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STANDARDISATION OF ONE OF TRADITIONAL 'DASHMULA': SHALPARNI (DESMODIUM GANGETICUM LINN. DC. FAMILY: LEGUMINOSAE) THROUGH PHYTOCHEMICAL AND HISTOLOGICAL EVALUATION

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ABSTRACT

Desmodium gangeticum (L.) DC. Belonging to Leguminosae commonly known as Salparni one of laghupanch -mool from traditional Ayurvedic *'dashmool'*contains sterols, flavonoids, pterocarpan, lipids, glycolipids and alkaloids. Advances in microscope technology like Scanning Electron Microscope have increased the accuracy and capabilities of microscopy as a mean of botanical identification. The objective of this work to provide set of SEM histological characters that may serve as standard for identification. That helps for establishment of botanical biomarkers based on the major microscopic features observed in *D. gangeticum*. Secondly physicochemical parameter was evaluated. Phytochemical screening petroleum ether, chloroform, alcoholic and ethyl acetate extracts were performed. HPTLC fingerprinting of DG-AL and DG-ET extract in mobile phase Toluene:Ethyl acetate :Formic acid (6:4:0.3),scanned at 262nm shows 11 and 7 zone with good resolution.. DG-ET extract was marked qualitatively with Genistein isoflavone as standard at Rf 0.49. Stability of DG-ET in solution and over plate with time was checked shows persistent chromatogram. Stability during chromatography checked with 2D chromatogram shows all zone in diagonal line. This article focused for qualitative fingerprinting of genistein in extracts of shalparni dashmul and histological evaluation for development of quality parameter as standardize aspect.

KEYWORDS: *Desmodium gagneticum*, HPTLC fingerprinting, SEM images, Dashmula, Genistein.

INTRODUCTION

Every herbal formulation must be standardized as per WHO guidelines. The objective of WHO guideline is to define basic criteria for evaluation of quality, safety and efficacy of herbal medicines. Government and health authorities of various nations have taken initiative for standardisation of botanical medicines. As per study commissioned by the Associated Chamber Of Commerce and Industry (ASSOCHAM), herbal products become very popular now a day and found uses in medicinal treatment across the world.[1-3]

Desmodium gangeticum DC.(*Hedysarum gangeticum* Linn.) belonging to Leguminosae, known as Ekamula, Shalani, Shalipatri, Shaliparni in Sanskrit ;Saplan, Salun in hindi; Ranphal in Marathi; Gitanaram inTelgu, ticktree in English, serengan in Malaysia, I-nio in Thai. Normal habitat of lower Himalayan region and throughout plains of India. It is an undershrub 0.6 -1.2 m. high, stems irregularly angled, pubescent with appressed white hair; leaves ovate- oblong ,1-3 foliate,green above, paler beneath with dense soft whitish appressed hairs ;flowers axillary racemes 15-30 cm long, white or violet in colour; pods sub falcate, indehiscent, clothed with minute hooked hair.[4-6]

Literature survey shows: the sterols N, N-dimethyltryptamine, 5-methoxy-N, Ndimethyltryptamine, their oxides and other derivatives have been isolated from aerial parts.[8] Three pterocarpenoids like gangetin, gangetinin and desmodin are the major chemical constituents of the roots.[9]

Twelve alkaloids comprising of four broad structural types ie. Carboxylated and decarboxylated tryptamines, β -carbolines and β-phenethymines have been isolated from different parts of it.[10] Flavonoids: Genistein and 2-hydroxy genistein,8-C-Prenyl-5,7,5'-trimethoxy-3',4'methylenedioxyflavone are reported.[10]

Gangetin, a pterocarpan, shows anti-fertility activity by affecting alkaline phosphatase activity in uterine fluid. Parts used as whole and root is one of the ingredients of the 'Ayurvedic preparation': 'Dashmoolaristha', 'Dashmoola kwath' and 'Dashmoola kadha' used as antipyretic, bitter tonic, anthelmintic, in asthma, bronchitis, in typhoid, in

neuromuscular disorder, cardiac disorders, dysentry and 'Shalparnyadi churna'- herbal compound was assessed for irritable bowel syndrome in clinical trial.[4-6]

Present study was under taken for standardisation of *Desmodium gangeticum* as raw material, ingredient of various Ayurvedic and herbal formulation. The study was focused over stem part using Scanning Electron microscopy and HPTLC for fingerprinting of flavonoids in ethyl acetate extract. Literature study do not show biomarking with genistein isoflavone for this drug and it has potential pharmacological properties. Therefore, selected for marking of this drug. Simultaneously stability study of extract was done.

MATERIALS AND METHODS

Stem of *D. gangeticum*, phloroglucinol, Conc. HCl, Glycerine, Soda lime, Pet. Ether, Chloroform, Ethanol, ethyl acetate, Sulphuric acid Silica Gel G TLC plate (Merck), Toluene, Ethyl acetate, Formic acid Trinacular microscope-Olympus CX21i, Scanning Electron microscope- PhilipXL-30 SEM, Sputter coater Sc 764, Linomet Syringe 5, TLC Scanner 5, Digistore- Reprostar 3.

Authentication, Collection, Drying and Sizing

Shalparani shrub was identified, collected from Igatpuri forest of Nashik District and its herbarium was prepared. It was deposited to Botanical survey Of India, Pune for authentication purpose.

Powder was prepared from entire dried shrub.

Physical parameter Evaluation: Moisture content, Total ash value, acid insoluble ash value, water soluble ash value, sulphated ash value, Extractive values: alcohol soluble, water soluble, Crude fibre content determination by Dutch method, were performed over powder of Stem of *D. gangeticum*. (as per WHO guideline: WHO Library cataloguing in publication Data -1998: Quality control methods for medicinal plant materials.[27,28,29]

Values were recorded and compared with standard value of The Ayurvedic Pharmacopoeia. as per **Table no.: 1.**

Microscopical Evaluation: Histology of stem and powder of *D. gangeticum* through trinocular microscope and Scanning Electron Microscope were studied as follow- Transverse section of lower portion of stem was taken, cleared with clearing agent ie. sodium

hypochlorite solution, mounted in glycerine water over glass slide and examined under trinocular microscope according to the standard method given in the textbook of pharmacognosy by TE.Wallis 1967.

Microchemical test Transverse section of stem have been treated with different reagents for the microchemical tests of cell wall content like lignin , cutin and for ergastic cell content like starch and calcium oxalate crystals.

Powder Characteristics: Slide Preparation- Fine powder of stem was boiled with soda lime solution for 10 minutes. It was filtered, washed with water and mounted in glycerin water, stained with phloroglucinol and conc. HCl and observed under microscope. Photographs are shown in **Plate no.: 1.a. to 1.i**.

Scanning Electron Microscope (SEM) -Tissue and powder preparation

Approximately 0.5 mm thin transverse section of dried stem and dried powder in thin layer were mounted on the aluminium holder stud using double sticky carbon tape. Stud with sample coated with gold particle using sputter coater SC 764 for about 20 min. to charge conductive. Further stubs were dried in oven at 60^0c for 3hours. Dried stubs were loaded to SEM holder. Vacuum was generated as $<5 \times 10^{-5}$ Pa. Tri-dimensional images of tissues were scan at different magnification mode. Photographs are shown in **Plate No.: 2.a to 2.k Extraction:** Dried powder was subjected for defatting using Pet. Ether (60-80^0 c) using Soxhlet assembly and successively extracted using chloroform and ethanol respectively. Alcoholic extract conc. to dried mass, made hydro alcoholic and fractionated with ethyl acetate. Ethyl acetate extract made concentrated, subjected for Phytochemical screening and HPTLC fingerprinting. Extracts were designated as Pet. ether Extract- DG-PE, Chloroform extract – DG-CE, Alcoholic extract- DG-AL, ethyl acetate extract – DG-ET.

Phytochemical screening by chemical test and TLC[27]

DG-PE, DG-CE, DG-AL, DG-ET were reconstituted with respective solvents and subjected for phytochemical screening using chemical tests for identification of class of compounds. As per **Table no.2.**

DG-AL and DG-ET for fingerprinting by HPTLC

Stationary phase: Silica gel G 254 (Merck)

Mobile Phase: Toluene: Ethyl acetate: formic acid (6:4:0.3)

Saturation Time: 10 min.

Scanning - 262 nm,

Sample : DG-AL, Dg-ET(1000ppm) in methanol.

Sample application: on Track -1,2 : DG-AL(5,10 µl); Track-3,4: DG-ET (5,10 µl)

Chromatogram scanned over TLC scanner densitometer, recorded as per **Table no.: 3** and photo documented as **Fig. no. :1.a. , 1.b., 2.a and 2.b.**

HPTLC fingerprinting of DG-ET along with Genistein isoflavone as biomarker:

Stationary phase: silica gel G 254 (Merck)

Mobile Phase: Toluene: Ethyl acetate: formic acid (6:4:0.3)

Saturation Time: 10 min

Scanning of densitometer: At 262 nm

Sample: Dg-ET in methanol

Standard: Genistein (Designated as: Ge-S, 50 ppm in methanol)

Sample application: on Track -1,2 : Ge-S (2,3 µl); Track-3,4: DG-ET (3.5,3.5 µl)

Chromatogram scanned over TLC scanner densitometer, recorded as **Fig. no. : 3.a,3.b**.

Stability Of DG-ET in solution, on plate[26]

Track no. 1: Sample DG-ET (applied) µl 3 hours prior to chromatography

Track no. 2, 4 (Twice): Sample DG-ET µl fresh applied prior to chromatography

Track no. 3: Sample DG-ET µl prepared 3 hours prior to chromatography (in solution)

Chromatogram scanned and recorded as **Figure no.: 4.a., 4.b.**

Stability Of DG-ET during chromatography[26]

Track no.: 1- Applied as spot: 5 µl

Developed as 2 D chromatogram: 1 D chromatogfram first developed, dried recorded and than turned 90 ^{o} and developed again. Chromatogram obtained as **Fig. No. 4.c.**

RESULT AND DISCUSSION

Authenticated herbarium was certified as *Desmodium gangeticum* (L.) DC. And voucher no. deposited as BSI/WRC/Tech. 2014/DVR-2.

As per The Ayurvedic pharmacopoeia Of India physico chemical values found to be in limit.

Table no. 1: Physical parameter[28,29] of stem Powder of *D. gangeticum*

Sr No.	Physical Parameter	% value Obtained	Value as per The Ayurvedic Pharmacopoeia[25]
1	Moisture content	8 % w/w	--
2	Total ash value	6% w/w	NMT 6%
3	Sulphated ash value	5% w/w	--
4	Acid insoluble ash value	0.5% w/w	NMT 2%
5	Water soluble ash value	5% w/w	---
6	Alcohol soluble extractive value	12% w/w	NLT 1%
7	Water soluble extractive value	14% w/w	NLT 6%
8	Crude fibre content	35% w/w	--

Transverse section of stem shows typical histological parameter such as --It shows central zone made up of large pith parenchyma, radiated with uni and biseriate medullary rays travels through concentric vascular bundle, Xylem towards centre of pith and phloem outer side. Xylem patch that covers major area of stem and shows lignified vessels, parenchyma and fibres. Phloem shows nonlignified parenchyma, sieve tubes and companion cells. Cortex zone shows two patches; one in irregular bulges underlie of epidermis filled with collenchyma, second as ground parenchyma. Typical discontinuous patches of lignified pericyclic fibres are also observed just periphery to phloem. Single layer of epidermal parenchyma encircles cortex, covered with thin cuticle. Also shows Uni and bi cellular covering warty trichrome in dermal layer.

Powder characteristics of entire herb of *D. gangeticum* studied through trinocular and SEM. It shows fragments of epidermal and cortical parenchyma with ergastic content, lignified pitted vessels, lignified fibres in group, scattered fragments of warty unicellular covering trichomes.

SEM Images of TS of Stem shows ergastic content ie. Starch grain (4-6-8 µ in dia.) and prismatic calcium oxalate crystals (10-20 µ in dia.) present in xylem and medullary parenchyma. It shows unicellular as well as bicellular covering trichrome (10-12 µ in width and 60 to 70 µ in length) in epidermal layer. Xylem vessels are 30-40 µ in dia.

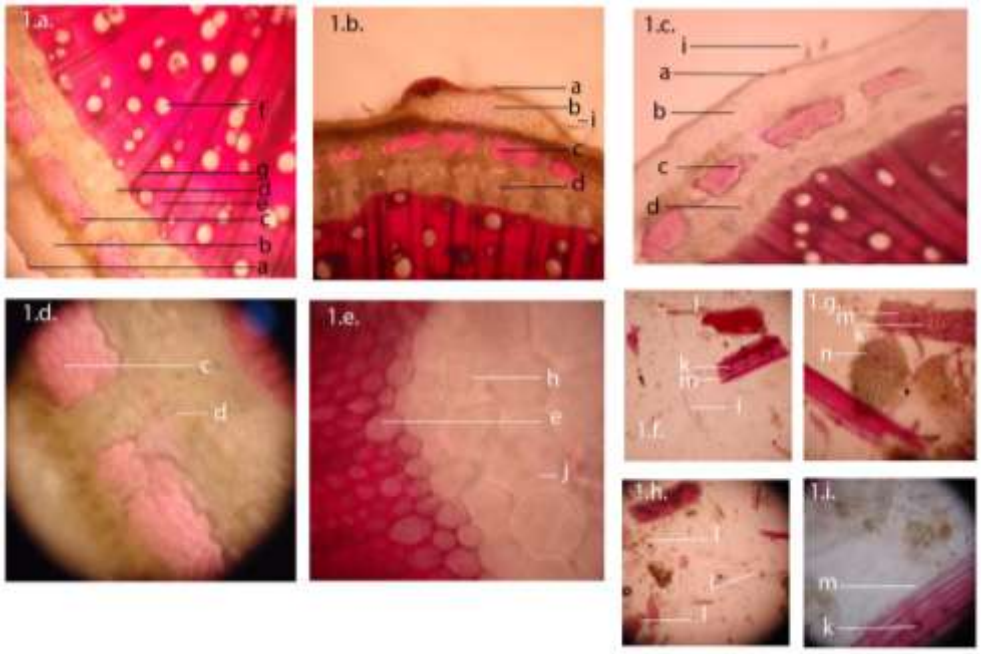
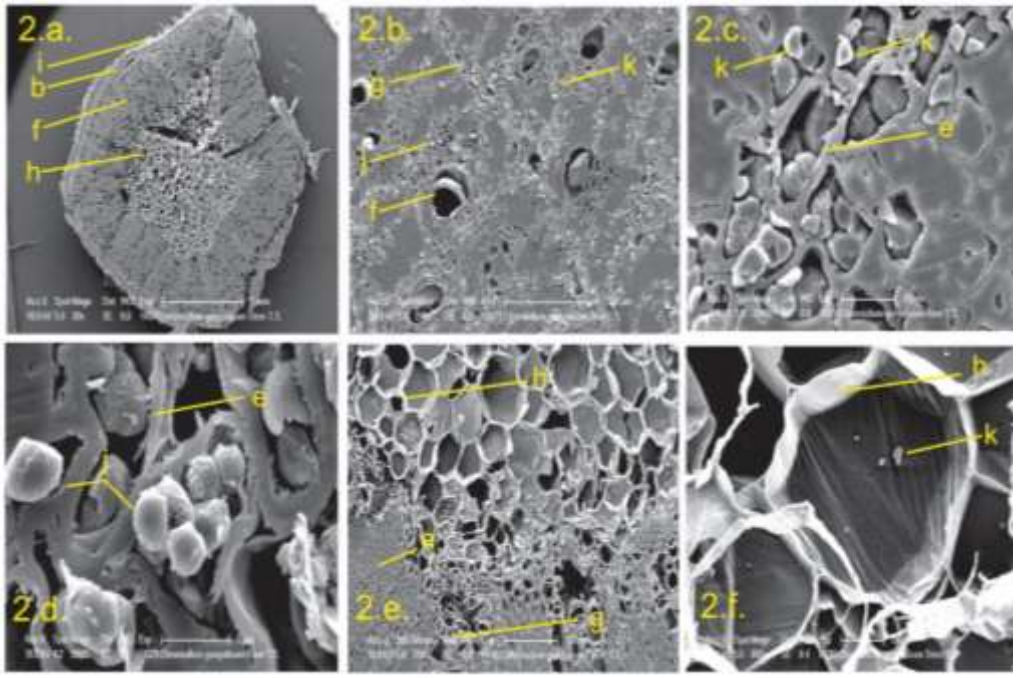


Plate No. : 1.a, 1.b.,1.c., - 10x-T.S. Of Stem of *D .gangeticum*, 1.d.-45x- pericycle patch, 1.e- 45x pith parenchyma, 1.h,1.i, 1.j,1.k-10x- Powder characteristic of *D. gangeticum*
Labels : a-epidermis, b-cortex ,c-pericyclic fibre, d-phloem , e- xylem parenchyma, f-xylem vessel, g- medullary rays, h- pith , i-covering warty trichome, j- starch grain, k-prismatic calcium oxalate crystal, l- pitted xylem vessel, m-fibers, n- parenchyma.



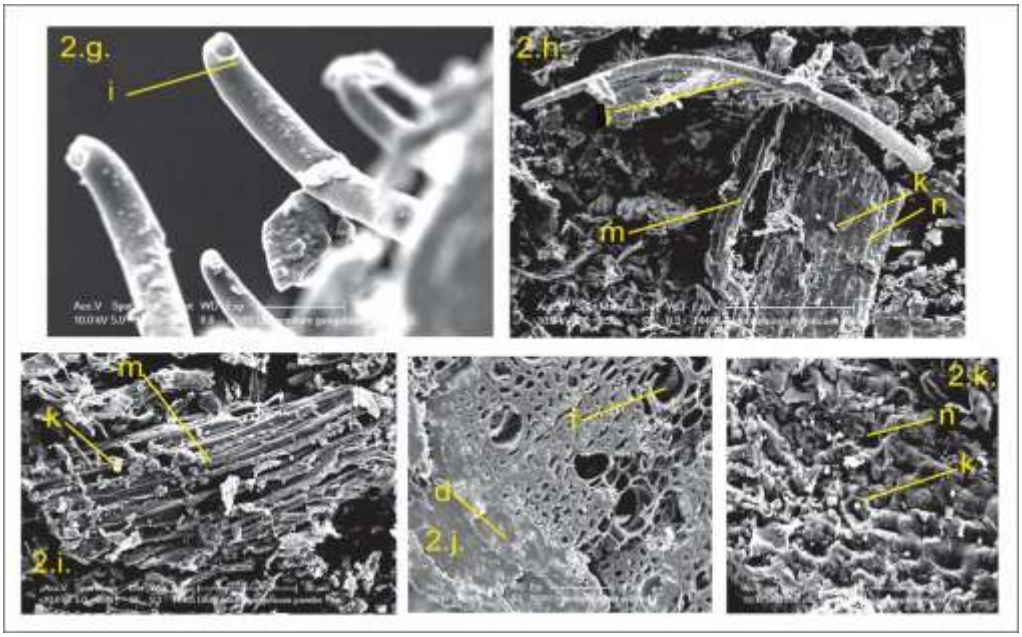


Plate no. : 2.a - SEM images of T.S. Of Stem of *D .gangeticum*, 2.b,2.c.,2.d.- SEM image of xylem zone with crystals and starch grain, 2.e,2.f .- SEM images of pith Zone, 2.g- SEM image of covering warty trichome, 2.h, 2.i, 2.k.- SEM images of powder of *D.gangeticum*, 2.j.- SEM images of Phloem and Xylem zone.

DG-PE, DG-CE, DG-AL and DG-ET extracts screened for phytochemical test shows presence of steroids, carbohydrate, alkaloid, tannin and flavonoids.

Table no: 2: Preliminary phytochemical screening of DG-PE, DG-CE, DG-AL and DG-Et[30]

Parameter	**DG-PE**	**DG-CE**	**DG-AL**	**DG-ET**
Colour	**Dark greenish brown**	**Dark green**	**Dark brown**	**Light brown**
Consistency	Semisolid	Solid	Solid	Solid
% yield w/w	1..09	1.08	4.76	1.42
Chemical test				
Glycosides	-	-	+++	+
Carbohydrate	-	-	+++	+
Steroids/terpene	+++	-	-	-
Flavonoids	+++	-	+++	+++
Tannins	-	-	+++	+++
Alkaloids	+	+++	-	-
DG-PE : *D. gangeticum* petroleum extract, DG-CE: : *D. gangeticum* chloroform extract, DG-AL: : *D. gangeticum* Alcoholic extract, DG-ET : : *D. gangeticum* ethyl acetate extract +++ : positive, - : negative				

Finger printing of DG-AL and DG-ET by HPTLC shows better resolution for given mobile phase as 11 and 07 spots respectively with following Rf and AUC.

Table No. 3: Qualitative Chromatogram for evaluation of DG-AL and DG-ET @262 nm

Sr. No.	DG-AL Track -1 (5 µl-1000 ppm)		DG-ET Track-3 (5 µl-1000ppm)	
	Rf	AUC	Rf	AUC
1	0.18 Rf	2338.6	0.19	1909.2
2	0.28 Rf	7955.3	0.28	7674.7
3	0.37 Rf	9159.2	0.38	7338.4
4	0.41 Rf	148.9	0.47	447.8
5	0.46 Rf	693.0	0.49	399.1
6	0.47 Rf	621.9	0.59	199.1
7	0.55 Rf	2137.7	0.75	1058.2
8	0.72 Rf	776.1	0.79	2196.4
9	0.76 Rf	816.3	-	-
10	0.81 Rf	1153.8	-	-
11	0.84 Rf	1745.9	-	-

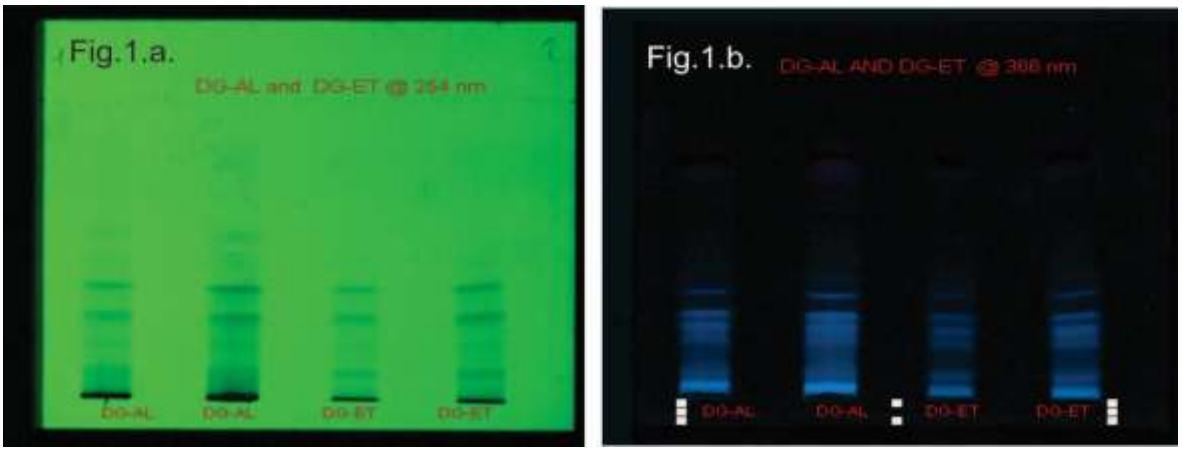


Figure No.1.a nd 1.b: Fingerprinting of DG-AL and DG-ET @ 254nm and @366 nm

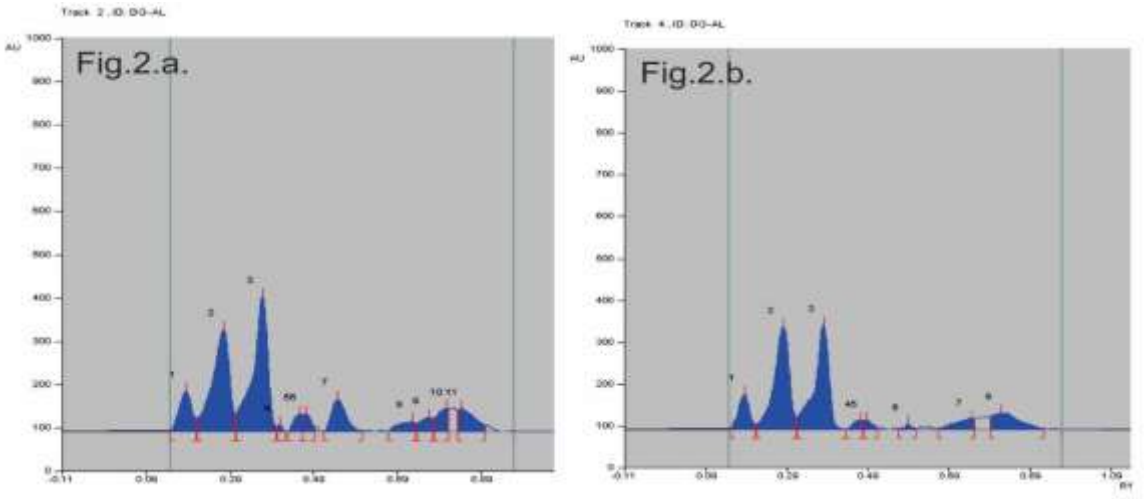


Figure No. 2.a and 2.b: Resolution chromatogram Of DG-AL and DG-ET

Further, DG-ET was screened for marking of flavonoid such as Genistein isoflavone. Genistein was selected as marker as reported in constituents, being flavonoid its better extracted out in ethyl acetate extract and being most potential isoflavone useful in osteoporosis, cardiovascular diseases and menopausal syndrome. Therefore, HPTLC fingerprinting of DG-ET along with Genistein isolflavone as biomarker was carried out. It shows better resolution with marking for Genistein at Rf 0.49.

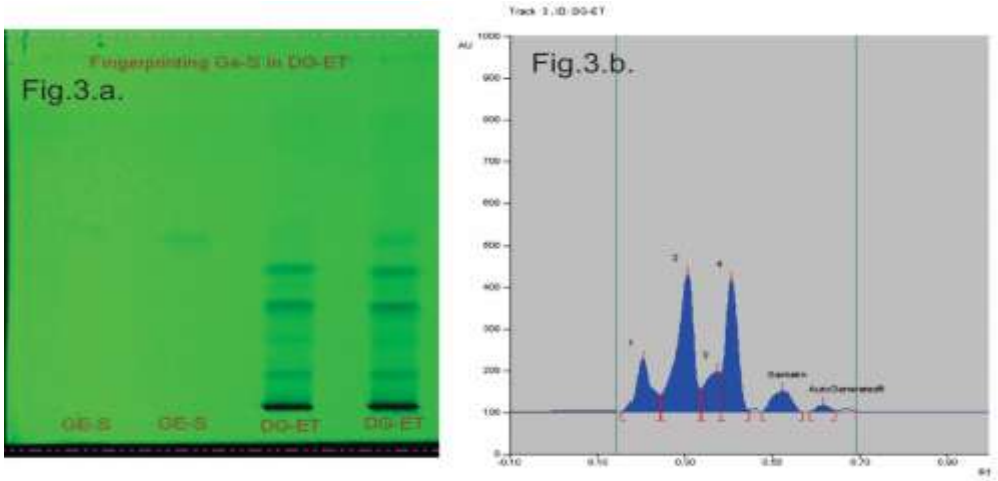


Figure No.3a. and 3.b: Resolution chromatogram of DG-Et with marking for Genistein

Stability study of DG-ET done with application of analyte with time interval shows no change in chromatogram of all 4 tracks designed for 3 hours stability in solution and 3 hours on the plate prior to chromatography.

Stability of analyte during chromatography was studied with 2 D development found acceptable as all zones are located on the diagonal connecting the application position with the intersection of the two solvent front.

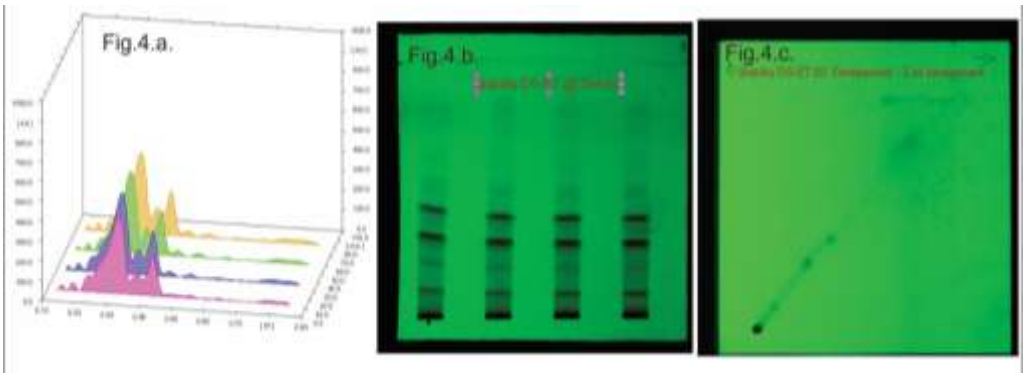


Figure No. 4: Chromatogram for stability Study Of DG-ET in solution, over plate and during chromatogram with 2 D development

CONCLUSION

Phytochemical screening shows presence of steroids, alkaloids, tannins and carbohydrates in respective extracts. HPTLC fingerprinting of DG-AL and DG-ET extract in mobile phase Toluene: Ethylacetate :Formic acid (6:4:0.3), scanned at 262nm shows 11 and 7 zone with good resolution. DG-ET was marked qualitatively with Genistein isoflavone as standard at Rf 0.49. Stability of DG-ET in solution and over plate with time was checked shows persistent chromatogram. Stability during chromatography checked with 2D chromatogram shows all zone in diagonal line. Genistein can be used as biomarker for standardisation of *D. gangeticum*.

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REFERENCES

1. Sharma A., Shanker C., Tyagi L., Singh M. and Rao V., Herbal Medicine for Market potential in India: An Overview'**.** Academic Journal of Plant Sciences, 2008; 1(2): 26-36.
2. Choudhary N and Sekhon B, An overview of advances in the standardization of herbal drugs. J.Pharm Educ Res, 2011; 2(2): 55-70.
3. Phillipsion, J.D., Ethnopharmacology and Western Medicine. J. Ethnopharmacol, 1989; 25: 61-72.
4. Nadkarnis K.M., Dr. K. M. Nadkarni's 'The Indian Materia Medica' Bombay Popular Prakashan, 1976; I: 612.
5. Kirtikar KR, Basu BD, Indian Medicinal Plants. 2nd ED. Edited, revised, enlarged and mostly rewritten by Blatter E, Calus JF and Mhaskar KS, Bishen Singh Mahendra Pal Singh, Dehradun, India, 1984; 1: 756.
6. Anonymous, 'The wealth of India' A dictionary of Indian Raw Materials and Industrial Product. Publication and Information Directorate, CSIR, New Delhi, 1988; 3: 19-20.
7. Ghosal S., Bhattacharya S K; 'Chemical and Pharmacological Evaluation of *Desmodium gangeticum'*. Planta Med., 1972; 22(8): 434-440.
8. Yadava R. N., Tripathi P.; 'A novel flavone glycoside from stem of Desmodium gangeticum'. Fitoterapia. 1998; 70: 213-214.

9. Purushothaman KK, Chandrasekharan S, Balakrishna K, Connolly JD Gangetinin and desmodin, two minor pterocarpanoids of Desmodium gangeticum. Phytochemistry. 1975; 14: 1129–1130.
10. Behari M, Varshney A. Sterols from Desmodium species. Indian Drugs. 1986; 23: 434– 435.
11. Kawale M. et al., 'Pharamcognostic characterization of Desmodium gangeticum (L.) DC.-An ayurvedic Medicinal plant'. 2012; 3(4): 113-120.
12. Mishra P.K., singh N., ahmad G., Dube A, and Maurya R.; 'Glycolipids and constituents from Desmodium gangeticum with antileishmanial and immunomodulatory activities. Bio organic and medicinal chemistry letters: 2005; 15: 4543-4546.
13. Meena A K, Rao M M, Kandale A, Sannd R, Niranjan U and Yadav A K, Standardisation of *Desmodium gangeticum*- A Tradition Ayurvedic Plant. Drug Invention Today, 2010; 2(2): 182-184.
14. Jabbar S, Khan MT, Choudhuri MS, The effects of aqueous extracts of Desmodium gangeticum DC. (Leguminosae) on the central nervous system. Pharmazie, 2001; 56(6): 506-8.
15. Govindarajan R, Rastogi S, Vijayakumar M, Shirwaikar A, Rawat AK, Mehrotra S, Pushpangadan P., Studies on the antioxidant activities of Desmodium gangeticum. Biol Pharm Bull., 2003; 26(10): 1424-7.
16. Rathi A, Rao ChV, Ravishankar B, De S, Mehrotra S., Anti- inflammatory and anti-nociceptive activity of the water decoction Desmodium gangeticum. J Ethnopharmacol. 2004; 95(2-3): 259-63.
17. Govindarajan R, Vijayakumar M, Shirwaikar A, Rawat AK, Mehrotra S, Pushpangadan P., Antioxidant activity of Desmodium gangeticum and its phenolics in arthritic rats. Acta Pharm., 2006; 56(4): 489-96.
18. Niranjan A., Tewari S.K., Phytochemical composition and antioxidant potential of Desmodium gangeticum (Linn.)DC. Natural Product Radiance, 2008; 7(1): 35-39.
19. Kurian GA, Paddikkala J., Administration of aqueous extract of Desmodium gangeticum L root protects rat heart against ischemic reperfusion injury induced oxidative stress. Indian J Exp Biol., 2009; 47(2): 129-35.
20. Kurian GA, Suryanarayanan S, Raman A, Padikkala J,. Antioxidant effects of ethyl acetate extract of Desmodium gangeticum root on myocardial ischemia reperfusion injury in rat hearts. Chin Med., 2010; 22: 5: 3.

21. Tsai J C, Huang G J, Chiu T H, Huang S S, Huang S C, Huang T H, Lai S C and Lee CY, Antioxidant activities of phenolic components from various plants of *Desmodium* species. African Journal of Pharmacy and Pharmacology, 2011; 5(4): 468-476.
22. Yadav AK, Singh SC & Gupta MM, A validated stability-indicating HPLC-PDA method for analysis of D*esmodium gangeticum*: an important ingredient of ayurvedic drug "dashmool".Journal of Liquid Chromatography & Related Technologies, 2012; 35(8): 1038-1052.
23. Anonymous, 'The Ayurvedic Pharmacopeia Of India', Government of India, Ministry Of Medicine and Homeopathy Welfare, Department Of Indian system Of medicine and homeopathy, New Delhi, Vol-III, Part-I, 1st edition, 2001; 179-180.
24. Eike Reich, Anne Schibli,' 'High Performance Thin Layer chromatography for the analysis of medicinal plants. Thieme New York, 2006; 245-246.
25. Khadabadi S S, Deore SL, Baviskar B A; 'Experimental Phytopharmacognosy: A Comprehensive guide., 2011, Nirali Prakasan: 4.9.
26. Indian Pharmacopoeia, Government of India Ministry of Health and Welfare, by the Indian Pharmacopoeia Commission, Ghaziabad, 2014; I: 98, 162, 277.
27. Mukherjee P K, 'Quyality control of Herbal Drugs: An Approch to evaluation of botanicals', Business Horizons Pharmaceutical Publishers, 2005; 189, 193.
28. Khandelwal K.R., 'Practical Pharmacognosy: Techniques and experiments'.Nirali Prakashan, 2011; 21st edition: 25.1-25.9.