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CHEMICAL STANDARDIZATION OF *DESMOSTACHYA BIPINNATA* (LINN.) STAPF. ROOTSTOCK

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ABSTRACT
Desmostachya bipinnata (Linn.) Stapf. is a member of Poacea family which is one of the largest families of the class monocotyledonae. The rootstock of the plant possesses medicinal properties and is used in Ayurveda and Siddha systems of medicine. Hence, rootstock was investigated for physico-chemical parameters, such as total ash, water soluble ash, acid insoluble ash, water soluble extractives, ethanol soluble extractives, alkalinity, pH, microbial load, heavy metal analysis and also chromatographic studies such as thin layer chromatographic photo documentation and high performance thin layer chromatographic finger print profiling.

KEYWORDS: *Eragrostis cynosuroides*, *Briza bipinnata*, diuretic, Tharuppai, Yagyabhūṣaṇa.

INTRODUCTION
The synonyms of *D. bipinnata* (Linn.) Stapf. are *Eragrostis cynosuroides* Beauv, *Briza bipinnata* L., *Eragrostis bipinnata* (L). K. Schum., *Eragrostis cynosuroides* Retz., *Stapfiola bipinnata* (L). Kuntza, *Uniola bipinnata* (L) L.[1] As per the Ayurvedic literatures, *D. bipinnata* is described as Yagyabhūṣaṇa, Sūcyagra in Sanskrit and is meant for any disorders, fevers, itching and diuretic.[2] In Siddha literatures, *D. bipinnata* is described as Tharuppai and is meant for all symptoms as above. It is consumed as kudineer for the ailment.[3,4] Authors aim to analyze two samples collected from different places, document their TLC profiles and HPTLC finger print profiles of chloroform and ethanol extracts of rootstock. The results could be considered for developing the pharmacopoeial standards and for quality control of rootstock of the plant.

MATERIALS AND METHODS
Plant Material
The plant material was collected from Dharmapuri district, Tamil Nadu while flowering during the month of August 2011. It was authenticated by Dr. R. Chelladurai, Botanist, Survey of Medicinal Plants Unit, Palayamkottai. Voucher specimen of the plant has been deposited in the Pharmacognosy department of Siddha Central Research Institute, Arumbakkam, Chennai-106. 100 g of each of the plant was coarsely powdered and stored in an airtight container till completing all the studies.

Reagents and chemicals
All the reagents were of analytical grade and Merck make.

Physico-chemical parameters
Loss on drying at 105°C, total ash, water soluble ash, alkalinity of water soluble ash, acid insoluble ash, water soluble extractives, ethanol soluble extractives, pH value (10% solution) and successive extraction with hexane, chloroform and ethanol were carried out as per the standard procedures.[5]

Heavy metal analysis
The lead, cadmium, arsenic and mercury are considered as heavy metals and their concentration in the plant samples were estimated using atomic absorption spectrophotometer.[6,7]

Microbial load and Pathogens
Enterobacteriacea, *E. coli*, *Salmonella spp. Pseudomonas aeruginosa*, *Staphylococcus aureus*, total bacterial count and total fungal count were determined as per the WHO methods.

Preparation of extract
4 g of the plant powder was packed in a thimble made of Whatman filter paper no.1 and loaded in the Soxhlet. 150 ml of AR chloroform was added into the flat bottom flask and boiled over a water bath until the complete extraction of phytochemicals. Then filtered, chloroform was distilled of. The residue was redissolved in

Chloroform and made upto 10 ml in a standard flask. The thimble with marc was dried in air and again extracted with ethanol. Filtered, ethanol was distilled of. The residue was redissolved in ethanol and made upto 10 ml in standard flask.

Solvent system

By trial and error method, many solvent systems were run and finalized as Toluene: Ethyl acetate: Formic acid (5:1.5:0.1, v/v/v) as suitable for chloroform extract and that of ethanol, it was chosen as Ethyl acetate: Methanol (10:1, v/v).

The thin layer chromatographic photo documentation of chloroform extract of *D. bipinnata* rootstock is shown in Fig. 2 and that of ethanol extract is shown in Fig. 3. The R_f value and their colour when viewed under UV 254 nm, UV 366 nm and after derivatization with vanillin-sulphuric acid reagent are presented in Table 3-5 respectively for chloroform extract and Table 6-8 respectively for ethanol extract. Both the extracts of two samples have similar TLC pattern indicating the presence of similar phytoconstituents.

Derivatizing agent

Vanillin-sulphuric acid reagent was prepared by dissolving one gram vanillin in the mixture of ethanol: sulphuric acid in the ratio 95:5 v/v and used for derivatization.

Instrument

For developing the TLC plate, the twin trough chamber (CAMAG) was used. Linomat IV (CAMAG, Muttenz, Switzerland) applicator was used for the application of the extract on a 6 x10 cm sized TLC plate as bands with 8 mm width and 6 mm distance between tracks. Aluminium plate precoated with silica gel $60F_{254}$ of 0.2 mm thickness (Merck) was used TLC plate. CAMAG's TLC scanner 030618 attached with WINCATS software were used for finger print analysis. CAMAG's visualizer was used for photo documentation at UV 254 nm, 366 nm; and in visible light after dipping in vanillin-sulphuric acid reagent followed by heating in an air circulated oven till the development of coloured spots.

Procedure for TLC & HPTLC

The extract was applied on the TLC plate as 10 µl and 15 µl bands of 8 mm width and 6 mm distance in between tracks using Linomat IV applicator and developed in the above mentioned solvent system. The developed TLC plate was air dried and photographs were taken under UV 254 and 366 nm. The plate was scanned under UV 254 nm, UV 366 nm using the scanner. The finger print was recorded. Then the plate was dipped in vanillin-sulphuric acid reagent, heated in an oven at 105°C till the development of coloured spots and photograph taken and again scanner for finger printing.

RESULTS AND DISCUSSION

The results of the physico-chemical analysis are pictorially represented in Fig. 1. The loss on drying of *D. bipinnata* collected from Trichy and Tuticorin were calculated as 8.32% and 8.16% respectively.

The total ash value of 3.13% and 3.30% says that the plant does not have higher amount of inorganic matter while the water soluble ash value of 1.25% and 1.15% represents that the about half of the total inorganic compound is soluble in -water. The alkalinity value of 0.25 and 0.30 ml of 0.1N HCl/g of the sample shows that the water soluble inorganic content is basic in nature. 6.84 and 6.82 are the pH values of *D. bipinnata* which indicate the slight acidic nature of the plant collected from both places. The extractive values by both cold and hot extraction methods says that the plant is containing less amount of phytoconstituents.

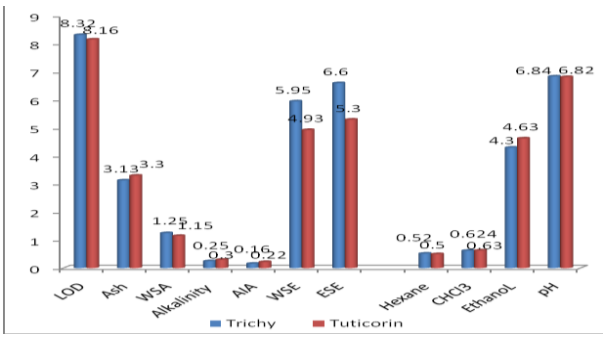


Figure 1. Graphical representation of physicochemical values of *D. bipinnata*

The microbial load and other pathogens as well as heavy metals, viz., lead, cadmium, mercury and arsenic of *D. bipinnata* are within the permissible limits. Obtained values are presented in Table 1 and Table 2 respectively.

The values of physico-chemical parameters greatly depends on the maturity of the plant, time of collection and place of collection. The above results shows that there is no much difference between the two samples collected from Trichy and Tuticorin.

The heavy metals, viz., lead, cadmium, mercury and arsenic as well as microbial load and other pathogens are within the WHO permissible limits. Hence this plant will be safe as an internal medicine.

Table 1. Heavy metal content of *D. bipinnata* rootstock

S. No.	Parameter	Value	WHO Limit (in ppm)
1.	Lead	Not detected	10
2.	Cadmium	Not detected	0.3
3.	Mercury	Not detected	1
4.	Arsenic	Not detected	3

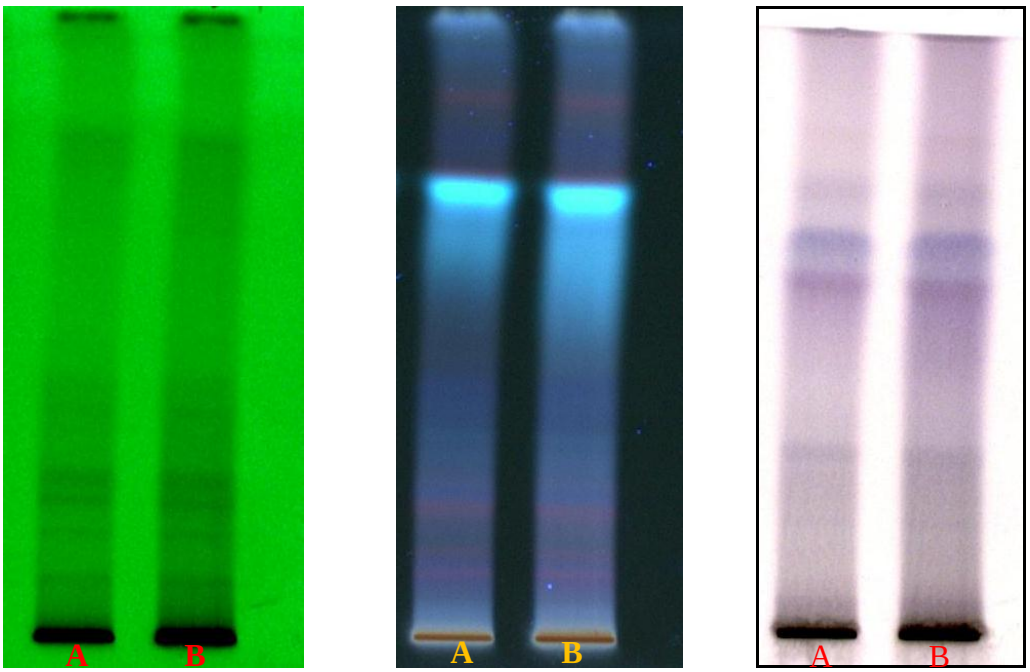
Table 2. Microbial load in *D. bipinnata* rootstock

S. No.	Parameter	Value	WHO Limit(CFU/g)
1.	Total Bacterial count	$<10^3$	10^5
2.	Total Fungal count	$<10^2$	10^3
3.	Enterobacteriacea	$<10^1$	10^3
4.	*E. coli*	Absent	10
5.	Salmonella spp.	Absent	None
6.	*Pseudomonoas aeruginosa*	Absent	Absent
7.	*Staphylococcus aureus*	Absent	Absent

The HPTLC finger print profile of chloroform extract of *D. bipinnata* rootstock under UV 254 nm is shown in Fig. 4 and that of ethanol extract is shown in Fig. 5.

Table 3. R_f and colour of spots of chloroform extract of *D. bipinnata* under UV 254 nm

S.No.	Trichy		Tuticorin	
	R_f	Colour	R_f	Colour
1.	0.01	Green	0.01	Green
2.	0.17	Green	0.17	Green
3.	0.22	Green	0.22	Green
4.	0.26	Green	0.26	Green
5.	0.36	Green	0.36	Green
6.	0.40	Green	0.40	Green
7.	0.80	Green	0.80	Green



UV 254 nm **UV 366 nm** **White light**

Figure 2. TLC photo documentation of 15 µl of chlroform extract of *D. bipinnata* rootstock. A. Trichy; B. Tuticorin.

Table 4. R_f and colour of spots of chloroform extract of *D. bipinnata* under UV 366 nm

S.No.	Trichy		Tuticorin	
	R_f	Colour	R_f	Colour
1.	0.01	Pink	0.01	Pink
2.	0.14	Pink	0.14	Pink
3.	0.21	Pink	0.21	Pink
4.	0.33	Blue	0.33	Blue
5.	0.71	Blue	0.71	Blue
6.	0.87	Pink	0.87	Pink

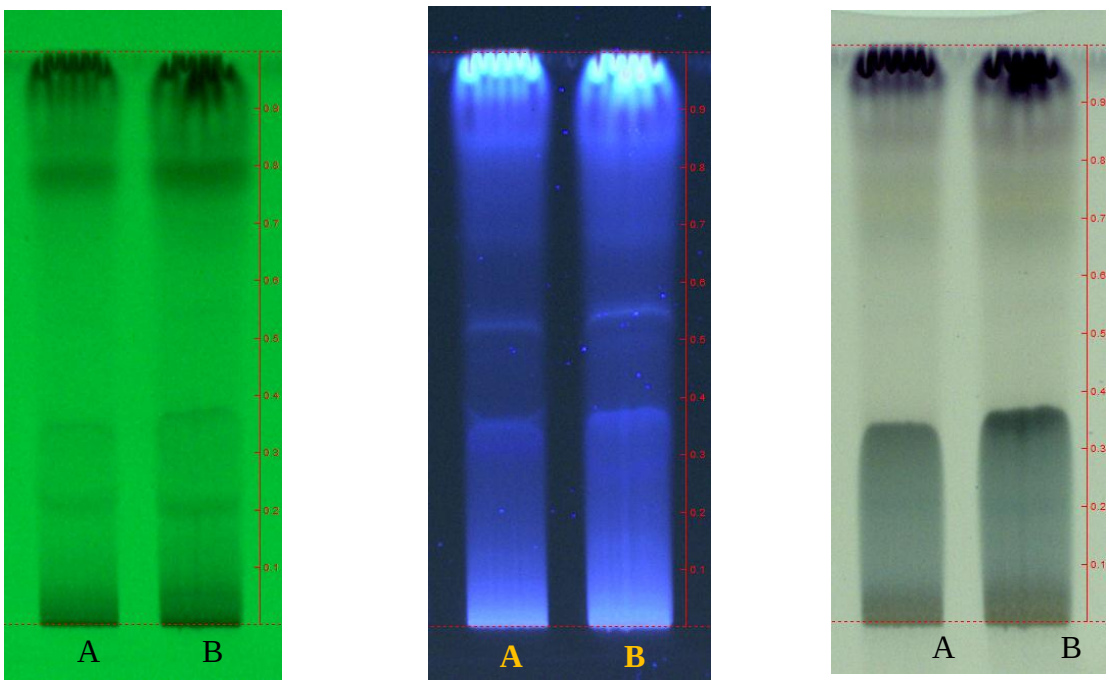
Table 5. R_f and colour of spots of chloroform extract of *D. bipinnata* under white light

S.No.	Trichy		Tuticorin	
	R_f	Colour	R_f	Colour
1.	0.05	Purple	0.05	Purple
2.	0.18	Purple	0.18	Purple
3.	0.29	Purple	0.29	Purple
4.	0.58	Magenta	0.58	Magenta
5.	0.64	Purple	0.64	Purple
6.	0.72	Pink	0.72	Pink
7.	0.80	Purple	0.80	Purple

The HPTLC finger print profile of chloroform extract of *D. bipinnata* showed a total of 13 peaks in which peaks at R_f 0.25, 0.79, 0.75 and 0.22 were the major peaks with areas 16.18%, 13.58%, 11.84% and 10.60%. Other peaks at R_f 0.09, 0.17, 0.36, 0.40, 0.60, 0.66 and 0.69 contributed to 1.54%, 3.42%, 6.96%, 5.10%, 4.12%, 6.85% and 5.55% respectively to the total area of all peaks.

The HPTLC The finger print profile of ethanol extract of *D. bipinnata* showed a total of 9 peaks in which peaks at R_f 0.97, 0.79 and 0.37 were the major peaks with areas 47.74%, 26.78% and 9.48%. Other peaks at R_f 0.05, 0.20, 0.31, 0.55, 0.68, 0.66 and 0.85 contributed to 1.47%, 4.14%, 3.82%, 1.79%, 0.87% and 3.92% respectively to the total area of all peaks.

The 3D chromatogram of both the tracks of chloroform and ethanol extracts indicated that both the samples collected from Trichy and Tuticorin resembles with each other in the tested solvent system and hence the phytoconstituents would be same.



UV 254 nm **UV 366 nm** **White light**

Figure 3. TLC photodocumentation of 15 µl of ethanol extract of *D. bipinnata* rootstock. A. Trichy; B. Tuticorin.

Table 6. R_f and colour of spots of ethanol extract of *D. bipinnata* under UV 254

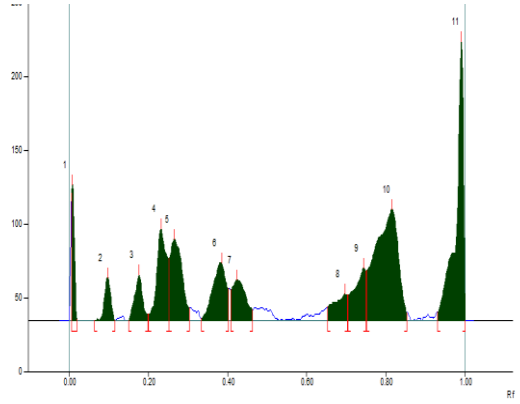
S.No.	Trichy		Tuticorin	
	R_f	Colour	R_f	Colour
1.	0.07	Green	0.07	Green
2.	0.27	Green	0.27	Green
3.	0.37	Green	0.37	Green
4.	0.54	Green	0.54	Green
5.	0.78	Green	0.78	Green

Table 7. R_f and colour of spots of ethanol extract of *D. bipinnata* under UV 366 nm

S.No.	Trichy		Tuticorin	
	R_f	Colour	R_f	Colour
1.	0.37	Greyish blue	0.37	Greyish blue
2.	0.55	Greyish blue	0.55	Greyish blue
3.	0.69	Grey	0.69	Grey
4.	0.75	Yellow	0.75	Yellow
5.	0.85	Grey	0.85	Grey

Table 8. R_f and colour of spots of ethanol extract of *D. bipinnata* under whitelight

S.No.	Trichy		Tuticorin	
	R_f	Colour	R_f	Colour
1.	0.20	Pale blue	0.20	Green
2.	0.33	Green	0.33	Green
3.	0.68	Green	0.68	Green
4.	0.73	Green	0.73	Green
5.	0.84	Green	0.84	Green



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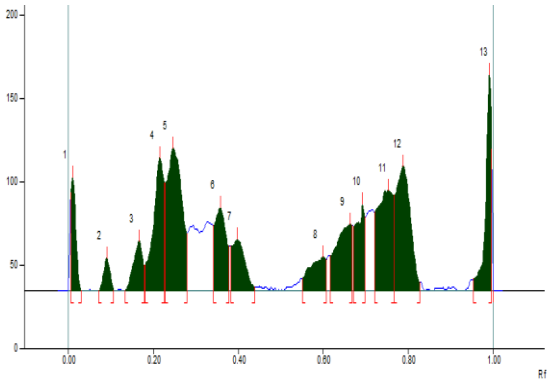

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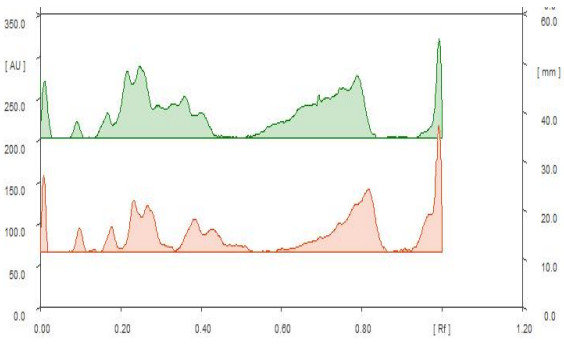


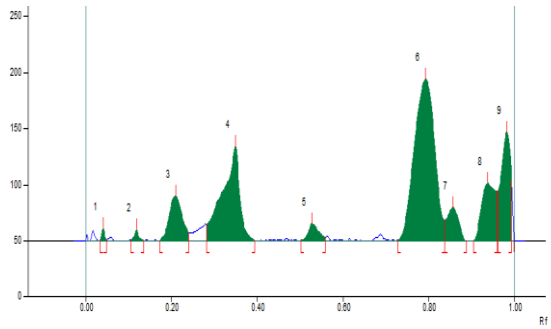
C

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	58.6 AU	0.01 Rf	67.8 AU	9.19 %	0.03 Rf	0.4 AU	722.2 AU	4.10 %
2	0.07 Rf	0.0 AU	0.09 Rf	19.6 AU	2.65 %	0.11 Rf	0.3 AU	270.9 AU	1.54 %
3	0.13 Rf	0.0 AU	0.17 Rf	29.8 AU	4.04 %	0.18 Rf	14.9 AU	602.9 AU	3.42 %
4	0.18 Rf	15.2 AU	0.22 Rf	79.8 AU	10.81 %	0.23 Rf	64.1 AU	1868.7 AU	10.60 %
5	0.23 Rf	64.2 AU	0.25 Rf	85.6 AU	11.59 %	0.28 Rf	34.3 AU	2853.4 AU	16.18 %
6	0.34 Rf	39.0 AU	0.36 Rf	49.6 AU	6.71 %	0.38 Rf	26.5 AU	1231.4 AU	6.98 %
7	0.38 Rf	26.5 AU	0.40 Rf	30.9 AU	4.18 %	0.44 Rf	1.9 AU	899.5 AU	5.10 %
8	0.55 Rf	7.3 AU	0.60 Rf	20.3 AU	2.75 %	0.61 Rf	18.7 AU	725.8 AU	4.12 %
9	0.62 Rf	20.9 AU	0.66 Rf	39.6 AU	5.36 %	0.67 Rf	39.1 AU	1384.1 AU	7.85 %
10	0.67 Rf	38.5 AU	0.69 Rf	51.8 AU	7.02 %	0.70 Rf	43.8 AU	979.1 AU	5.55 %
11	0.72 Rf	47.1 AU	0.75 Rf	60.0 AU	8.13 %	0.77 Rf	56.8 AU	2087.4 AU	11.84 %
12	0.77 Rf	57.0 AU	0.79 Rf	74.5 AU	10.09 %	0.83 Rf	4.9 AU	2394.6 AU	13.58 %
13	0.95 Rf	7.0 AU	0.99 Rf	129.1 AU	17.48 %	1.00 Rf	84.7 AU	1614.4 AU	9.15 %

D

Figure 4. HPTLC Finger print profile of chloroform extract of *D. bipinnata.*

A. Trichy; B. Tuticorin; C. 3D chromatogram of both; D. R_f and peak area table



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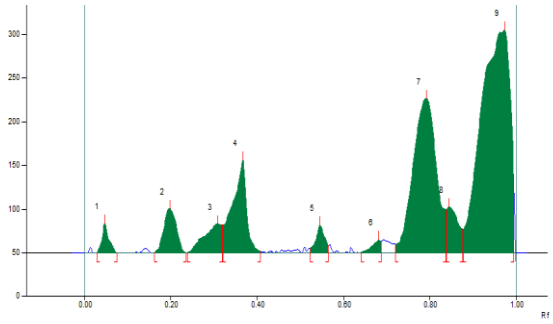

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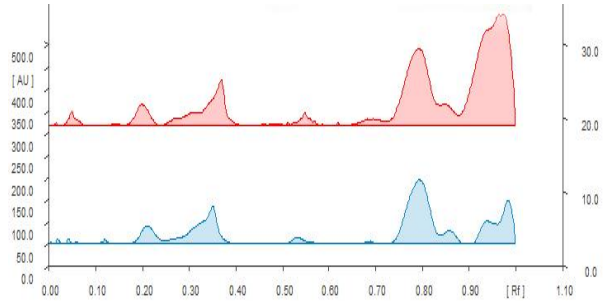

C

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.03 Rf	0.2 AU	0.05 Rf	34.4 AU	4.55 %	0.08 Rf	0.0 AU	454.7 AU	1.47 %
2	0.16 Rf	0.1 AU	0.20 Rf	50.8 AU	6.71 %	0.24 Rf	0.1 AU	1282.4 AU	4.14 %
3	0.24 Rf	0.2 AU	0.31 Rf	33.4 AU	4.41 %	0.32 Rf	31.8 AU	1182.3 AU	3.82 %
4	0.32 Rf	31.7 AU	0.37 Rf	106.1 AU	14.03 %	0.41 Rf	1.6 AU	2937.5 AU	9.48 %
5	0.52 Rf	5.5 AU	0.55 Rf	31.5 AU	4.17 %	0.57 Rf	8.0 AU	553.9 AU	1.79 %
6	0.64 Rf	0.4 AU	0.68 Rf	15.1 AU	1.99 %	0.69 Rf	13.3 AU	268.1 AU	0.87 %
7	0.72 Rf	9.7 AU	0.79 Rf	176.9 AU	23.39 %	0.84 Rf	48.9 AU	8297.0 AU	26.78 %
8	0.84 Rf	49.2 AU	0.85 Rf	52.6 AU	6.95 %	0.88 Rf	26.5 AU	1215.7 AU	3.92 %
9	0.88 Rf	26.6 AU	0.97 Rf	255.6 AU	33.79 %	1.00 Rf	80.1 AU	14791.0 AU	47.74 %

D

Figure 5. HPTLC Finger print profile of ethanol extract of *D. bipinnata*

A. Trichy; B. Tuticorin; C. 3D chromatogram of both; D. R_f and peak area table.

CONCLUSION

From the physico-chemical analysis it is inferred that both the samples of *D. bipinnata* rootstock have similarities in all parameters except in water and ethanol soluble extractives. However, pharmacopoeial standards can be resulted from the lower values of water and ethanol soluble extractives. From the TLC and HPTLC studies it is understood that chloroform and ethanol extracts of both samples are having similar TLC pattern indicating the resemblance in the chemical constituents.

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