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Comparative phyto-pharmacognostical evaluation of *Desmodium gangeticum* (L.) DC and *Desmodium velutinum* (Willd.) DC root

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Abstract
Desmodium gangeticum (L.) DC (*Shalaparni*) is one among the *Dashamoola* group of drug and is extensively used in Ayurveda. The high demand of this drug makes it necessary to find and promote the usage of equally efficient alternate species. *Desmodium velutinum* (Willd.) DC is a medicinal plant which is used instead of *D. gangeticum* in southern parts of India. The study is an attempt to compare the roots of these two species Phyto-Pharmacognostically. The morphology, microscopy, physic-chemical parameters, preliminary phytochemical screening and HPTLC studies were done and compared. *Desmodium gangeticum* (L.) DC. and *Desmodium velutinum* (Willd.) DC both from Fabaceae family is erect undershrubs peculiarly differing in the leaf shape and texture. Microscopically both showed similar characters with presence of calcium oxalate crystals, pitted vessel and starch grains in root powder. Physico-chemical, phytochemical evaluation and HPTLC studies of both samples were comparable with the presence of carbohydrates, proteins, tannins, alkaloids and glycosides in both samples. Hence on the basis of present phyto-pharmacognostical study it can be concluded that *D. velutinum* may be considered as a substitute of *D. gangeticum*.

Keywords: *Desmodium gangeticum* (L.) DC, *Desmodium velutinum* (Willd.) DC, *Shalaparni*, phyto-pharmacognostical study

1. Introduction
Desmodium gangeticum (L.) DC. Is a medicinal plant extensively used in Ayurveda, Folk, Homeo, Sidha, Tibeten and Unani system of Medicine [1]. It is a sub erect diffusely branched undershrub with 1-1.5 m height, woody branched stem, uni foliate, broadly ovate leaves raceme inflorescence with small purple flowers and pods [2, 3, 4]. It is widely distributed throughout India and is known by the common names- *Pivari*, *Shalaparni* (Hindi), *Salvan* (Marathi), *Orila* (Malayalam), *Murelehonne* (Kannada) etc [5]. It is the accepted source of *Shalaparni*, one of the ingredients of much used *Dashamoola* group of drugs in Ayurveda [6]. Ayurveda classical texts mention the usage of this in various formulations and as single drug. *Shalaparni* having *Tikta-Madhura Rasa*, *Ushna Veerya*, *Madhura Vipaka*, *Guru-Snigdha Guna* and *Tridoshahara* properties has *Balya*, *Rasayana*, *Vrishya* & *Brihmana* actions [7]. It is indicated in *Jwara*, *Swasa*, *Krimi*, *Arsa*, *Athisara* etc. Charaka Samhita, Susruta Samhita, Ashtanga Sangraha & Ashtanga Hridaya mention 139, 64, 165 & 98 formulations of *Shalaparni* respectively [8]. According to National Medicinal Plants Board, *Desmodium gangeticum* comes in the list of 178 high volumes traded medicinal plants (ie; > 1000 MT per year) [9]. This high demand of *Shalaparni* makes it necessary to find and promote the usage of equally efficient alternate species for use, especially which is geographically available in different areas. Though identified as *D. gangeticum*, many other species are found to be used as *Shalaparni* in different parts of India. *Desmodium velutinum* is one such species which is used instead of *D. gangeticum* in southern parts of India [10]. *Desmodium velutinum* is a popular plant Used as traditional medicine in India and Africa for Diarrhoea, Vomiting, Swellings, Blood urination etc [11]. This plant is being used by 17 tribes in India at states namely Maharashtra, Kerala, Madhya Pradesh, West Bengal and Orissa [12]. Being from the same family *Fabaceae*, it shares many common morphological characters with *D. gangeticu*m. *D. velutinum* is an erect undershrub of 1.5 to 2 m height with terete branches which is densely clothed with pubescent hairs. Leaves are unifoliolate, usually ovate with broadly cordate base. Inflorescence is racemes with purple flowers [4, 13, 14]. Although it is marketed and used as *Shalaparni* due to the morphological similarity, studies on the comparative evaluation of these species is not reported yet. Root being the useful part of *Shalaparni*, this study aims to compare the roots of both *D. gangeticum* and *D. velutinum* phyto-pharmacognostically.

2. Materials and methods
2.1 Collection and authentication
Desmodium gangeticum and *Desmodium velutinum* were collected from natural habitat of Nilambur, Kerala. (Lattitude- 11.2748, Longitude- 76.2250, Altitude- 183 ft). The authentications of the drugs were confirmed at Pharmacognosy department of Gujarat Ayurveda University by comparing its characters with the literature mentioned in various floras. Herbaria were deposited to Pharmacognosy museum, IPGT & RA, Gujarat Ayurveda University with Specimen Numbers: IPGT & RA PhM 6318/ 2020-2021 & IPGT & RA PhM 6280/ 2019-2020 for future reference. The roots were washed in running tap water to remove unwanted materials and some of them preserved in FAA (Formalin 90:

Acetic acid 7: Alcohol 3) for microscopic investigation. The remaining were chopped into small pieces, air dried for 2 weeks and ground into powder.

2.2 Pharmacognostical study
Morphological characters were studied by observing the root as such and also with the help of dissecting microscope. Organoleptic characters like colour, touch, taste and odour were recorded. Free hand sections of the preserved root were taken and observed under the microscope for the presence of ergastic and cell contents. The section was cleared with chloral hydrate and then was stained with phloroglucinol and hydrochloric acid to observe the lignifications of the cell wall if any. Photographs were taken. Powder characters were also observed for 60# powder according to the standard guidelines of practical Pharmacognosy [15, 16].

2.3 Physico-chemical and Phytochemical parameters
Loss on drying, extractive values, Ash value, Acid insoluble ash etc. were carried out in triplicate as per the guidelines of The Ayurvedic Pharmacopeia of India and the mean value is taken [17]. Qualitative analysis was performed to detect primary and secondary metabolites in water and alcohol extracts of roots.

2.4 HPTLC study
Methanolic extract of roots were exposed to HPTLC study. The solvent system used for the study is toluene: ethyl acetate (9:1)
Chromatographic conditions: Application mode was CamagLinomatV, Development Chamber used was of Camag Twin trough Chamber. Pre coated Silica Gel GF254 plates were used. Chamber Saturation was done for 30 min. Development Time was 30 min. The plate was scanned in Camag Scanner III with Deuterium lamp, Tungstan Lamp as detectors and Wincats software was used for data analysis.

3. Results and discussion
3.1 Macroscopy of Roots
The root of *Desmodium gangeticum* is hard and woody, externally it is yellowish brown in colour and internally light yellow, fracture short. Rootlet scars were seen on the surface. The root of *Desmodium velutinum* is branched taproot with a width of about 0.5 to 2 cm. It is externally dark brown to pale brown and internally creamish white in colour. The surface shows longitudinally striated with thin papery scales of external cork with rootlet scars. Fracture short. Macroscopically these species can be differentiated by the size and shape of roots. *D. gangeticum* has taproot system with prominent spreading fibrous wiry lateral rootlets, where *D. velutinum* has well developed and stout taproot with bunch of strong lateral rootlets appearing as a strong nest like taproot system. On organoleptic evaluation both the roots have slight characteristic smell and bitter taste. (Table 1)

3.2 Microscopy
***Desmodium gangeticum* DC**
The diagrammatic section of root is circular in outline; consist of outermost cork, wide cortex and centrally wide xylem covered with phloem. (Figure 1) Detail section shows, outer most thin layer tissue which appears as light yellowish brown strip, consists of 1-2 rows of rectangular tangentially elongated cork cells. Inner to the cork, cortex is broad and composed of several rows of thin walled, oval to circular tangentially elongated parenchyma cell embedded patches of lignified fibers either in groups or isolated. Phloem a narrow zone composed of narrow polygonal to rectangular thin walled tangentially elongated moderately compressed parenchymatous cells encircling the central wood portion. Xylem mainly consists of vessels, parenchyma and fibers. The medullary rays mostly uni to triseriate. The presence of prismatic crystals of calcium oxalate, starch grains, pitted vessels and fibres observed during the powder microscopy of *D. gangeticum.* (Figure 2)

***Desmodium velutinum* (Willd.) DC**
The diagrammatic section of root shows outermost layer of cork, zone of cortex scattered with groups of fibres followed with phloem and centrally wood. (Figure 3)
Detail section of root shows, an outer most cork consisting of 9– 10 layers of yellowish brown coloured, mostly rectangular at places broken outwardly and with radial wavy walls being tangentially elongated, lignified cells. Cortex is composed of 4 to 6 layers of oval, tangentially arranged, thick walled, parenchymatous cells, scattered fibres present singly or in groups; Phloem composed of sieve elements, parenchyma and fibres traversed by phloem rays covering central wood. Xylem composed of vessels, tracheids, fibres and parenchyma traversed by rays. Medullary rays mostly multiseriated. Starch grains and prismatic crystals of calcium oxalate, fibres and pitted vessels observed during powder microscopy. (Figure 4)
The root part of both *Desmodium* species showed almost similar tissue structures, reserved contents and metabolites. The sections outlined with outermost cork, wide cortex followed with central xylem encircled by phloem. Specifically the medullary rays observed mostly uni and triseriate in *D. gangeticum*, while multiseriated in *D. velutinum.* The characters like simple and compound starch grains, pitted vessels, fibres and crystals of calcium oxalate shows similarity in both samples.

3.3 Physico-chemical and phytochemical study
Physico-chemical analysis
The detailed results of physicochemical parameters are described in table 2 along with calculated deviation. The root powder of both plants was found to be devoid of any foreign matter, which may be due to the good collection and storage practices followed. Ash value is useful in determining the quality and purity of drug. It indicates the presence of silicates, carbonates and phosphates as inorganic contents [18]. Ash value and acid insoluble siliceous matter was found to be relatively very less in both root samples indicating low foreign inorganic matter. However *D. gangeticum* showed slightly high values of both ash (2.77 ± 0.14) and acid insoluble ash content (0.14 ± 0.06) than *D. velutinum*, which may signify high level of inorganic matter, sand and silica content in it than the other. The extractive values of the crude drug provide an indication of the extent of polar, medium polar and non-polar components present in it [19]. The methanol and water soluble extracts found to be more in root of *D. gangeticum* than *D. velutinum*. The alcohol soluble extractive value which indicates the presence of polar constituents like phenols, alkaloids, steroids etc. found more in *D. gangeticum* (4.68 ± 0.04). In both samples the methanol

soluble extractive was found to be more than water soluble extractive indicating more solubility and presence of the polar contents in both the species. As the difference between the extractive values of both species is not large to identify any difference in their efficacy in different dosage forms, these can be used as alternates.

Phytochemical analysis

Preliminary phytochemical analysis was done for the identification of different chemical constituents in the samples. The results of phytochemical analysis of methanol and water soluble extracts of *D. gangeticum* and *D. velutinum* are compiled in table 3. Tannin and flavonoids were absent in Methanol soluble extracts of both samples. Carbohydrates, proteins, alkaloids and glycosides were present in root of both plants. The astringent and bitter taste of both samples can be attributed to the tannin and alkaloid content.

3.4 HPTLC analysis

The chromatograms were recorded as densitographic profile under UV radiation at short UV (254 nm) and long UV (366 nm).

The methanol extract *D.gangeticum* root showed 10 and 4 peaks at 254 and 366 nm respectively, whereas the *D. velutinum* root showed 13 and 14 peaks at 254 and 366 nm respectively. Common Rf values 0.67 and 0.86 for *D. gangeticum* and 0.03, 0.11, 0.16, 0.22, 0.35, 0.44, 0.51, 0.69 and 0.92 for *D. velutinum* were found under UV range of 254 nm and 366 nm. The samples showed common Rf values of 0.34, 0.56, 0.78, 0.86 and 0.90. The Rf values are mentioned in table 4.

The 3D graphs and peak display at UV ranges are depicted in figure 5 and 6. The spectral comparison has shown in figure 7. The common Rf values indicates the presence of same chemical components and this may be due to the same genus and family, while others may be used as markers to identify the peculiar species of *Desmodium*.

Table 1: Organoleptic characters of root powder of *D. gangeticum* and *D. velutinum*

Character	*D. gangeticum*	*D. velutinum*
Colour	Brown	Light brown
Odour	Characteristic	Characteristic
Taste	Bitter	Bitter
Touch	Fibrous	Fibrous

Table 2: Preliminary Physico-chemical analysis of *D. gangeticum* and *D. velutinum* root powder

Serial number	Parameters	*D. gangeticum* Results (%w/w)	*D. velutinum* Results (%w/w)
1	Loss on drying	7.52 ± 0.13	7.96 ± 0.38
2	Ash value at 450°C	2.77 ± 0.14	2.32 ± 0.33
3	Acid insoluble ash value at 450°C	0.14 ± 0.06	0.09 ± 0.08
4	Water extractive value	2.8 ± 0.76	2.07 ±0.35
5	Alcohol extractive value	4.68 ± 0.04	3.68 ± 0.60

*Values are means of three independent analysis ± Standard deviation

Table 3: Preliminary phytochemical analysis of root powders of *D. gangeticum* and *D. velutinum*

Sr.no	Active constituent	Test (water & methanolic extract)	*D. gangeticum*		*D. velutinum*	
			Water Extract	Methanol Extract	Water Extract	Methanol Extract
1.	Carbohydrate	Molish's test	+	+	+	+
2.	Protein	Biuret test	+	+	+	+
3.	Amino acids	Ninhydrin test	+	+	+	+
4.	Steroids	Salkowski test	_	_	_	_
5.	Glycosides	KellarKilliani test	+	+	+	+
6.	Saponins	Foam test	+	_	+	_
7.	Alkaloids	Dragendorff's test	+	+	+	+
8.	Tannins	$FeCl_3$	+	_	+	_
9.	Flavonoids	Lead acetate test	+	_	+	_

"+": Positive, "-": Negative

Table 4: High performance thin layer chromatography studies of methanolic extracts of *D. gangeticum* and *D. velutinum* at 254- 366 nm

Solvent system	Sample (MeOH extract)	254 nm (short UV)		366 nm (long UV)	
		No. of spots	Rf value	No. of spots	Rf value
Toluene: Ethyl acetate (9:1)	*Desmodium gangeticum*	10	0.03, 0.08, 0.18, 0.34, 0.56, 0.63, 0.67, 0.78, 0.86, 0.90	4	0.02, 0.67, 0.86, 0.93
	Desmodium velutinum	13	0.03, 0.11, 0.16, 0.22, 0.35, 0.38, 0.44, 0.51, 0.56, 0.69, 0.79, 0.87, 0.92	14	0.03, 0.11, 0.16, 0.22, 0.35, 0.39, 0.44, 0.51, 0.68, 0.69, 0.86, 0.89, 0.92, 0.98

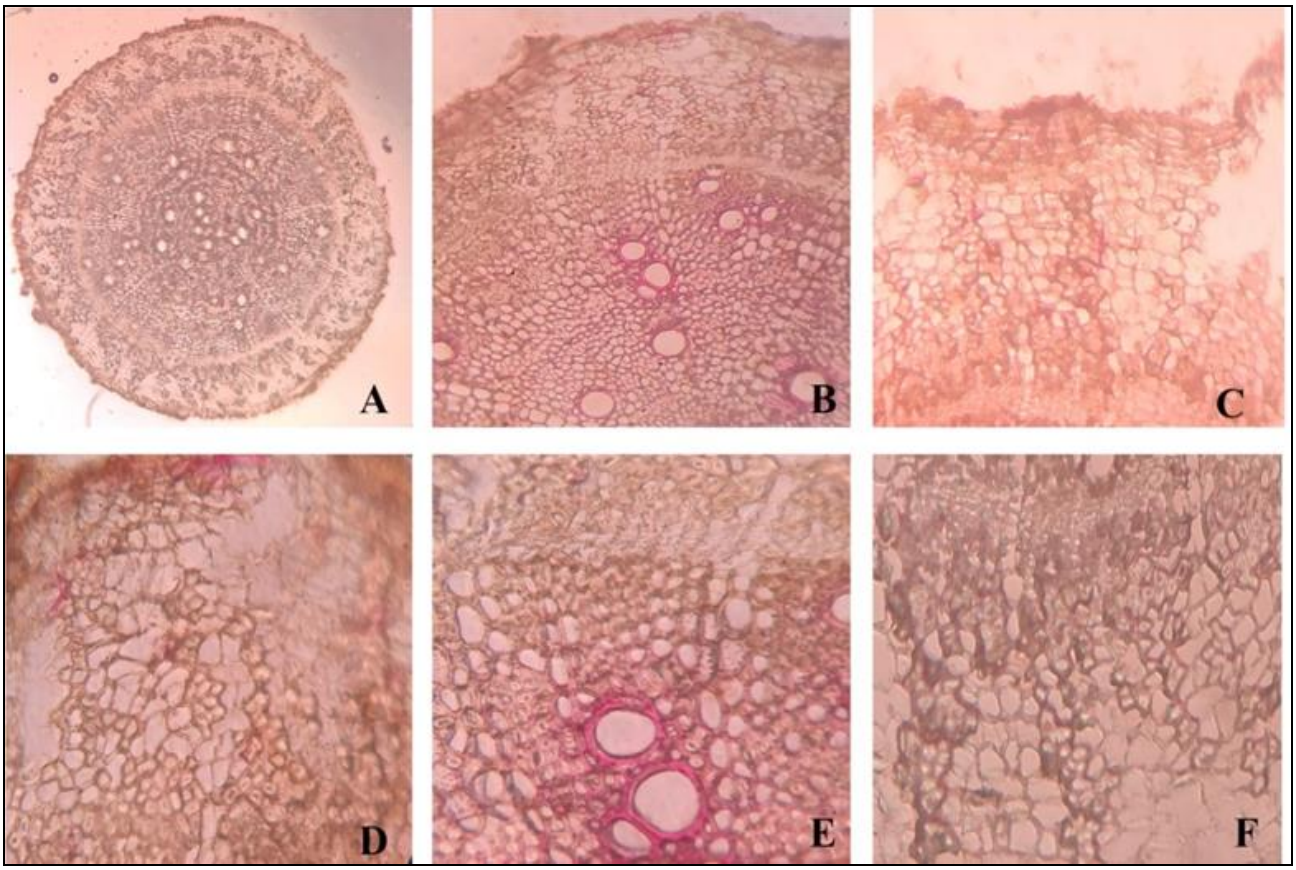

Fig 1: Microscopy of *Desmodium gangeticum* (L) DC Root

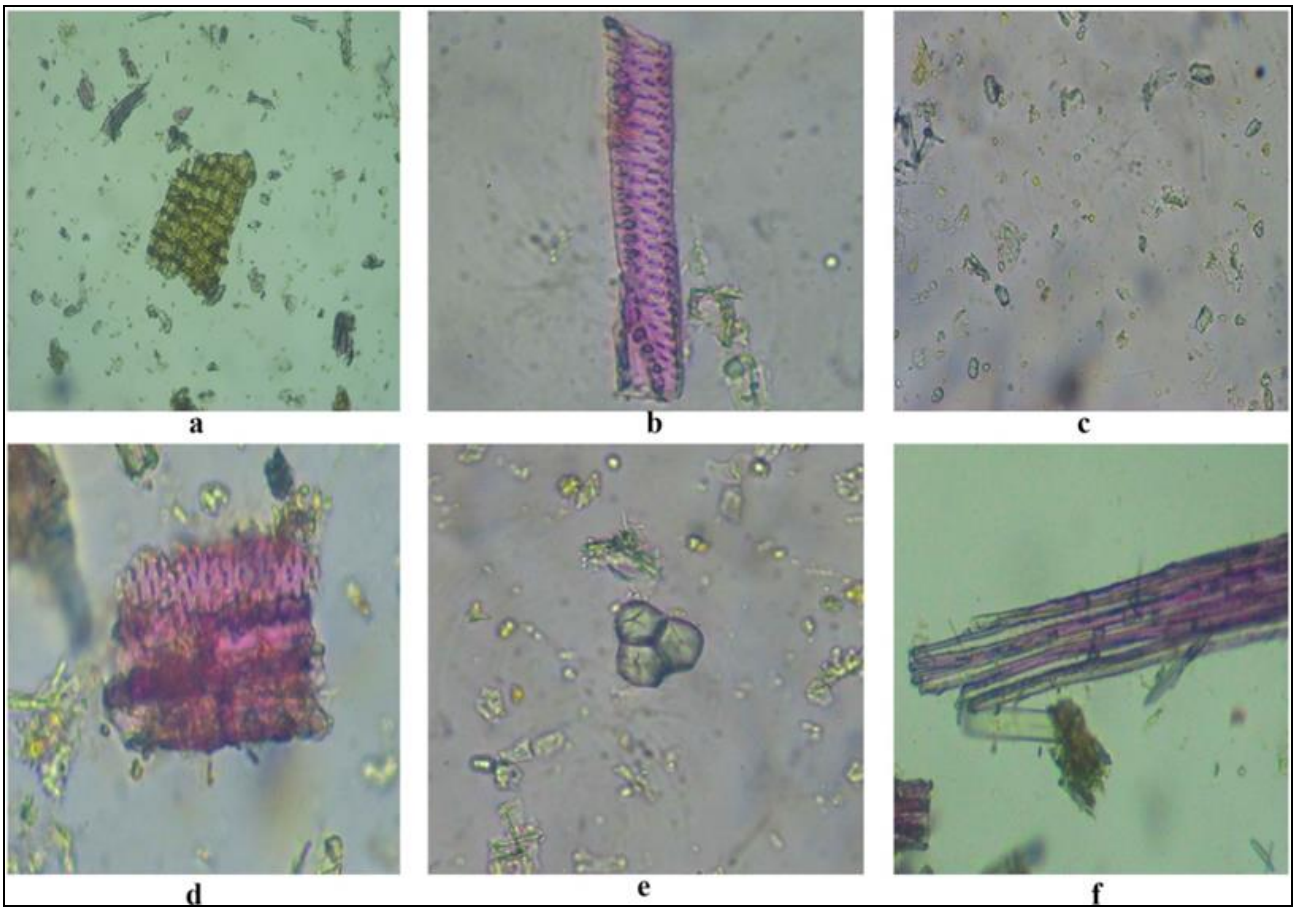

Fig 2: Powder microscopy of *Desmodium gangeticum* (L.) DC Root

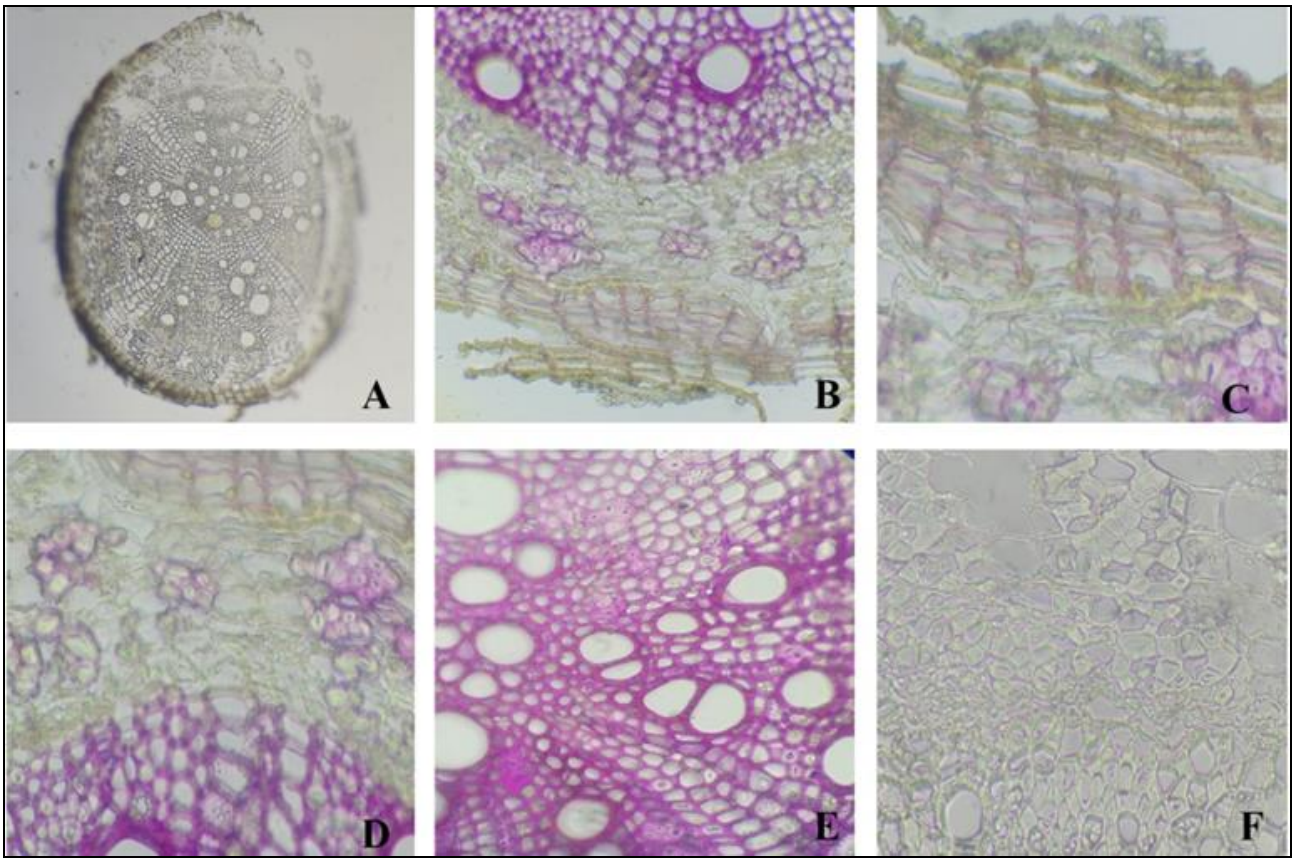

Fig 3: Microscopy of *Desmodium velutinum* (Willd.) DC Root

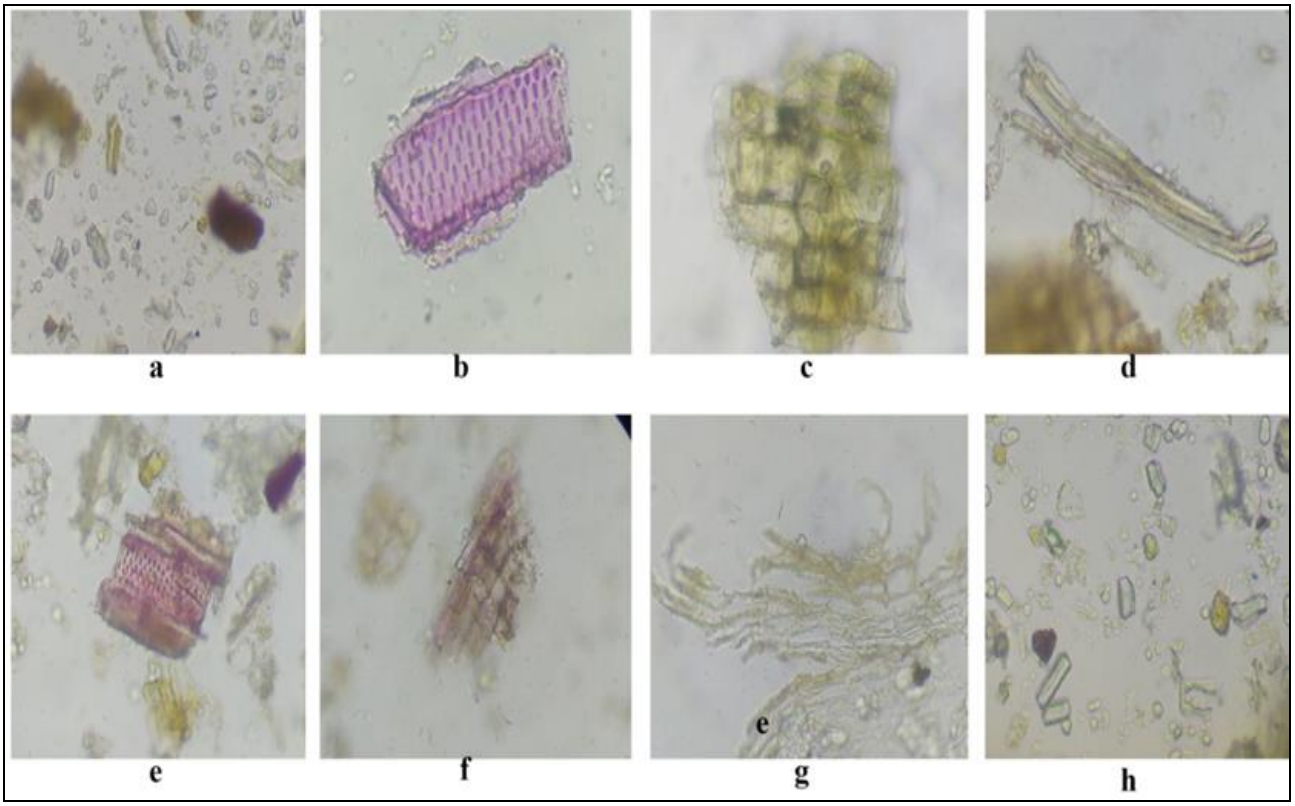

Fig 4: Powder microscopy of *Desmodium velutinum* (Willd.) DC Root

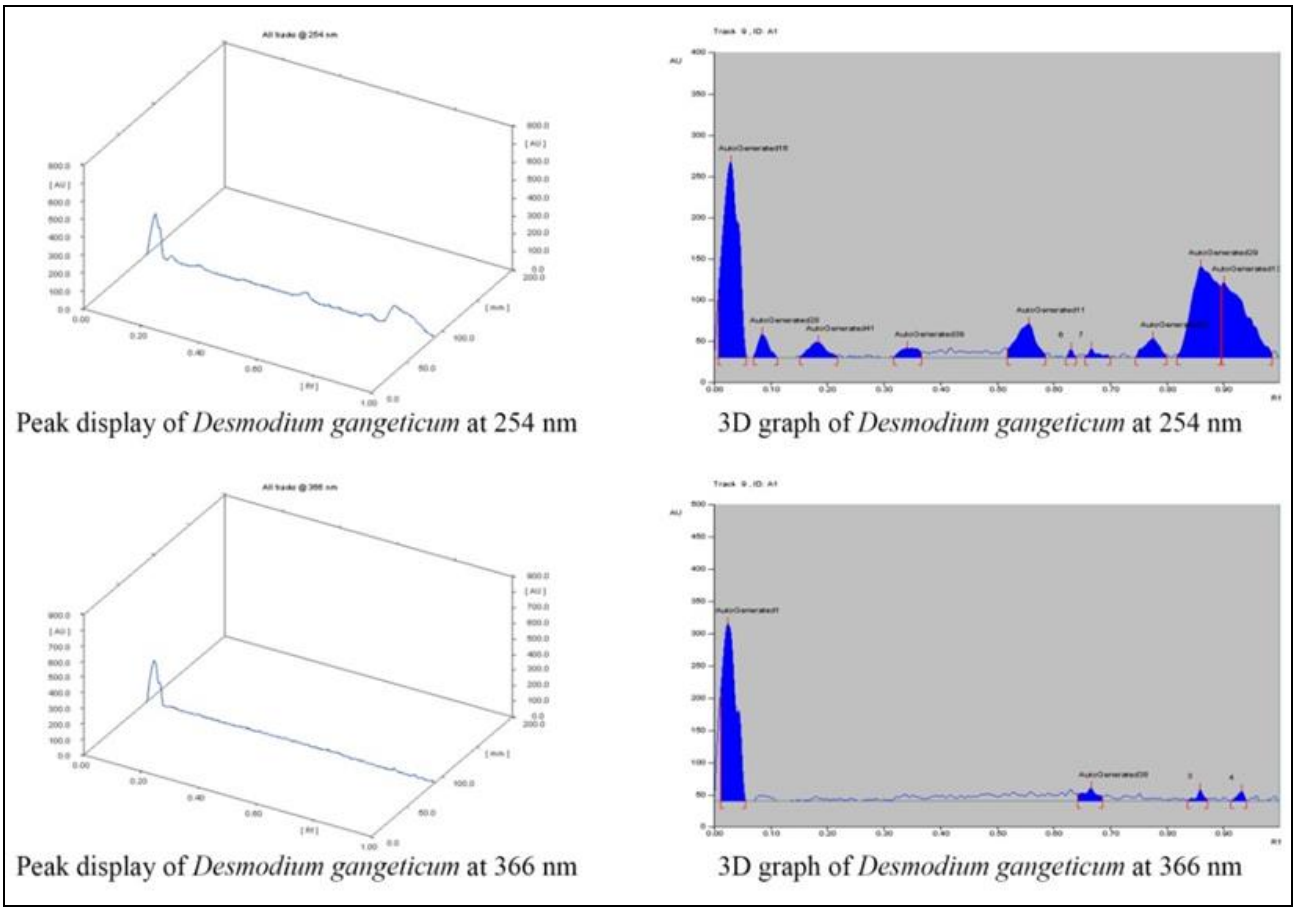

Fig 5: HPTLC Profile of *Desmodium gangeticum* (L.) DC

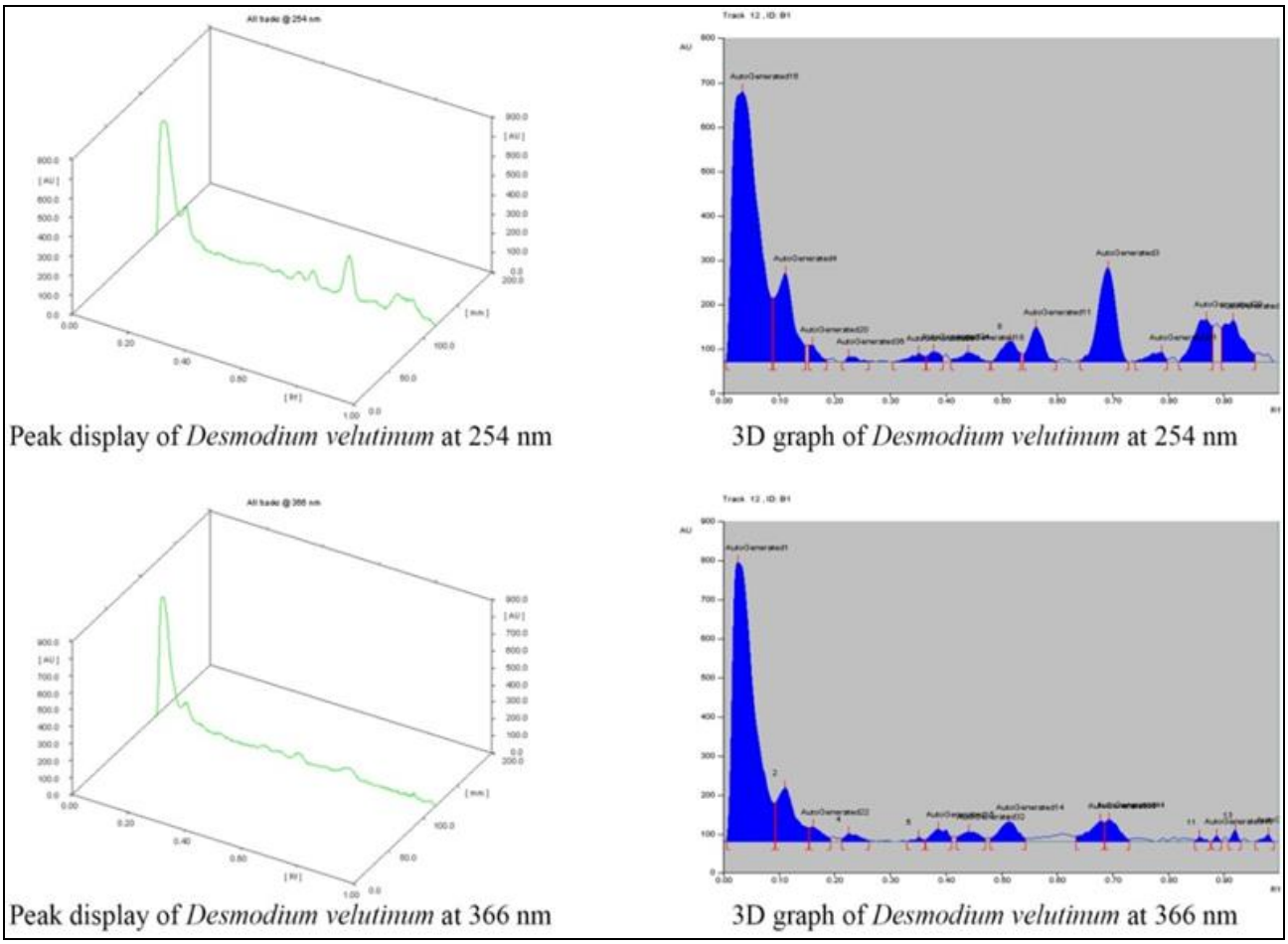

Fig 6: HPTLC Profile of *Desmodium velutinum* (Willd.) DC

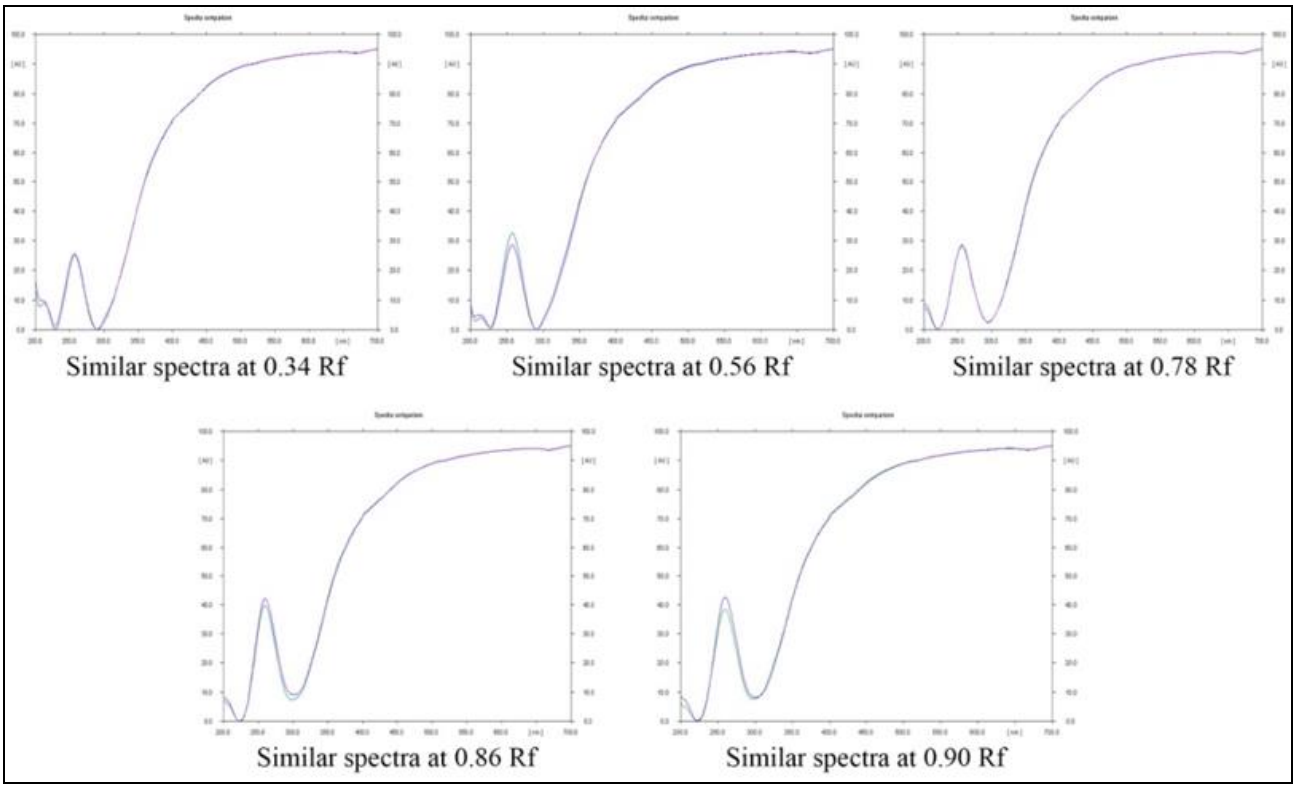

Fig 7: Spectral comparison of *Desmodium gangeticum* (L) DC & *Desmodium velutinum* (Willd.) DC

5. Conclusion

The morphological and microscopical study done reveals that both *Desmodium gangeticum* (L.) DC and *Desmodium velutinum* (Willd.) DC shows almost similar characters. Both these samples gave almost comparable results in physic-chemical, preliminary phytochemical and HPTLC studies. Therefore, based on the phyto-pharmacognostical comparison of roots, it can be concluded that *D. velutinum* (Willd.) DC may be used as substitute of *D. gangeticum* (L.) DC.

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