ISSN 2456-4990

J Ayu Med Sci

Quarterly Journal for Rapid Publication of Researches in Ayurveda and Other Traditional Medicines

Journal of Ayurveda Medical Sciences

J Ayu Med Sci | 2017 | Vol 2 | Issue 4 (Oct – Dec)

www.jayumedsci.com

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Journal of Ayurveda Medical Sciences
Quarterly Journal for Rapid Publication of Researches in Ayurveda and Other Traditional Medicines
Original Article

Pharmacognostic evaluation of *Desmodium oojeinense* (Roxb.) H. Ohashi - Stem bark

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ABSTRACT

Introduction: *Desmodium oojeinense* (Roxb.) H. Ohashi, (Syn. *Ougeinia oojeinensis* (Roxb.) Hochr. is a tree belonging to family Fabaceae. Bark is reported to be astringent, acrid, cooling, anti inflamatory, sadorrificand rejuvenating and used in the treatment of diabetes. ***Method:*** Detailed macroscopy , microscopy, histochemical tests, fluorescense analysis, behaviour of powdered drug with different chemical reagents were performed for pharmacognostic evaluation of stem bark by following standard methods. ***Results:*** Stem bark showed presence of rhytidomes, groups of stone cells, resin ducts, uni and biseriate medulary rays, starch grains, prismaic crystals of calcium oxalate. Histochemical tests, behaviour of drug with different chemical reagents and fluorescense analysiss howed the presence of lignin, resin, oils and crystals which would prove to be a unique parameters for identification of the drug. ***Conclusion:*** Findings of this study will be helpful for identification and authentication of stem bark.

KEYWORDS Fluorescense, Microscopy, *Ougeinia oojeinensis*, Pharmacognosy.

PICTORIAL ABSTRACT

ARTICLE HISTORY **Received** 14.11.2017 **Accepted** 03.01.2018

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CITE THIS RESEARCH AS Nirawane RB, Gurav AM, Rao G, Mangal AK, Narayanam S. Pharmacognostic evaluation of *Desmodium oojeinense* (Roxb.) H. Ohashi - Stem bark. J Ayu Med Sci 2017;2(4):261-8.

DOI 10.5530/jams.2017.2.33

1. Introduction

Desmodium oojeinense (Roxb.) H.Ohashi, (Syn. *Ougeinia oojeinensis* (Roxb.) Hochr.)is a tree belonging to family Fabaceae. It is native of India and also found in Nepal. Bark is astringent, acrid, cooling, sadorrific, depurative, stypic and rejuvenating[1]. Bark is reported to posseses antiinflamatory[2], antispasmodic[3], hypoglycemic and hypolipidemic activity and useful in the management of diabetes[4-6]. It is used to treat diarrhoea, dysentery and used as fish poison and febrifuge[7-10]. It is also useful in anaemia, leucoderma, ulcer and biliousness[10]. Methanolic extract of bark contains alkaloids, terpenoids, flavonoids, glycosides, tannins, saponins, proteins and carbohydrates.[11]

Pharmacognostic and phyto chemical evaluation of bark has been reported[12] but the detailed study was needed to standardize the bark drug as per the standards laid down by Ayurvedic Pharmacopoeaia of India and guidelines provided by WHO for medicinal plants.

2. Materials and methods

2.1 Plant material

Stem bark and flowering twigs of *D. oojeinense* were collected from the plant growing in the campus of Abasaheb Garware, Art and Science College, Pune, Maharashtra, India.

2.2 Identification and authentication of plant material

Plant material was identified and authenticated with the help of the Flora[13]. Plant material was also compared with the herbarium speciemen available in the Botanical Survey of India, Western Circle, Pune.

2.3 Preparation of herbarium

Herbarium of the plant speciemen were prepared and deposited in the herbarium section of the RAIFR, Pune with voucher speciemen number 4526/2014[14].

2.4 Preservation of wet sample

Freshly collected and thoroughly washed bark pieces were kept in a glass bottle containing a mixture of formalin: glacial acetic acid: 70% ethyl alcohol [10:5:85][15].

2.5 Powder preparation

Shade dried, stem bark pieces were made in to powder using grinding mill; passed through #60 sieve and kept in airtight container for further analysis.

2.6 Macroscopic characterization

Macroscopic characters like fracture, shape, size,colour, taste, odour of bark and powder were determined by naked eyes[16].

2.7 Microscopic characterization

Free hand transverse sections (TS) of bark were taken and stained with Safranin and Phloroglucinol, followed by Hydrochloric acid. Micro photographs were snapped with the help of Deno Capture 2.0 version 1.4.2.D, the versetile digital microscope[16].

2.8 Histochemical and fluorescense analysis

Dried bark powder was used for the analysis of histochemical, physico-chemical aspects such as behaviour of powder, fluorescense analysis[17].

2.9 Determination of physicochemical parameters

Physicochemical parameters namely, loss on drying, ash value, acid insoluble ash, water soluble ash, water soluble extractive, alcohol soluble extractive were performed as per the standard procedures[18].

2.10 Preliminary qualitative analysis

Preliminary qualitative tests were performed by following standard methods[16].

2.11 Thin layer chromatography

Thin layer chromatography of aqueous and methanolic extract of *D. oojeinense* - stem bark was developed using solvent system toluene: ethyl acetate: acetic acid: methanol (5:3:1:1) which was saturated for 45 minute in CAMAG twin trough chamber. Both extractswere applied manually on TLC Silica gel 60 F_{254} Aluminum coated plate and run up to 8 cm. Plates were observed under day light, ultra violet light at 254 nm and 366nm and subsequently derivatized with Iodine vapour and anisaldehyde-sulphuric acid. Developed band colours and retention factor (R_f) were recorded. Photo documentation was done with the help of digital SLR Canon camera.

Figure 1. Macroscopic features of *Desmodium oojeinense* stem bark

1.1 Natural habit

1.2 Stem

1.3 Stem bark

1.4 Transversly cut surface of bark

3. Results and discussion

3.1 Organoleptic Character of Powder

Stem bark powder is light brown in colour, coarse, fibrous, odourless, initially slightly sweet and then slightly astringent.

3.2 Macroscopy

Mature pieces of bark are flat or slightly curved in shape, 5 to 6 cm in length, 2 to 2.5 cm in width and 4 to 8 mm in thickness. Outer surface of bark is powdery, slightly rough, and creamy to light brown in colour. There are small patches, oozing brown resin. Powdery layer alternating with rough surface transversely up to rhytidome; occasionally longitudinally cracked. Inner surface rough, yellowish brown, due to oozing of resin, splintery fractured (Figure 1).

3.3 Microscopy

Diagrammatically,transverse section of bark showed a rhytidome outside which occupies half area of entire bark with scattered groups of fibres, resin ducts and biseriate medullary rays. Rhytidome alternating with lignified irregular cells of powdery layer followed by cork. The innermost layer is phloem traverse with groups of fibres, resin duct, biseriate medullary rays and patches of compressed phloem (Figure 2.1).

Detailed T.S. of bark shows outer region of rhytidome composed of 6 to 7 rows of rectangular cells of cork, followed by 2 to 3 rows of groups of stone cells and sclerides. Pentagonal, hexagonal or polygonal cells of rhytidome traverse with groups of thin walled fibres, compressed phloem, groups of empty resin duct and biseriate medullary rays. Parenchymatous cells and medullary rays embedded with starch grains and prismatic crystals of calcium oxalate. Rhytidome alternating with 6 to 8 layers of irregular, lignified cells of powdery layer, followed by 6-7 layers of rectangular cells of cork. Below this group 2-5 layers of of stone cells were observed. Secondary phloem made up of somewhat polygonal to oval shaped cells traverse with groups of thin walled fibres, groups of resin duct, compressed cells of phloem and biseriate medullary rays. Phloem parenchyma and medullary rays embedded with simple, compound, oval starch grains and prismatic crystals of calcium oxalate (Figure 2.2 and 3).

3.4 Powder Microscopy

Powder under microscope showed different anatomical characters. Details of characters recorded are given in Figure 2.3 and 4.

3.5 Histochemical and fluorescense analysis

Powder drug was analysed for histochemical tests for detection of lignin, aleurone grains, oils, mucilage and crystals. Analysis revealed that bark contains lignin, resins, oils, starch and calcium oxalate crystals which are the unique characters of bark (Table 1).

Powder drug was treated with different chemical reagents and observed in day light and ultra-violet light and inferences was recorded in Table 2. Drug colour changed after reacting with chemical and observed under 254 and 366 nm which is would be characteristics for particular drug and would be beneficial to identify the bark of *D. oojeinense*.

Behaviour of drug with different chemical reagent showed that drug immidiately move down in Glacial acetic acid, 5% $FeCl_3$ solutions whereas powder colour changed in concentrated H_2SO_4, HNO_3 and HCl solutions. Details of observations are exhibited in Table 3.

3.6 Determination of physicochemical parameters

Results obtained from physico- chemical parameters such as, loss on drying, total ash, acid insoluble ash, water soluble ash, water and alcohol soluble extracts are depicted in Table 4.

3.7 Preliminary qualitative analysis

Preliminary analysis revealed that bark contain, carbohydrates, saponins, anthroquinone, glycoside, tanins, phenol and organic acids. Details of the result given in Table 5.

3.8 Thin layer chromatography

TLC of methanolic extract developed 7 spots in 254 nm, 1 spot in 366 nm, 8 spot in anisaldehyde-sulphuric acid and 12 spots in Iodine. Whereas, single spot developed in aqueous extract at 0.72 R_f observed in 254 nm, and after derivatized with anisaldehyde-sulphuric acid and Iodine. Details are shown in Table 6 & 7 and Figure 5.

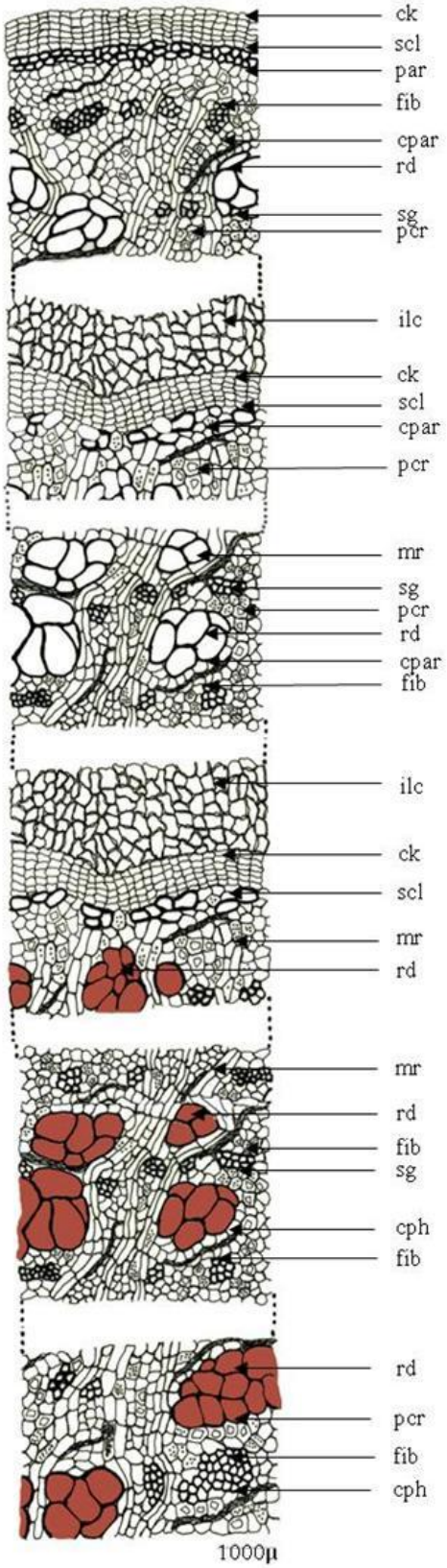
Table 1. Histo-chemical analysis of *Desmodium oojeinense* stem bark

Test	Chemical	Observation	Result
Lignified cell walls	Phloroglucinol + HCl	Pink to cherry red colour	+
Cuticular cell walls	Sudan red -III	Orange red or red	-
Aleurone grains	Iodine	Yellowish brown to brown	-
Fats, fatty oils, volatile oils and resins	Sudan red- III	Orange red to red	+
Mucilage	Ruthenium red	Pink	-
Starch	Iodine	Blue or reddish blue	+
Calcium oxalate crystals	Hydrochloric acid	Dissolved	+
Calcium carbonate crystals	Hydrochloric acid	Dissolved with effervescence	-

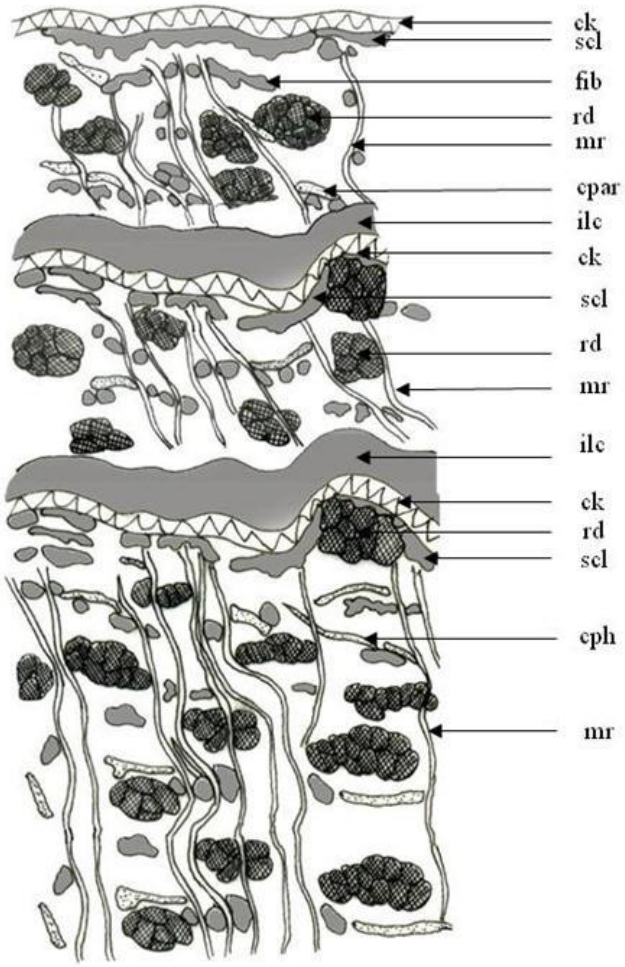
Table 2. Fluorescence analysis of *Desmodium oojeinense* stem bark

Test	Day light	254nm	366nm
Powder as such	Fawn	Fawn	Brown
Powder + H_2O	Umber	Greyish sepia	Olivaceous
Powder + HCl	Sepia	Dark mouse grey	Fuscous Black
Powder + HNO_3	Sepia	Isabelline	Fuscous Black
Powder + H_2SO_4	Dark brown	Black	Greenish Black
Powder + Glacial Acetic acid	Dark brick	Brown vinaceous	Chestnut
Powder +50 % HCl	Brown	Chestnut	Sepia
Powder +50% HNO_3	Brown	Chestnut	Sepia
Powder +50% H_2SO_4	Brown	Chestnut	Fuscous Black
Powder +50% G. Acetic acid	Reddish Brown	Dark mouse grey	Fuscous Black
Powder + 1N NaOH	Dark Brown	Dark mouse grey	Fuscous Black
Powder +1N KOH	Dark Brown	Dark mouse grey	Fuscous Black
Powder +5 % Iodine	Reddish Brown	Brown	Fuscous Black
Powder +5 % $FeCl_3$	Olivaceous	Isabelline	Dark libid
Powder + Liquid NH_3	Dark brick	Dark mouse grey	Fuscous Black

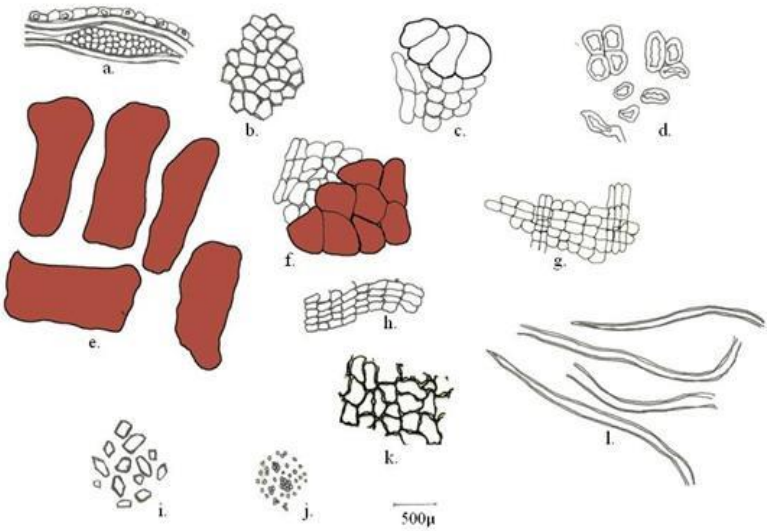
Figure 2. Camera lucida drawings of *Desmodium oojeinense* stem bark



2.2 Detailed TS of stem bark



2.1 Diagrammatic TS of stem bark



2.3 Powder characters

a. Fragment of radially cut medullary rays with prismatic crystals of calcium oxalate, b. fragment of cork cells in surface view, c. transversally cut fragment of phloem with resin cells, d. stone cells and sclerieds, e. isolated resin cells, f. transversally cut fragment of rhytidoma with resin cells, g. fragment of tangential cut medullary rays, h. fragment of cork cells in sectional view, i. prismatic crystals of calcium oxalate, j. simple, compound, oval starch grains, k. fragment of irregular lignified cells of powdery layer, l. thin walled, simple, sharp fibres.

ck- cork; cpar- compressed parenchyma; cph- compressed phloem; fib- fibres; ilc- irregular lignified cells; mr- medullary rays; par- parenchyma; pcr- prismatic crystals of calcium oxalate; rd- resin duct; scl- sclerenchyma; sg- starch grains.

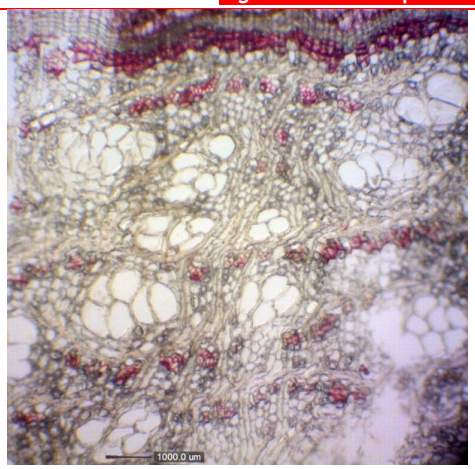
Table 3. Behaviour of *Desmodium oojeinense* stem bark powder with different chemical reagents

Test	Observations
Conc. H_2SO_4	a) Powder floats on surface. b) On shaking powder move down up to 1.5 cm and remains suspended. c) Powder colour changed from brown to dark brown.
Conc. HNO_3	a) Powder floats on surface. b) On shaking few particles move down slowly and majority of particles remains suspended. c) Powder colour changed from brown to yellowish orange.
Conc. HCl	a) Powder Floats on surface. b) Few particles slowly move down. c) On shaking majority of particles move down and remain suspended and few settled down. d) Powder colour changed from brown to dark brown
Glacial Acetic acid	a) Powder immediately moves down. b) No colour change in powder
5% I_2 water	a) Powder floats on surface. b) On shaking few particles settled down and few remain suspended and solution became turbid.
5% $FeCl_3$	a) Powder immediately settled down. b) Colour changed from brown to black.
5% NaOH	a) Powder floats on surface. b) Few particles move down. c) On shaking particles move down and settled at the bottom; few remains suspended and no change in colour.
5% KOH	a) Powder floats on surface. b) Few particles moves down. c) On shaking particles moves down fastly and settled down at bottom and few remains suspended and no change in colour.

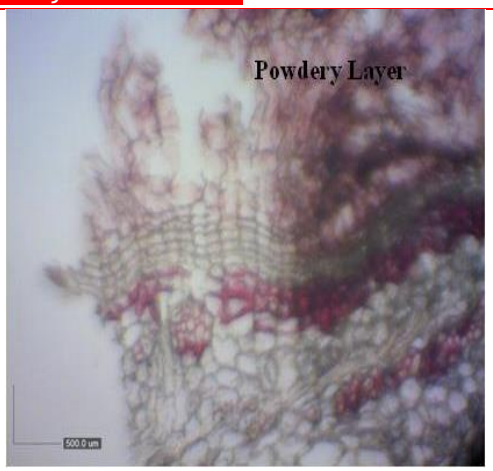
Table 4. Physico-chemical constants of *Desmodium oojeinense* stem bark

Parameter	Result (%)
Loss on drying	10.98
Total Ash	16.57
Acid insoluble ash	0.06
Water soluble ash	2.12
Water soluble extractive	26.43
Alcohol soluble extractive	10.13

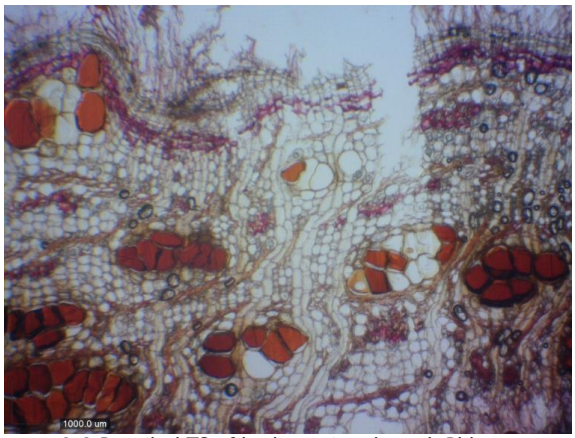
Figure 3. Microscopic features of of *Desmodium oojeinense* stem bark



3.1 Detail transverse section of bark passing through Rhytidome

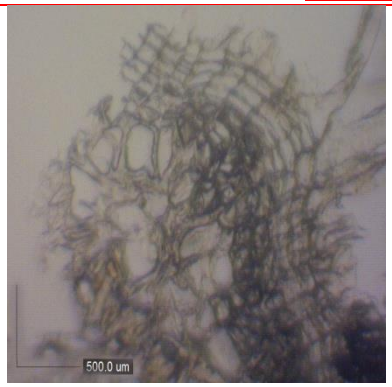


3.2 Powdery Layer



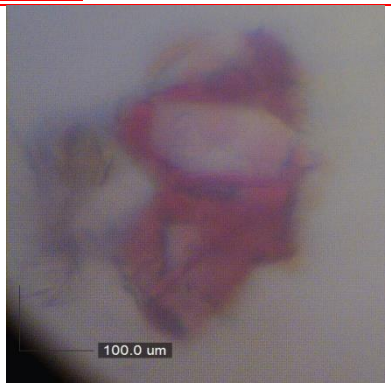
3.3 Detailed TS of bark passing through Phloem

Figure 3. Powder microscopy of of *Desmodium oojeinense* stem bark

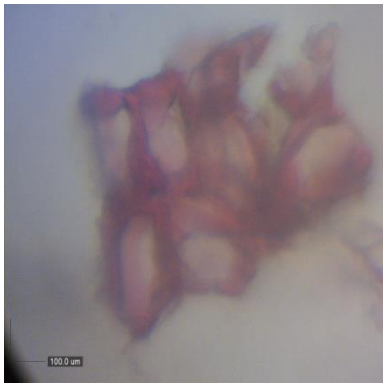


3.1 Fragment of cork cells in sectional view

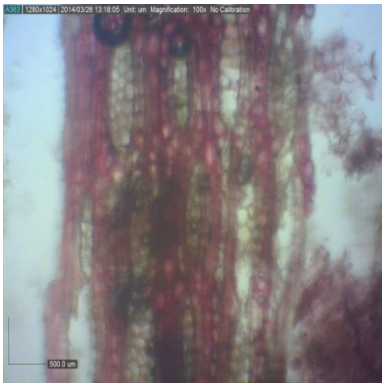
3.2 Fragment of cork cells in surface view



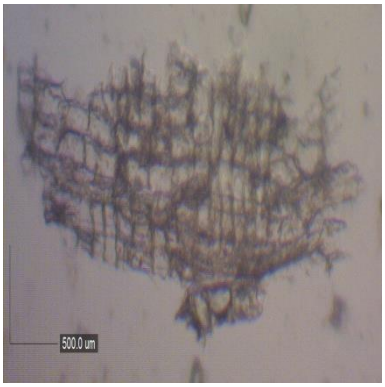
3.3 Stone cells and sclerieds



3.4 Stone cells and sclerieds



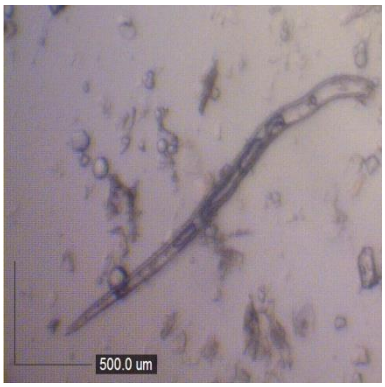
3.5 Fragment of radially cut medullary rays



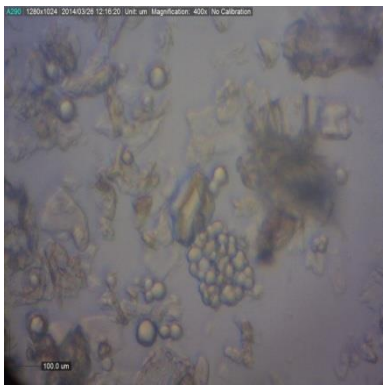
3.6 Tangential cut medullary rays

3.7 Isolated resin cells

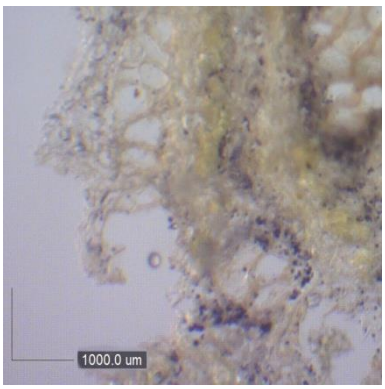
3.8 Thin walled, simple, sharp fibres



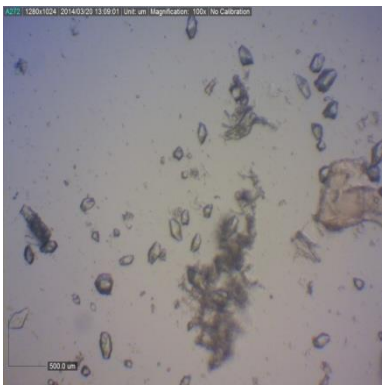
3.9 Thin walled, simple, sharp fibres



3.10 Simple, compound, oval starch grains



3.11 Simple, compound, oval starch grains stained with iodine



3.12 Prismatic crystals of calcium oxalate

Table 5. Preliminary qualitative analysis of *Desmodium oojeinense* stem bark

Phyto-constituent	Tests Performed	Extracts	
		Aqueous	Methanolic
Carbohydrates	Molish's test	+	--
Reducing sugar	a) Fehling's test	+	+
	b) Benedict's test	+	+
Pentose sugar	Phloroglucinol Reag. test	+	+
Hexose sugar	a)Tollen's Phloroglucinol test	+	+
	b)Cobalt Chloride test	+	+
Protein	a) Biuret test	_	_
Amino acid	a)Ninhydrin test	_	_
	b)Test for cysteine	_	_
Steroids	a)Libermann-Burchard test	_	_
	b)Salkowaski reaction	_	_
Glycoside	General test	_	_
Cardiac Glycosides	a) Legal test	_	_
	b) Keller-Killiani test	_	_
Anthroquinone Glycoside	a)Borntrager's test	_	+
	b) Modified Borntrager's test	_	+
Saponins	a)Foam test	+	--
	b) Lead Acetate solution test	+	--
Coumarin Glycoside	a)Aromatic odour test	_	_
	b) Fluorescence test	--	_
Flavonoids	a) Shinoda test	--	_
	b) Lead acetate test	_	--
Alkaloids	a) Dragendorff's test	_	_
	b) Mayer's test	_	_
	c) Wagner's test	_	_
Tanins	a) Lead acetate solution test	+	--
	b) Gelatine sol. test	_	--
Phenol	a) Neutral $Fecl_3$test	--	+
	b) Indophenol reaction	_	_
Starch	Iodine test	_	_
Organic acids*	Oxalic acid (In NH_4OH Extrat)	+	

-- not performed, *extracted with NH_4OH, _ absent, + present

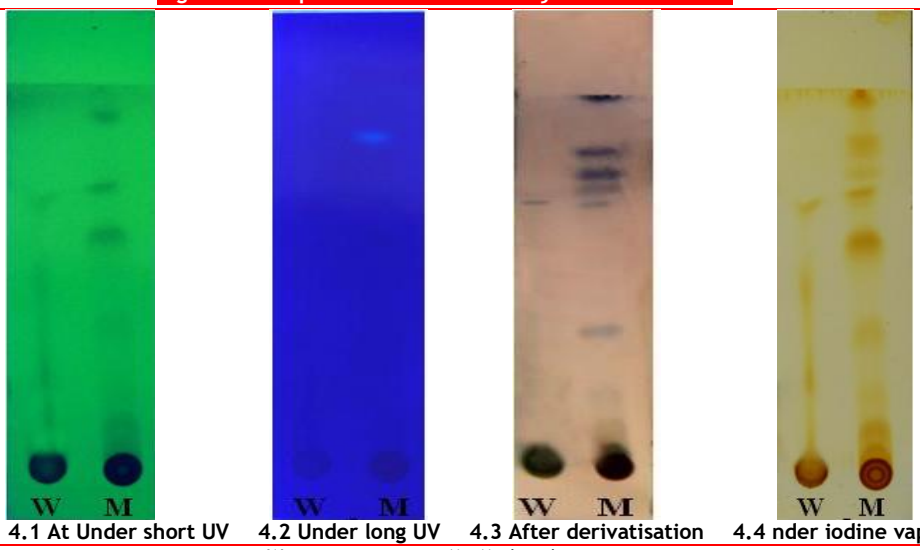
Table 6. Thin layer chromatography of methanolic extract of *Desmodium oojeinense* stem bark

254 nm		366 nm		Anisaldehyde sulphuric acid		Iodine	
R_f	Band colour	R_f	Band colour	R_f	Band colour	R_f	Band colour
-	-	-	-	0.04	Green	-	-
-	-	-	-	-	-	0.06	Brownish yellow
-	-	-	-	0.11	Yellowish brown	0.12	Brownish yellow
0.15	Black	-	-	-	-	-	-
-	-	-	-	0.19	Brown	-	-
0.21	Black	-	-	-	-	0.21	Yellow
-	-	-	-	0.31	Faint Navy blue	-	-
-	-	-	-	-	-	0.34	Yellow
0.36	Black	-	-	-	-	0.37	Yellow
0.40	Black	-	-	-	-	-	-
-	-	-	-	-	-	0.60	Brownish yellow
0.62	Black	-	-	-	-	-	-
-	-	-	-	0.71	Navy blue	0.71	Yellow
0.74	Black	-	-	0.74	Navy blue	0.74	Brownish yellow
-	-	-	-	0.79	Purple	0.78	Brownish yellow
-	-	-	-	0.85	Purple	0.85	Brownish yellow
-	-	0.89	Light blue	-	-	0.89	Brownish yellow
0.92	Black	-	-	-	-	-	-
-	-	-	-	-	-	0.97	Brownish yellow

Table 7. Thin layer chromatography of aqueous extract of *Desmodium oojeinense* stem bark

254 nm		366 nm		Anisaldehyde sulphuric acid		Iodine	
Rf value	Band colour	Rf value	Band colour	Rf value	Band colour	Rf value	Band colour
0.72	Black	-	-	0.72	Navy blue	0.72	Brownish yellow

Figure 5. TLC profile of *Desmodium oojeinense* stem bark



4.1 At Under short UV **4.2 Under long UV** **4.3 After derivatisation** **4.4 nder iodine vapors**

W - aqueous extract; M - Methanol extract

4. CONCLUSION

The results of this studies would be useful for identification, authentication and standardization of stem bark of *Desmodium oojeinense* (Roxb.) H. ohashi and also to detect the spurious adulteration in the genuine drug.

ACKNOWLEDGEMENTS Authors are thankful to Prof. Sharyu Sathe, Head of Department of Botany, Abasaheb Garware Arts and Science College, Pune for allowing to use genuine stem bark from their Botanical Garden.

SOURCE OF SUPPORT Nil

CONFLICT OF INTEREST Authors declare no conflict of Interest

CONTRIBUTORS Mrs. Rekha B Nirawane performed all experimental work in pharmacognosy, phytochemical parameter, Camera lucida drawings, TLC and any other laboratory work. Dr Arun M Gurav contributed to the planning and execution of research work, literature survey for article, drafting and finalization of article as per the format. Dr. Gajendra Rao contributed to vetting and suggestions on drafting. Dr. Anupam K Mangal and Dr. N Shrikanth edited the manuscript contents to acceptable form.

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