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Research Article

Pharmacognostic Characterization of *Desmodium gangeticum* (l.) DC - an Ayurvedic Medicinal Plant

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ABSTRACT

Desmodium gangeticum (L.) DC. (Family: Fabaceae) is an important medicinal herb commonly known as Salparni. It is widely used in Ayurveda formulations for the treatment of various ailments and neurological disorders. Pharmacognostic evaluation including examination of morphological and microscopical characters, ash values, extractive values, powder characteristic, bitterness value, foreign matter and HPTLC profile was carried out to set them as diagnostic indices for the identification/ validation of the raw material and standardization of its formulations in fixing quality control parameters.

Keywords: *Desmodium gangeticum*, pharmacognosy, standardization, anatomical features, HPTLC profile.

INTRODUCTION

Desmodium gangeticum commonly known as Salparni, is widely used medicinal herb. It is used in 'Ayurvedic' preparations like 'Dashmoolarishta', 'Dashmoolakwaath' and for treatment of nervous disorders. It is of great therapeutic value in treating typhoid, piles, inflammation, asthma, bronchitis, and dysentery[1]. It was reported to contain various classes of bioactive principles such as flavonoid glycosides, pterocarpanoids, lipids, glycolipids, lactones [2, 3] and alkaloids [3, 4] from the whole plant. The group of pterocarpanoid which includes major pterocarpanoid Gangetin and minor pterocarpanoid Gangetinin and Desmodin were isolated from hexane extract of roots and considered main active compounds [5].

Owing to various flavonoids and alkaloids present in the roots and aerial parts of the plant, the plant is found to possess various biological activities such as anti-inflammatory and anti-nociceptive activity [6], Anti-ulcer, cytoprotective and anti-secretary activity [7] hypocholesterolemic [8], anti-diabetic activity [9] and anti-leishmanial activity [10]. The aqueous extract of *D. gangeticum* has been reported to have strong anti-writhing and central nervous system (CNS) depressant activity [11], in improving memory [12] and in healing different types of wounds [13, 14]. It has also been found to be a promising candidate for the management of dementia and Alzheimer disease [12].

The extensive uses of *D. gangeticum* by different pharmaceutical industries coupled with the recent revival of interest in herbal medicine have led to an ever-increasing demand of this species. It has therefore become essential to search for a possible substitute for this species and to ensure the quality of the raw drug by pharmacognostic investigations. The situation has become more adverse as there is no detailed pharmacognostic data available on this species; it has become extremely important to make effort towards standardization of the plant material to be used as medicine, to maintain safety and efficacy of the formulations. Therefore the present work has been undertaken to establish various pharmacognostical and phytochemical parameters which could serve as a measures of authentication and quality control for commercial samples of the crude drug. In addition the detailed microscopy of the aerial parts of the plant (stem and leaf) had also been studied and documented which will be useful to pharmaceutical industries for the authentication of their commercial samples.

MATERIALS AND METHODS

Plant material: The plants of *Desmodium gangeticum* were collected from Sabarkanta forest of Gujarat, India and domesticated in the DMAPR experimental field, Anand, Gujarat (Fig. 1A). Plant material was dried under a shed, powdered and stored at ambient temperature for the further physicochemical and phytochemical analysis. After identification and confirmation the herbarium of the voucher specimens was deposited at NBRI, Lucknow.

Morphological studies:The organoleptic characters like size and shape, colour, surfaces, venation, petiole, lamina apex, margin, base, texture, odour and taste were noted [15, 16] of the fresh leaf, stem, flowers and roots of *D. gangeticum*. The Morphological features were photographed using digital camera (DSC W220 – Sony Corp., Japan) while the macroscopic observations were done under stereoscopic microscope (Olympus BX50)

Anatomical studies: Free hand sections of the freshly collected plant materials (leaf, petiole, stem and root) were taken using sharp razor blades and stained with (0.5 %) Tolueidene blue [17] while fine grinded powder of the root was also examined using microscope (Olympus BX50). The quantitative microscopy on the anatomical

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Table 1. Micro-morphological characters of *D. gangeticum* leaf.

Parameter		Range
Stomata Number	Adaxial surface	3-5-6 / mm^2
	Abaxial surface	7-10-11 / mm^2
Stomatal Index	Adaxial surface	14-16-17 / mm^2
	Abaxial surface	21-25-27 / mm^2
Vein islet Number	--	15-20-23 / mm^2
Vein termination number	--	9-13-17 / mm^2
Palisade Ratio	--	5.30 – 7.20

section and the epidermal strips of the fresh leaf of the plant to determine the palisade ratio, stomatal index, vein islet and vein termination number were carried out as per the methods of Indian Pharmacopoeia [18].

Physico-chemical studies: The total ash content, acid-insoluble ash, water soluble ash, extractive values, powder fineness, bitterness value and foreign matter were made from the shade dried plant material as per the methods of Indian Pharmacopoeia [18]. The fluorescence behavior of plant powder was observed and recorded in daylight and ultraviolet light at 254 and 366 nm [19].

Phyto-chemical evaluation: For preliminary phytochemical studies, 2 g of powdered material was extracted by cold extraction (keeping overnight on shaker) successively with hexane, chloroform, methanol, butanol and water. All the extracts were air dried while the water extract was obtained by boiling with distilled water for 2 h, filtering, concentrating and drying in an oven at 40-50°C. The presence of various phytoconstituents viz. alkaloids (Dragendorffs test), anthraquinone (Borntrager's test), flavonoids (Shinoda test), steroidal glycosides (LB test), proteins (Biuret test), reducing sugars (Fehling solution test) and saponins (foam test) were determined by prescribed methods [20, 21].

Table 2. Powder analysis of *D. gangeticum* root.

Parameters	Results
Powder fineness/sieve size	Moderately coarse
Foreign matter	None
Acid insoluble ash*	0.50%
Total ash*	6.25%
Water soluble ash*	2.70%
Bitterness value	Negligible

**Mean value of triplicate analysis*

HPTLC phyto-chemical profile: The densitometry HPTLC analysis and TLC fingerprinting was carried with powdered material 1g each of root and aerial (stem + leaves) parts. The material was refluxed with 50 ml methanol for 30 min over a water bath, finally concentrated and filtered to a volume of 10 ml. An aliquote of 4 and 6 µl of the concentrated filtrate was applied on TLC plates (Merck, precoated silicagel G60 F_{254}) using HPTLC (CAMAG Linomat-IV applicator). The plate was developed in a solvent system toluene : chloroform : methanol (5 : 8 : 3 v/v), derivatized using 5 % H_2SO_4 and finally heated at 105°C for 5 min for the development of the bands.

Table 3. Extractive value (%) of *D. gangeticum* root.

Extractive Values* (%)			
Ether	Chloroform	Alcohol	Water
4.0	1.8	5.2	7.4
3.65	1.3	5.65	6.70
3.65	1.3	6.7	8.35
4.94	1.8	7.34	10.97

*Mean value of triplicate analysis

RESULTS

Plant morphology: The plant is a sub-erect, diffusely branched under-shrub up to 2-3 ft height, stem woody, branched, irregular angled, covered with white hairs (Fig.1A, B). Leaves are unifoliate or trifoliate, with ovate, oblong to lanceolate in shape measuring 3-3.5 x 2-2.5 cm in size. The apex is acute or acuminate with wavy margins (Fig. 1 C-E). Pinna is light green in colour, with some yellowish green patches on it. In addition scarious stipules (6-8 mm) are located at the base of petioles that are triangular in shape and 1-2 cm in length. Flowers small pink to purple in colour, arranged in terminal or axillary raceme which after fertilization form pods, having 5-8 seeds with curved beak like ends (Fig. 1H, I). The flower is complete with five hairy sepals (2 mm in size), triangular in shape; five petals (4 mm), violet or white in colour, arranged papilionaceously and Androeciums (9+1) present around the single carpel. The flowering occurs from October to December. Pods were compressed, many-jointed, 12-20 x 2 mm in size, deeply indented on the lower edge and slightly indented on the upper edge. The taproot is poorly developed and large number of primary roots developed from very close positions which are 20 -50 cm long, 0.4 -1.2 cm thick, cylindrical in shape, light yellow in colour and smooth in texture (Fig. 1B).

Plant micro-morphology: The presence of paracytic type of stomata is observed in the leaf, with very low density on both of its adaxial (3-6/mm^2) and abaxial (7-11/ mm^2) surfaces (Fig. 1F). Moreover, the leaves possess uniseriate trichomes (hairs) with slightly curved beaklike

Figure 1. A. The plants of *D. gangeticum* domesticated at the DMAPR, Anand **B.** Roots of *D. gangeticum* showing taproot, poorly developed and large number of primary roots **C.** Leaf of *D. gangeticum* with wavy margins **D.** acute to acuminate leaf tip of *D. gangeticum* **E.** Leaf venation pattern in *D. gangeticum* **F.** The presence of paracytic type of stomata on the leaf of *D. gangeticum* **G.** The presence of trichomes on the leaf of *D. gangeticum* **H.** Flower of *D. gangeticum* **I.** Pods of *D. gangeticum* with curved beak like ends.

Table 4. Fluorescence analysis of *D. gangeticum* root powder.

Procedure	Day light	254 nm	366 nm
Powder as such	Light brown	Dark brown	Light brown
Powder + Nitric acid	Orange yellow	Brown	Yellowish black
Powder + Hydrochloric acid	Brown	Dark brown	Green
Powder + 50% Sulphuric acid	Brownish black	Dark black	Green
Powder + glacial Acetic acid	Reddish brown	Brownish	Greenish black
Powder + Methanol	Brownish	Bluish black	Greenish black
Powder + 1N Sodium hydroxide	Brown	Brown	Green

apex. The midrib and vein region of the leaf was fully covered by the trichomes (Fig. 1G). Besides, the stomatal number and stomatal index values, both were higher at the abaxial surface (7-11/ mm^2 and 21-27/ mm^2) than that of the adaxial surface (3-6/ mm^2 and 14-17/ mm^2) of the leaf. The vein islet number, vein termination number and palisade ratio were 15-23/ mm^2, 9-17/ mm^2 and 5.3-7.2 respectively (Table 1).

Stem anatomy: Stem appears angular in shape when young but after attaining maturity it becomes irregularly cylindrical. The presence of trichomes was observed at the angular ends of the stem. The single layered epidermis consists of thick walled, transversely elongated cells covered with a thick layer of cuticle. In the cortical region 5-6 layered, thick walled, parenchymatous cells are present below the epidermis while angular parts of the stem showed the presence of some sclerenchymatous cells. The vascular bundles are deltoid in shape in the cortical region, fused with one another to form a continuous cylinder over the large pith. The central pith consists of parenchymatous cells that are circular to polygonal in shape with an increase in their size from the periphery towards the center (Fig. 2A).

Leaf anatomy: Occurrences of short and curved unicellular trichomes are observed on the midrib and veins of leaf lamina. Both, the upper and lower epidermis layers are composed of single layer of small cells which are thick walled, circular to elliptical in shape followed by 4-5 layered parenchymatous cells in the cortex of the midrib region. In the central region, 3-5 vascular bundles are arranged in triangular shape with the xylem tissues place towards the upper side and phloem on the lower side. In the blade part, spongy mesophyll tissue is divided into two layers of palisade tissue (Fig. 2B). The vascular bundles are conjoint collateral and open type besides; both the upper and lower epidermis possesses paracytic type of stomata.

Root anatomy: A thick layer of dead tissues which are hard and dry called periderm are found present at the peripheral position of root. The presence of lenticels is also observed where the bark was broken down as it protects the inner tissues viz. epidermis which is parenchymatous in nature and cork which are lignified. Inside the cork region compressed and thin walled cells of the cortex are present. Secondary phloem tissue is found to be present in patches or elements which are separated from very thick layer of secondary xylem by the cambium. Also the one or two cell layered radially elongated bands called medullary rays is found to differentiate the secondary xylem of the root and it extend from the primary xylem up to secondary phloem.

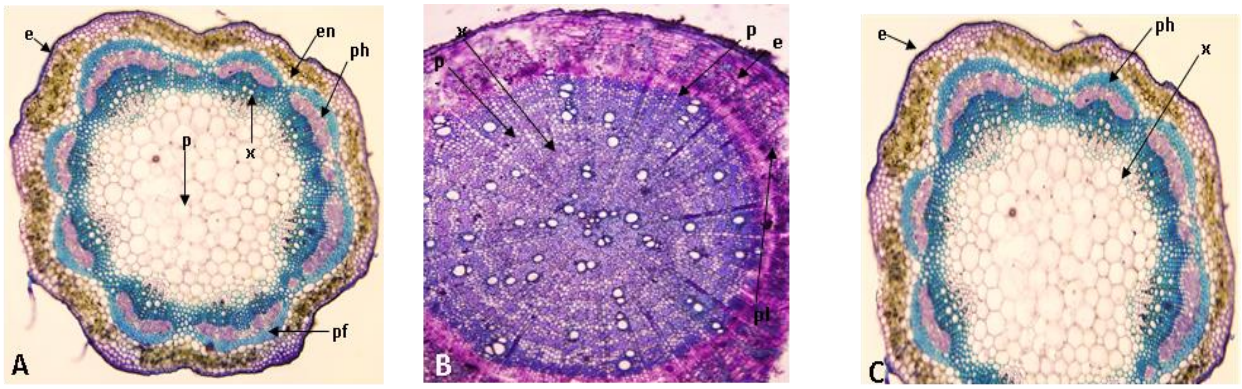

Figure 2.
A. T. S. of the stem of *D. gangeticum*
B. T. S. of the leaf of *D. gangeticum*
C. T. S. of the root of *D. gangeticum*.
e - epidermis, en – endodermis, p – pith, pf – phloem fibre, ph - phloem, pl – palisade tissue, x – xylem.

Table 5. Preliminary phytochemical analysis of *D. gangeticum* root.

Phyto-constituent	Petroleum Ether	Hexane	Chloroform	Methanol	Water
Alkaloid	+	-	+	+	+
Glycosides	-	-	-	-	-
Anthraquinones	-	-	-	-	-
Flavonoids	+	-	+	+	-
Proteins/ Amino acids	-	-	-	+	+
Tannins	-	-	-	-	-
Saponins	-	-	-	-	-
Steroids	-	-	-	-	+
Terpenoids	-	-	-	-	+

(+ Present; - Absent)

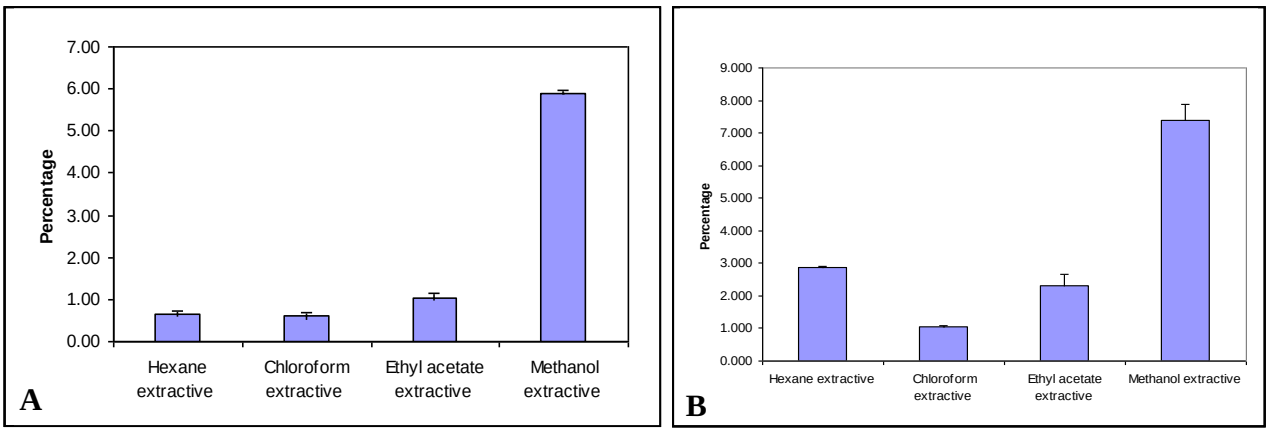

Figure 3. **A.** Successive extractive values of roots in different solvents of *D. gangeticum.* **B.** Successive extractive values of roots in different solvents of *D. gangeticum.*

Table 6. HPTLC profile data of methanolic extract of aerial parts of D. gangeticm.

Peak	Start position (Rf)	Start Height (AU)	Max position (Rf)	Max Height (AU)	Max %	End position (Rf)	End Height (AU)	Area (AU)	Area %
1	0.07	0.1	0.09	35.6	6.32	0.11	16.3	514.1	3.95
2	0.11	16.6	0.12	21.0	3.72	0.13	2.3	278.2	2.14
3	0.31	0.3	0.34	10.8	1.91	0.37	0.1	240.5	1.85
4	0.38	0.0	0.42	78.3	13.87	0.45	0.2	1680.3	12.92
5	0.46	0.1	0.48	13.2	2.33	0.51	0.9	208.6	1.60
6	0.54	6.3	0.59	66.5	11.78	0.62	19.3	1991.0	15.30
7	0.62	19.8	0.65	94.7	16.79	0.68	0.3	1936.7	14.89
8	0.68	0.1	0.71	76.9	13.64	0.74	6.5	1550.9	11.92
9	0.74	6.8	0.75	10.0	1.78	0.77	0.0	134.7	1.04
10	0.78	0.2	0.86	157.2	27.86	0.89	3.8	4474.7	34.40

However, in the center, primary xylem bundle are accumulated (Fig. 2C).

Powder study of root: The powder of the root appeared cream brown in colour, without any aroma and taste (Table 2). Under the microscopes large numbers of vessel elements possessing simple pits were observed. The presence of starch grains were also observed in the powder.

Physico-chemical properties of root powder :The harvested roots were 20–50 cm long, 0.4-1.2 cm thick, odourless, cylindrical with slightly tapering on both ends and bitter in taste. Its younger roots were somewhat lighter in colour, while older roots were deep to almost dark brown. When the root was peeled and dried irregular longitudinal furrows developed. The root powder showed coarseness and there was no foreign matter present in it. The total ash content was around 6.25%, with 0.50% acid insoluble ash and 2.70% water soluble ash. Moreover the bitterness value of root material was also found to be negligible (Table 2). Among the different solvent extracts such as ethyl acetate, chloroform, alcohol and water used for extractives values; the non-polar solvents indicating considerable content of polar compounds in roots with a maximum in water extract (Table 3; Fig. 3A, B).

Fluorescence analysis of root powder: The fluorescent properties exhibited by root powder examined under various treatments and observed in normal day light and ultra violet light. The powder appeared brown in normal day light and at 254 nm while at 366 nm most of the test resulted in black or green colour (Table 4).

Preliminary phyto-chemical analysis of root powder:Preliminary phytochemical investigation was undertaken for the identification of different types of chemical constituents present in the roots. Screening of petroleum ether, chloroform, methanol and water extracts indicates the presence of alkaloids and flavonoids while, anthraquinonens, tannins, saponins and glycosides were totally absent in all the extracts used. Moreover steroids and terpenoids showed their presence in water extract only, however hexane extract had not phytoconstituent present in it (Table 5).

HPTLC profile: Of the various solvent systems tried, the one containing toluene : chloroform : methanol (5 : 8 : 3 v/v) gave the best resolution (Fig. 4). In the methanolic extract of aerial parts of *D. gangeticum* demonstrated the presence of ten different compounds, of which the tenth compound constituted 34 % of the crude extract at Rf value 0.78 (Fig. 5A, Table 6), while in the methanolic extract of root a total of five different compounds were identified of which two compounds measured at Rf value 0.55 and 0.68 constitute around 30 % each of the total extract (Fig. 5B, Table 7).

DISCUSSION

Desmodium gangeticum is one of the important ingredients of Dasmula kwatha of Ayurveda. The name

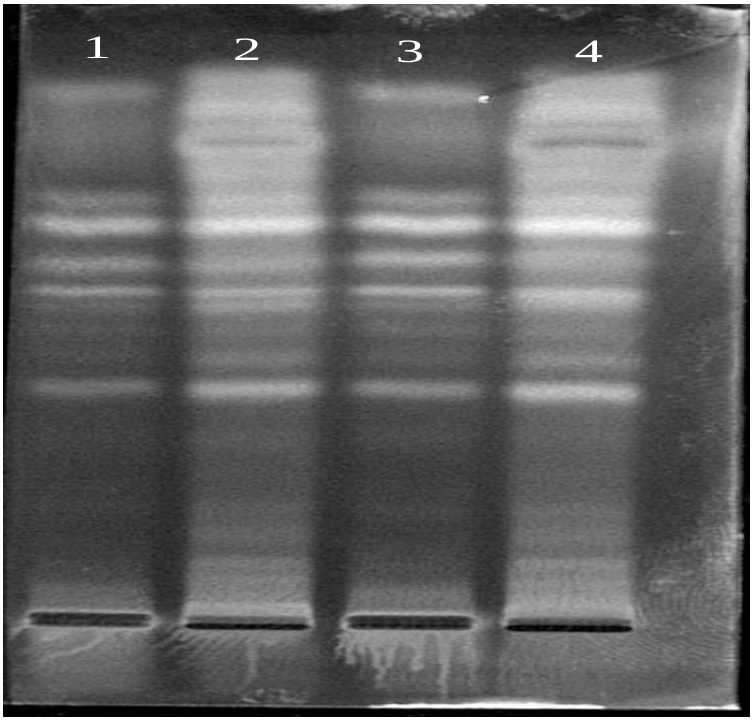

Figure 4. HPTLC chromatogram of root and aerial parts of *D. gangeticum* viewed under 366nm. The plates were developed using 5 % H_2SO_4. **1** - root extract (4 µl), **2** - root extract (6 µl), **3**- aerial parts extract (4 µl), **4**- aerial parts extract(6 µl)

Table 7. HPTLC profile data of methanolic extract of *D. gangeticm* root.

Peak	Start position (Rf)	Start Height (AU)	Max position (Rf)	Max Height (AU)	Max %	End position (Rf)	End Height (AU)	Area (AU)	Area %
1	0.38	2.8	0.42	50.9	16.82	0.46	4.7	1173.8	16.30
2	0.55	16.6	0.60	81.6	26.99	0.62	19.5	2158.1	29.97
3	0.62	19.6	0.65	50.2	16.61	0.68	7.6	1229.5	17.07
4	0.68	7.8	0.72	87.0	28.76	0.74	23.7	1990.4	27.64
5	0.74	24.0	0.76	32.7	10.81	0.79	1.1	649.6	9.02

Dasmula signifies the mixture of roots of ten different plants. The species *D. gangeticum* is a plant that has been confused with other species due to their relative similarities, therefore many a times some other materials are mixed or adulterated during the preparation of medicines. As standardization of a crude drug is an integral part of establishing its correct identity for its inclusion in herbal pharmacopoeia. For which pharmacognostic parameters and standards must be established [22]. The results of the present investigations could, therefore serve as a basis for proper identification, collection and investigation of the plant. The macro and micro morphological features of the various plant part described, distinguished it from other members of the genera. The vein islet and vein termination numbers are relatively constant for plants and can be used to differentiate closely related species [23]. The physical constant evaluation of the drugs is an important parameter in detecting adulteration or improper handling of drugs. The moisture content of the drug is not too high, thus it could discourage bacterial, fungal or yeast growth. Equally important in the evaluation of crude drugs, is the ash value and acid-insoluble ash value determination. The total ash value is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign inorganic matter such as metallic salts and/or silica [23]. As there is no pharmacognostic/ anatomical work on record of this much valued traditional drug, the present work was taken up with a view to lay down standards which could be useful to detect the authenticity of this medicinally useful plant. HPTLC profiles and densitometric details presented here will serve equally well for proper identification of the crude drug of *D. gangeticum.*

CONCLUSION

In conclusion, the pharmacognostic features examined in the present study that includes morphological, anatomical, physico-chemical and HPTLC fingerprinting, may serve as a tool for identification/ validation of the raw material and standardization of its formulations in fixing quality control parameters as well as answer to the latest GMP norms and FDA guidelines on standardization of herbal drugs.

ACKNOWLEDGEMENT

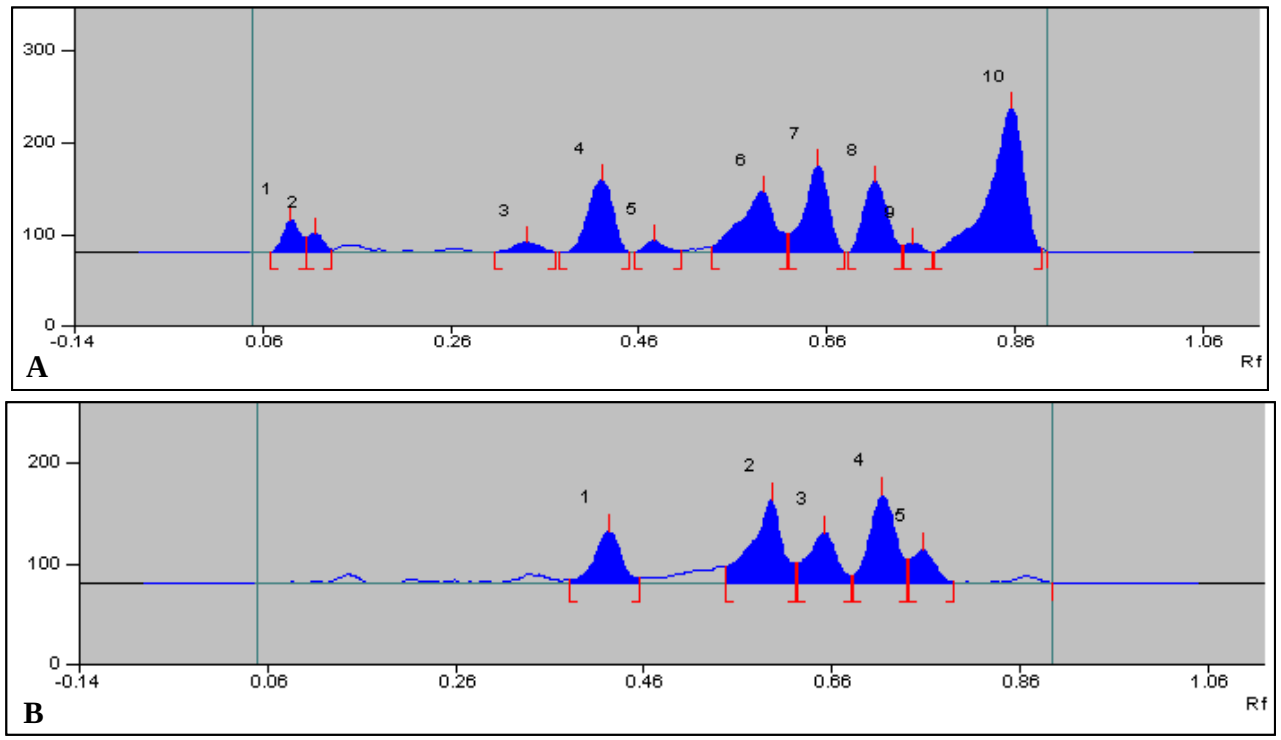

Figure 5. A. HPTLC profiling of methanolic extract of aerial parts of *D. gangeticm*. **B.** HPTLC profiling of methanolic extract of *D. gangeticum* root

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