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Pharmacognostical and phytochemical blueprint of *Abroma augusta* L. stem bark

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Uses of *Abroma augusta* L. stem and root are mentioned in traditional texts where the presence of the alkaloid, betaine in these parts is known. The study was undertaken to generate a pharmacognostical and phytochemical blueprint of *Abroma augusta* stem bark and detection of the bioactive alkaloid, betaine in it. Authenticated plant materials were subjected to pharmacognostical, physicochemical, and phytochemical studies. HPTLC, HPLC, and FTIR were used for chemical fingerprinting of the plant materials. Diagnostic features of *A. augusta* stem bark such as organoleptic evaluation, powder microscopic characters, fluorescence profile with various reagents were established. Phytochemical screening of different solvent extracts showed the presence of alkaloids, steroids, saponins, tannins, phenolics, glycosides, but fewer terpenoids, flavonoids, and carbohydrates. This was rationalized by FTIR spectroscopy of the chloroform extract that gave maximum extractive yield. HPTLC and HPLC fingerprint profiling with marker identification was generated. The alkaloid, betaine was isolated and identified by mass spectrum. The botanical and chemical screening suggested that *A. augusta* stem bark may be a potential substitute for the root or stem of the plant. However, further, bio-evaluations are required to ascertain its possible clinical applications. The generated profile may serve as a reference document in future for identification and authentication of the plant material.

Keywords: *Abroma augusta*, Betaine, FTIR, HPLC, HPTLC, Isolation, Microscopy.

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Introduction

Abroma augusta (L.) L. f., synonymous with *Abroma angulata* Lam. (Ulatkambal in Hindi and devil's cotton in English) is a wildly grown hairy shrub in tropical Asia, South Africa, Eastern Africa and Australia,[1] and is also cultivated for ornamental purpose[2]. Use of the whole plant or its parts (roots, root barks, and leaves) against various ailments has been mentioned in folk medicines[3-5]. A mother tincture of the plant is also claimed to be an effective homoeopathy treatment of type –II diabetes mellitus[6,7]. Some of the medicinal properties *viz.* antidiabetic[8], anti-inflammatory[9], wound healing[10], hypolipidemic[11], antimicrobial[12], antioxidant[13], anti-sinusitis[14] of various parts of *A. augusta* have also been demonstrated in animal models. Nevertheless, its utility against gynaecological and urinary disorders is perhaps one of the most important physiological attributes of this plant. It can control menstrual disorders, reduce uterus leucorrhoea, and exert contractile action on the uterus. Hence it is used as an emmenagogue and for the treatment of dysmenorrhoea, amenorrhorea, sterility, and other menstrual disorders[3].

The phytoconstituents, singularly and/ or in combination(s) contribute to the medicinal attributes of any plant. A host of chemical compounds including steroids, sugars, terpenes, aliphatic alcohols/ diols etc. have been isolated from various parts of the plant[1,14]. Amongst these, an alkaloid, abromine[15], isolated from the roots was later found to be identical with betaine (trimethylglycine) based on melting point data[16]. Betaine is a methyl group donor for transmethylation in several vital biochemical reactions[17]. Moreover, because of its zwitterionic and hydrophilic nature, it also functions as an osmolyte that may account for the curative action of *A. augusta* against gynaecological and urinary problems as well as of some other health benefits. Indeed, the efficacy of betaine in preventing oxidative stress-induced diseases[18-21], menstrual and urinary disorders[22], wound healing[23], as well as antimicrobial activity[24], have been demonstrated in animal models.

The authors envisage that the use of *A. augusta* stem bark for herbal drug development would spare the rest of the plant for harnessing its other health benefits. However, to the best of the authors' knowledge, the *A. augusta* stem bark has never been used for any biological study. The pharmacognostical

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and phytochemical characteristics of plants are very important in developing herbal drugs. Hence, in this study, the phyto-pharmacognostic characteristics of *A. augusta* stem bark that may serve as a standard profile in authentication and identification of the plant part were investigated. In addition, given the health benefits of betaine, special attention was given to isolate the alkaloid from *A. augusta* stem bark and unequivocally identified it for the first time using mass spectroscopy.

Materials and Methods

Material and reagents

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

Plant materials collection and authentication

Fresh matured portions of *A. augusta* stem were collected from the natural habitat of Madhabpur village, East Midnapur (21° 52′ 32.2″ N, 87° 35′ 4.9″ E), West Bengal in early March 2018 and authenticated by Dr. M. N. Das, ex-scientist, Dept. of Pharmacognosy, Central Ayurveda Research Institute for Drug Development, Kolkata, West Bengal. Stem bark portions (2 to 10 cm) from the peripheral shoot region were separated for study. The voucher specimen (voucher no. CARIDD/DP-08118) was deposited in the Department of Pharmacognosy of the institute and is available for reference.

Plant sample processing

The plant material (stem bark fragment) was washed with aqueous 70% (v/v) ethanol and dried at an ambient temperature (24–27 °C). A small portion of the air-dried plant sample was used for macroscopic, organoleptic, and anatomical (transverse section) studies, while the rest of the plant materials were pulverized with a grinder (National SM 2000). The whole and powdered plant samples were stored at room temperature in airtight, light-resistant containers as per guidelines of Ayurvedic Pharmacopoeia of India[25,26]. The finely (sieved in 60 mesh) powdered sample was used for powder microscopy, while the coarsely powdered samples were used for phyto-pharmacognostic examinations and chromatography analyses.

Macroscopy of plant material

The organoleptic parameters *viz*. texture, shape, size, colour, etc. of the plant material were noted by naked eye observation with a simple microscope (Olympus OIC DM).

Cytomorphology of plant material

The dried stem bark samples were transversely sectioned with a clean, sharp diamond edge blade. The selected sections were mounted on slides in 50% glycerine and observed under a binocular compound microscope (Olympus OIC-07964) at 10× and 40× magnifications. The Camera Lucida drawings of cellular details were obtained using a standard drawing prism attachment of the microscope.

For powder microscopy, finely powdered samples (~2 g) were separately treated with different solutions *viz.* aqueous saturated chloral hydrate (for maceration), 50% glycerine, phloroglucinol in conc. HCl (for staining lignified tissues) and 0.02 N iodine reagent (for starch grains), mounted on slides with glycerine following a standard protocol and observed under the binocular compound microscope (Olympus OIC-07964) at 10× and 40× magnifications. The Camera Lucida drawing of cellular details was obtained as above[25]. Photomicrographs of different cellular structures and inclusions were also taken using a Magcam DC14 camera attached to an Olympus CX21i trinocular compound microscope.

Fluorescence analysis

Different solvents (including distilled water) or reagents (5 mL, each) were added to the coarse sample powder taken into clean test tubes (~0.5 g in each tube), which were shaken well and allowed to stand for about 20-25 minute. The individual solutions were observed under normal white daylight and UV (254 and 365 nm) light for their characteristic colours and compared with the standard colour chart[27,28].

Physicochemical evaluation

The physicochemical parameters like ash values, loss on drying, extractive values, and pH value of the plant material were determined as per World Health Organisation guidelines[29].

Phytochemical screening

The coarsely powdered plant materials were subjected to Soxhlet extraction for 3 hours, separately with petroleum ether, chloroform, ethyl acetate, ethanol and water. The individual extracts were evaporated to dryness and stored at ~4 °C in an airtight container for screening the presence of secondary metabolites[28].

Biochemical characterisation

Total phenolics and tannins contents were determined using Folin-Ciocalteu reagent[30]. The proximate alkaloidal assay was done using bromocresol green reagent[31] and total flavonoids contents were measured with modified ferric chloride reagent[32].

Fingerprint analysis

Given that the chloroform extract of the plant materials gave the maximum extractive value, the same was used for the fingerprinting analysis. For this, the plant material (2 g) was extracted with chloroform (20 mL) using a Soxhlet apparatus. The extract was filtered and used for fingerprinting analysis. A chloroform solution of betaine, isolated during the course of the present work (*vide infra*) was used for comparison.

HPTLC fingerprinting

The extract (3 µL) and betaine solution were applied in the form of 8 mm bands, 15 mm from the bottom of a 10 cm × 10 cm preactivated aluminium supported precoated silica gel $60F_{254}$ plate, with the help of an ATS-4 applicator attached to a CAMAG HPTLC system. The plate was developed in a pre-saturated twin trough chamber using the mobile phase as hexane: ethyl acetate: formic acid (6:4:0.1, v/v) to a distance of 8 cm, dried for 5 minutes at an ambient temperature. Images of the developed plate were captured under 254 and 366 nm UV light. Densitometric scanning[33] of the developed plate at 366 nm was performed for the identification of betaine. An image was also captured using visible light after derivatising the plate with aqueous 20% sulphuric acid[34].

HPLC fingerprinting

This was carried out with HPLC equipment (Perkin Elmer model PE 200 series), equipped with quaternary LC-2010 AHT VP pumps, a variable wavelength programmable UV/VIS detector, SPD-10AVP column oven and Class-VP software for analysis. The chromatographic separation was performed using a Phenomenex C_{18} (250 mm × 4.6 mm, 5 µm particle size) column at 25 ºC. The optimized mobile phase was found to be methanol-0.1% aqueous orthophosphoric acid 90:10 (v/v) a flow rate of 0.6 mL/min. An autosampler with an injection volume of 20 µL was used for sample loading and the peaks were detected at 254 nm UV.

FTIR fingerprinting

A drop of the extract was placed between two potassium bromide pellets to obtain a thin layer, which was analysed with a FTIR spectrophotometer (Agilent Cary 630).

Betaine isolation

The coarse *A. augusta* stem bark powder (10 g) was taken in chloroform (100 mL) and refluxed for 3 hours. The mixture was filtered and concentrated on a water bath up to 5 mL, an aliquot of the extract applied on a 2" wide strip of a precoated silica Gel $60F_{254}$ plate and developed with hexane: ethyl acetate: methanol (8:1:1) as the mobile phase. The alkaloid region on the plate was located at R_f ~0.56 by spraying the plate with Dragendorff's reagent. For isolation, the rest of the chloroform extract was applied on 16 plates using a 500 µL syringe and the plates developed with the aforesaid mobile phase. Strips of the plates at R_f ~0.56-0.57 were cut, individually extracted with chloroform, the extracts pooled, filtered, and concentrated on a hot water bath to obtain betaine as a whitish solid, as characterized by mass spectroscopy.

Results and Discussion

Macroscopic characters

The fragmented stem bark is dark brown and 0.2 to 0.3 cm thick. The outer surface is blackish brown and longitudinally wrinkled with small warty markings, sometimes with cork exfoliates from the outer surface. The inner surface is whitish-yellow and finely longitudinally striated, slimy and tough, but not brittle without any characteristic odour and taste. When soaked in cold water for 3-4 days, the bark produces slimy mucilage which can be extracted (Fig. 1).

Cytomorphological characters

The transverse sections (T. S.) of mature stem bark show periderm, consisting of 6-8 layers of cork, 1-2 layers of phellogen and a 3 to 4 of phelloderm. Cork 6-9 cell layered with tangentially elongated thin-walled, suberized cork cells. The phelloderm with parenchymatous, thin-walled, circular or elliptical to polygonal cells, and are characterized by irregular divisions, large mucilaginous cavities, starch grains and rosette crystals of calcium oxalate. Pericyclic fibres are present. The secondary phloem is wedge-shaped with sieve tubes, companion cells, and phloem parenchyma, alternating with the strands of phloem fibres traversed by medullary rays. In transverse sections, the phloem parenchymatous cells are polygonal. The fibres are thick-walled, lignified, the extremities being sharply pointed or chisel-like or truncated. The walls of the fibres are generally entire, but in some cases, dentations

Fig. 1 — Photograph of fragmented *A. augusta* stem bark.

occur on one side of the tapering ends. Anatomically the stem bark resembles the root bark containing more or less similar cytomorphological pattern except for some differences i.e., cork layer is wider in root bark which is devoid of pericyclic fibres, chloroplast, collenchymatous cortex etc[35]. When soaked in cold water for 3- 4 days, the bark produces slimy mucilage which can be extracted (Fig. 2).

Fine powders are yellowish-brown with no distinguishable odour and taste; show the presence of a group of polygonal angular lignified cork cells in layers, starch grains, rosette and prismatic crystals of calcium oxalate, profuse fibres in a group, fragmented phloem tissue, rhytidoma and groups of parenchymatous cell with rosette crystals are also observed. Stem bark powder characters (Fig. 3 and 4) are more or less similar to that of root bark[35].

Fluorescence analysis

The qualitative fluorescence analysis of coarse dried plant powders treated with different reagents provides useful information on the presence of chromophoric compounds in them. In the present studies, the *A. augusta* stem bark powders showed white and bright creamish fluorescence with 1 N HCl, methanol plus 50% HNO_3, and 50% HCl respectively

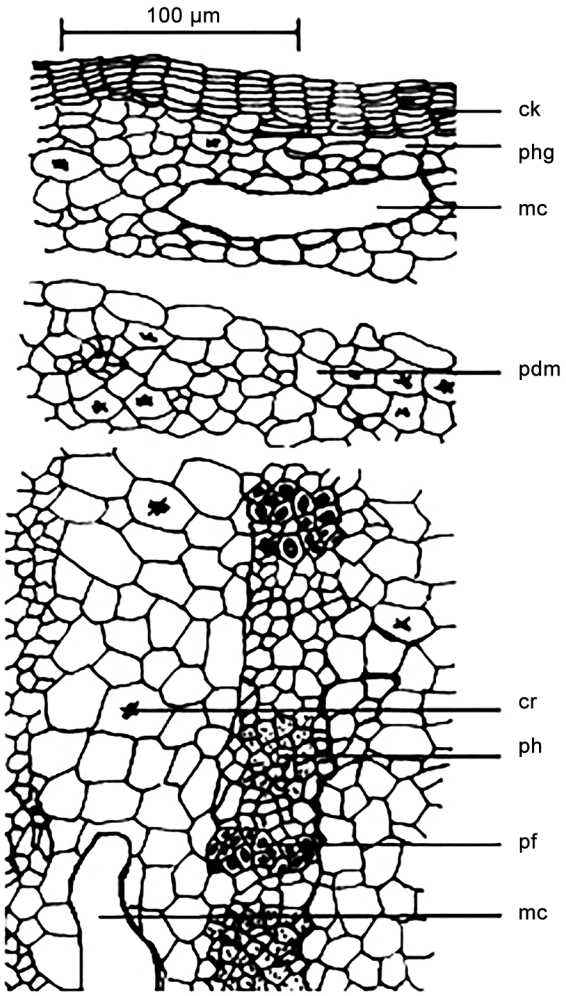

Fig. 2 — Cellular anatomy (T. S.) of *A. augusta* stem bark. [ck- cork; phg- phellogen; mc- mucilage canal; cr- crystal; pdm- phelloderm; ph- Phloem; pf- Phloem fibre]

under 366 nm UV light. No fluorescence was observed under normal daylight and short UV (254 nm) light, indicating a very less amount of chromophores in the sample (Table 1). The fluorescence characters of the root barks show very less similarities with the stem bark[35]. The appearance of fluorescence character is distinguishably different when powder mounted with methanolic NaOH.

Phytochemical screening

Phytoconstituents are the natural bioactive compounds found in plants. It works with nutrients and fibres to form an integrated part of the defence system against various diseases and stress conditions. They are divided into two groups like primary and secondary metabolites, according to their functions in plant metabolism. Primary metabolites consist of common sugars, amino acids, proteins and

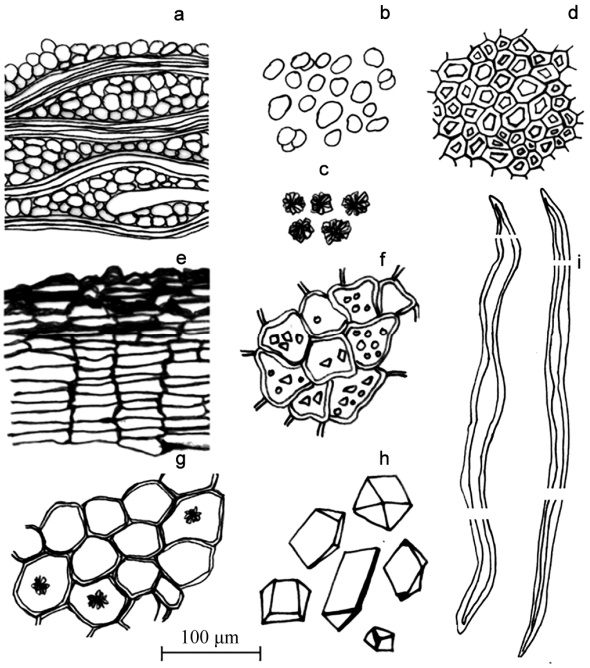

Fig. 3 — Powder study (Camera Lucida drawing) of *A. augusta* stem bark. a) fibres associated with parenchymatous cells, b) starch grains, c) rosette crystals, d) cork cells in group, e) cork cell layers, f) reddish brown parenchyma with prismatic crystals, g) parenchyma with rosette crystals, h) prismatic crystals of Ca-oxalate, and i) Aseptate fibres.

chlorophyll while alkaloids, terpenoids, flavonoids, tannins etc. contribute as the secondary metabolites. In the present study, different qualitative tests were carried out with the *A. augusta* stem bark samples after extraction with various solvents. The results of the phytochemical screening (Table 2) revealed the presence of alkaloids, steroids, saponins, tannins, phenolics and glycosides, but fewer terpenoids, flavonoids and carbohydrates. The tannins, phenolics and glycosides were primarily present in the protic, polar solvents. The presence of major alkaloid betaine is not reported[35] from the stem bark. In addition to that, carbohydrates and long-chain fatty acids are present in the root bark.

Biochemical characterization

The results of the proximate estimation of phenolics, tannins, flavonoids, and alkaloids are shown in Table 3. The sample was enriched with phenolics and alkaloids, while its total flavonoids content was very poor. The data are consistent with our fluorescence analysis and qualitative screening results (Tables 1 and 2 respectively). The root bark was reported[35] to have aliphatic alcohols and aromatic alcohol. The presence of long-chain fatty acids is also reported.

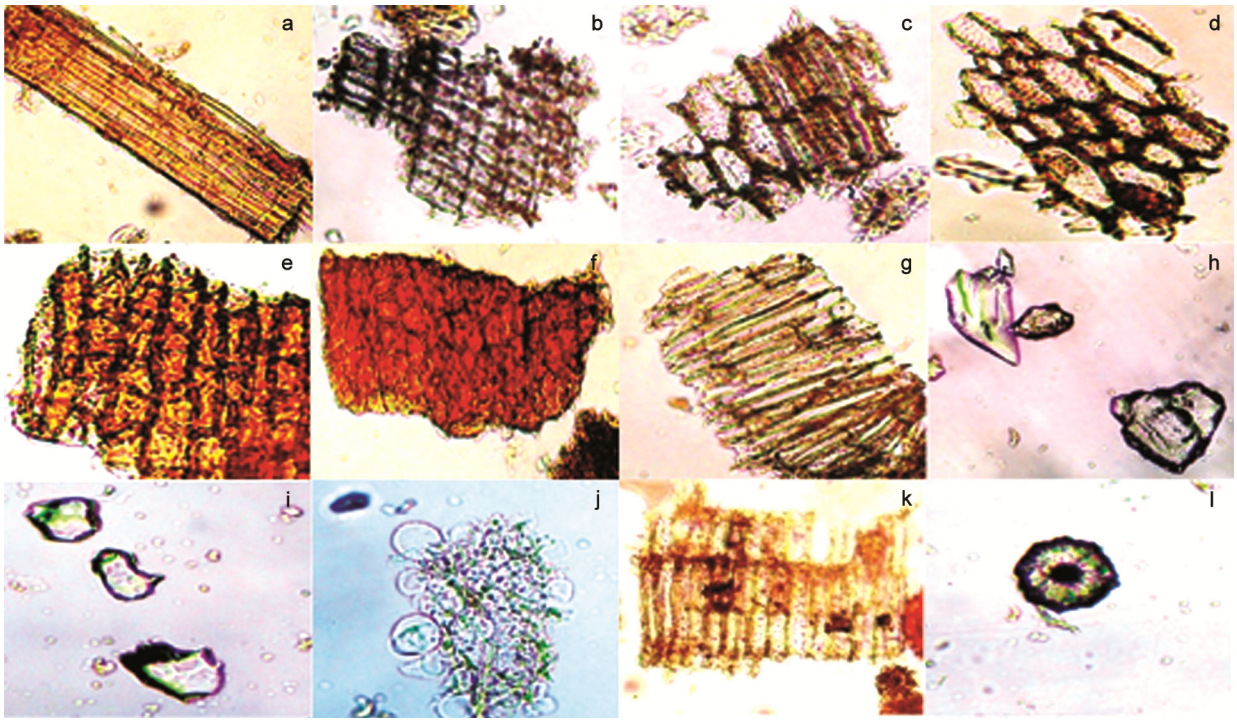

Fig. 4 — Photomicrograph of *A. augusta* stem bark powders. a) group of fibres, b) group of cork cells in layers, c,d) pitted schlerenchyma, e,f) squarish thick walled parenchyma with golden reddish brown content, g) rhytidoma, h,i) prismatic crystals of Ca-oxalate, j) starch grains, k) phloem tissue with rosette crystals, and l) rosette crystal of Ca-oxalate.

Table 1 — Florescence analysis of *A. augusta* stem bark powders

S. No.	Reagents	Daylight	UV (254 nm)	UV (365 nm)
1	Distilled water	No colour	White	White '-ve'
2	1 N HCl	No colour	Pale creamish with pink tinge	White '+ve'
3	1 N NaOH	No colour	Pale bluish gray	Gray '-ve'
4	50% HCl	No colour	Pale creamish with yellow tinge	Bright cream '+ve'
5	50% H_2SO_4	Greyish	Bluish gray	Pale creamish with gray tinge '-ve'
6	Methanol+50% HNO_3	No colour	Light pinkish yellow	White '+ve'
7	Methanol + 1 N NaOH	No colour	Greyish	No colour'-ve'
8	Aqueous extract	No colour	White	White '-ve'
9	Methanolic extract	No colour	Pale bluish gray	White '-ve'
10	Pet. ether extract	No colour	No colour	White '-ve'
11	Ethyl acetate extract	Greyish	Bluish gray	Faint grayish '-ve'

Table 2 — Phytochemical screening of *A. augusta* stem bark

Phytochemical class	Hexane	Chloroform	Acetone	Ethyl acetate	Methanol	Ethanol	Water
Alkaloid	-	+	+	+	-	+	-
Flavonoid	-	-	-	-	-	+	-
Glycoside	-	+	-	-	-	+	+
Polyphenol	-	-	-	-	+	+	+
Phenolic		-	-	-	+	+	+
Saponin	+	-	+	-	-	-	-
Steroid	+	+	-	+	-	+	-
Tannin	-	-	-	+	-	+	+
Terpenoid	-	+	-	-	-	+	-
Carbohydrate	-	+	-	-	-	+	-

Table 3 — Biochemical characterization of *A. augusta* stem bark.[a]

Parameters	Percentage
Total phenolics	2.21 ± 0.39%
Total flavonoids	0.51 ± 0.02%
Total tannins	0.88 ± 0.02%
Total alkaloids	1.38 ± 0.37%

[a]Values are expressed as Mean±S.D.

Physicochemical evaluation

Evaluation of the physicochemical parameters of the plant samples showed (Table 4) its total ash value as 8.635%, water-soluble and acid insoluble ash contents as 1.383 and 2.135% respectively, while its weight loss on drying was 1.342%. The very low moisture of *A. augusta* stem bark suggested that it can be stored at an ambient temperature without much spoilage. The extractive values of different solvents for the plant samples revealed maximum and least extraction by chloroform and hexane respectively. Except for hexane, the extraction yields of the respective solvents were not significantly different under both cold and hot conditions. However, the extraction yield in hexane was higher under hot maceration. Notably, all these values differ significantly from that reported earlier with *A. augusta* roots[13]. Based on the best phytoconstituents yield in chloroform extract, the same was used for the subsequent fingerprinting analyses. From the literature[35] it has been observed that total ash values are a little lesser in the root bark. Alcohol soluble extractive is on a little higher side for stem bark than that of root bark, while vice versa is true for water-soluble extractive.

HPTLC fingerprinting and identification of betaine

The HPTLC conditions for the best separation of the phytoconstituents were optimized using pre-activated HPTLC silica gel 60 F_{254} plates and different combinations of polar and apolar solvents as the mobile phases (data not shown). The best result was obtained with hexane: ethyl acetate: formic acid (6:4:0.1, v/v) as the mobile phase, which showed four bands at R_f values of 0.26, 0.42, 0.62, 0.71 when visualized under UV at 254 nm. At 366 nm, five bands at R_f values 0.37, 0.52, 0.58, 0.63, 0.69 were seen, while several bands at R_f 0.03, 0.11, 0.18, 0.29,

Table 4 — Physico-chemical evaluation of *A. augusta* stem bark[a]

Physico-chemical parameters	Percentage	
Loss on drying (LOD)	1.34±0.32	
Ash values		
Total ash value	8.64±0.21	
Acid insoluble ash value	2.14±0.31	
Water soluble ash value	1.38±0.39	
Sulphated ash	1.93±0.37	
pH value (10% aq. suspension)	6.12±0.27	
Extractive values	Cold extraction	Hot maceration
Hexane	1.02±0.32	1.98±0.32
Acetone	6.91±0.35	6.85±0.39
Chloroform	12.8±0.20	12.9±0.29
Ethyl acetate	7.10±0.31	6.35±0.31
Methanol	7.92±0.31	7.22±0.23
Alcohol	10.57±0.32	10.52±0.37
Water	9.34±0.29	9.78±0.32

[a]Values are expressed as Mean±S.D.

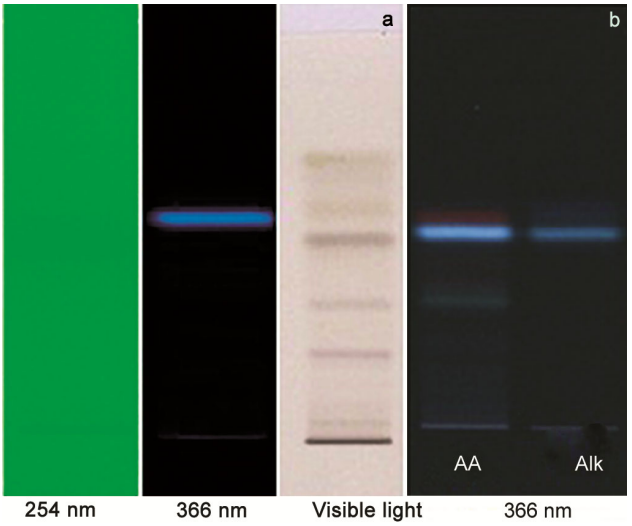

Fig. 5 — (a) HPTLC profiles of *A. augusta* stem bark chloroform extract. (b) Comparison of the HPTLC profiles of the extract and chloroform solution of betaine, visualized at 366 nm.

0.46, 0.54, 0.61, 0.67) were seen after derivatisation and followed by exposure to visible light (Fig. 5a). Since the qualitative phytochemical analysis of the extract revealed the presence of alkaloids, we compared the HPTLC profiles of the extract and betaine (one of the known chemical constituents of the plant) by visualizing the plates at 366 nm. As shown in (Fig. 5b), both the samples showed bluish bands at R_f = 0.52. The densitometric scanning of the chromatogram (Fig. 6a-b) showed different peaks (bands) of phytoconstituents, wherein the 3rd peak was found to be betaine with R_f value of 0.52.

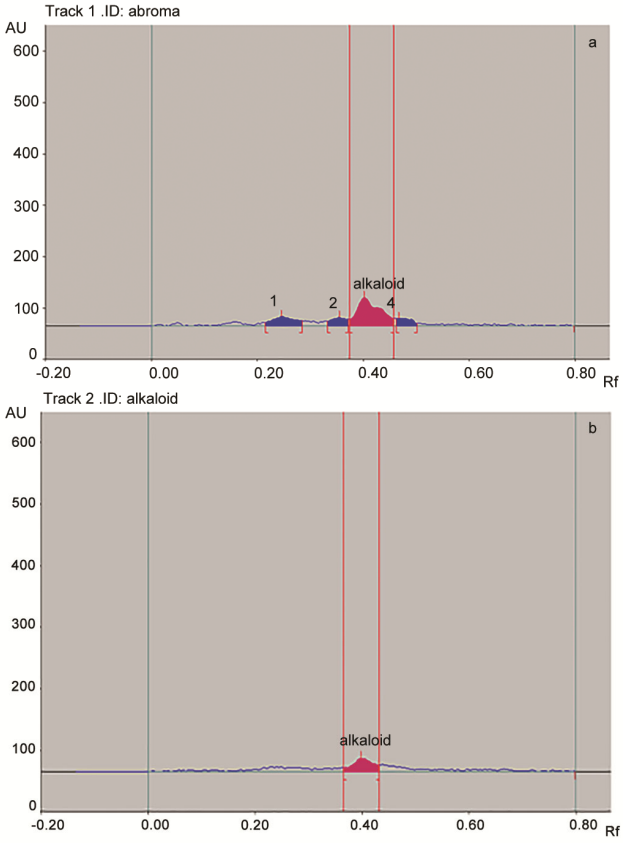

Fig. 6 — Densitogram display of HPTLC profiles of (a) *A. augusta* stem bark chloroform extract. (b) betaine. The alkaloid, betaine is shown in red histograms.

HPLC fingerprinting

An HPLC method was developed for the best resolution of the chemical constituents of the

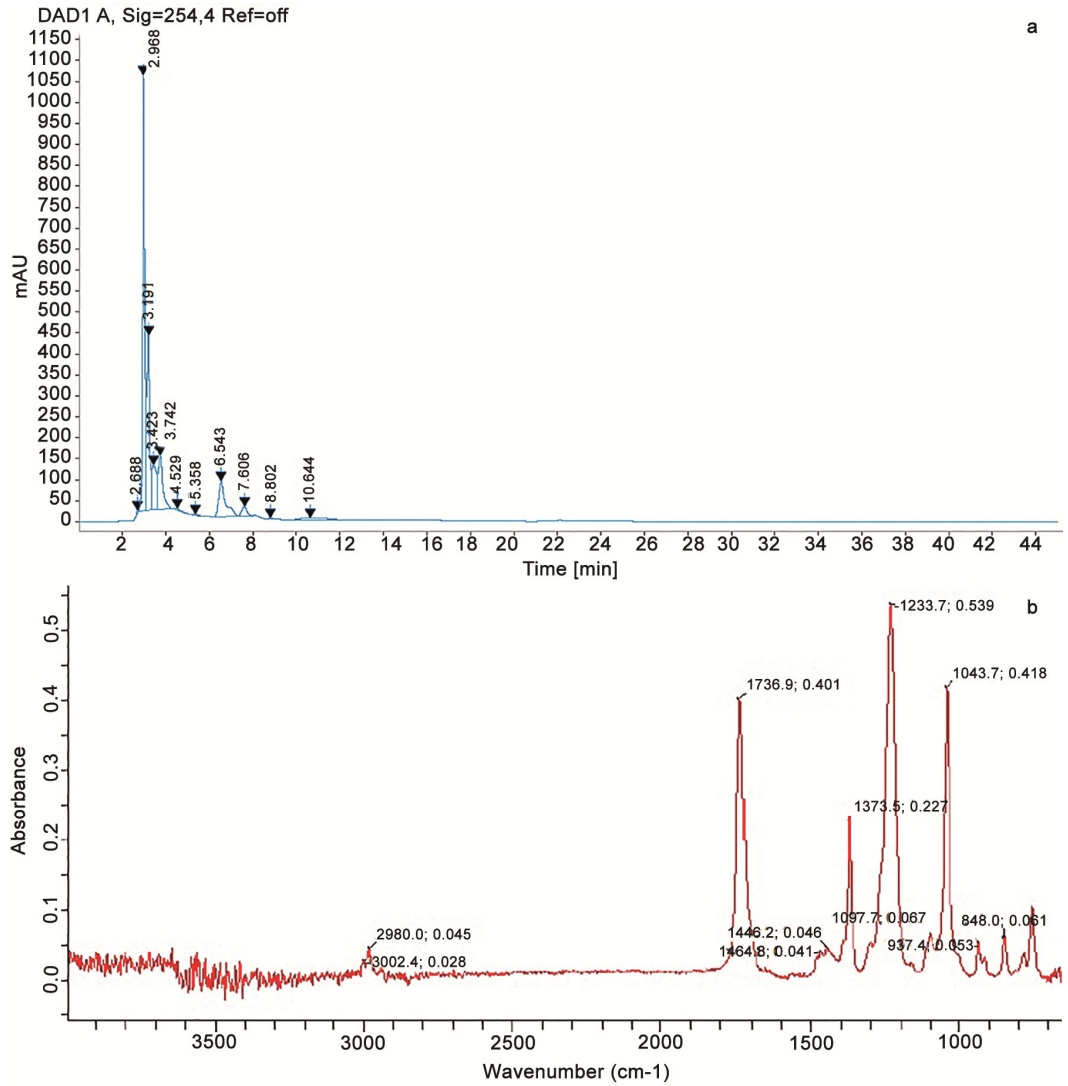

Fig. 7 — HPLC chromatogram (a) and FTIR spectrum (b) of *A. augusta* stem bark chloroform extract.

A. augusta stem bark chloroform extract, under UV (254 nm) detection. The HPLC fingerprint analysis showed (Fig. 7a) with nine peaks with retention times 3.19, 3.42, 3.74, 4.53, 5.36, 6.54, 7.61, 8.80 and 10.64 min respectively. This was comparable to the HPTLC profile of the extract after derivatization and white light detection. The relative ratios of the peaks of the HPLC and HPTLC chromatograms, determined from the areas under the curves are represented in Tables 5 and 6 respectively.

Table 5 — Relative ratios of the HPLC peaks[a]

S. No.	Peak retention time (Minute)	Relative ratio (%)
1	2.688	0.1699
2	3.191	37.5071
3	3.423	16.2432
4	3.742	18.7551
5	4.529	0.2669
6	5.358	0.1831
7	6.543	18.4100
8	7.606	3.5610
9	8.802	0.3390
10	10.644	4.5578

[a]The peaks were recorded by visualising the chromatogram spots at 254 nm.

FTIR fingerprinting

The FTIR spectrum of the chloroform extract is shown in (Fig. 7b). The absorption spectrum revealed distinct peaks at 1373.6, 1233.7 and 1043.7 cm^{-1} indicating the presence of compounds with C-N or C-O bonds. The very low intensity peaks in the region of 2800-3000 cm^{-1} indicated lack of C-H bonds suggesting absence of long-chain alkanes/alkene and their derivatives. The peak at 1736.9 cm^{-1} may account for the C=O stretch of some carbonyl containing group (possibly betaine carboxyl).

Table 6 — Relative ratios of the HPTLC peaks[a]

S. No.	Rf values	Relative ratio (%)
1	0.37	10.14
2	0.52	13.63
3	0.58	36.38
4	0.63	25.14
5	0.69	14.71

[a]The peaks were recorded by visualising the chromatogram spots at 366 nm.

Isolation of betaine

Betaine was isolated from the chloroform extract of the plant material, which was subjected to HPTLC using hexane: ethyl acetate: methanol (8:1:1, v/v) as the mobile phase. The location of betaine at R_f ~0.56 was identified on one plate using Dragendorff's reagent, while other plates were used for its isolation. Betaine was obtained as a white solid in 0.003% yield from the stem bark. Its mass spectrum showed two peaks at m/z 117.08 ($[M]^+$, relative intensity 100%) and 118.08 ($[M+1]^+$, relative intensity 5%). The mass spectral pattern matched that of betaine given in the Wiley mass library, confirming its structure. The previous anomaly of its identification as abromine and betaine by two groups[15,16] might be because of inadequate characterization. Thus, one of the major findings of the present study is the proper characterization of the alkaloid from *A. augusta*.

Conclusion

The present investigations furnished a set of qualitative and quantitative phyto-pharmacognostic parameters along with HPTLC, HPLC, and FTIR; fingerprinting profile of *A. augusta* stem bark. These data can serve as diagnostic tools for the establishment of quality standards, authentication, and identification of the medicinally important plant, and help in compiling a suitable monograph of this. The stem bark contains an appreciable amount of phytochemicals such as alkaloids and phenolics, which may confer health benefits. In particular, its constituent betaine that is a known osmolyte may be responsible for the efficacy of the plant against gynaecological and urinary disorders. The stem bark can be easily identified by comparing the data of physicochemical characters. The fluorescence analysis of the powdered materials gave distinguishable characters which help identify the stem and root bark.

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