after derivatization	0.33, 0.46, 0.98	0.35, 0.46, 0.98

4. Conclusion

The present study carried out in *A. cadamba* to evaluate the possibilities of using small branches in place of stem bark will help sustainable utilization. Almost similar HPTLC fingerprint profile of stem bark and small branches of this plant indicates the presence of almost similar compounds in both the parts of this plant. Therefore small branches may be used in place of stem bark and vice-versa after comparison and confirmation of same pharmacological activities. The results of qualitative evaluation of HPTLC fingerprint profile will also be helpful in the identification and quality control of the drug and can provide standard HPTLC fingerprints with selected solvent system. The fingerprint HPTLC profile and can also be used as a reference for the proper identification/authentication of the drug.

5. Acknowledgement

Authors are thankful to CCRAS, New Delhi for financial assistance and Director General, CCRAS, New Delhi for providing facilities.

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