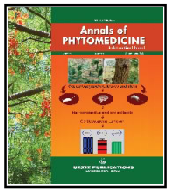
Annals of Phytomedicine 11(1): 613-618, 2022

DOI: http://dx.doi.org/10.54085/ap.2022.11.1.72

Annals of Phytomedicine: An International Journal

http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885



Original Article : Open Access

Authentication and quality evaluation of an Indian traditional medicinal plant: Salparni

Santosh Kumar*, Dinesh Kumar Yadav◆ and Richa Gautam**

Department of Pharmacognosy, SGT College of Pharmacy, SGT University, Gurugram-122505, Haryana, India

**Department of Botany, Government Girls Degree College, Kurawali, Mainpuri-205265, Uttar Pradesh, India*

***Department of Community Medicine, Hamdard Institute of Medical Sciences, New Delhi-110062, India*

Article Info

Article history

Received 20 January 2022

Revised 10 March 2022

Accepted 11 March 2022

Published Online 30 June 2022

Keywords

Desmodium gangeticum (L.) DC

Salparni

Desmodium velutinum

Desmodium triflorum

Pseudarthria vescida

Pharmacognosy

Abstract

The roots of *Desmodium gangeticum* (L.) DC. has been utilized as an official drug to the name of Salparni. However, the stems of *D. gangeticum* and the roots of some other species of Desmodium, *viz*., *D. velutinum*, *D. triflorum* and *Pseudarthria vescida* are also being used as Salparni. It is an essential indigenous traditional medicine for the treatment of various disorders like cough, bronchitis, fever, vomiting and as an antidote to a scorpion sting. Due to concerning adulteration issue and the standardization of Salparni, the study aimed to evaluate the pharmacognostic standardization of Salparni (*D. gangeticum* and its possible substitutes/adulterants). The results showed that all the three species of Desmodium can be differentiated based on the distribution pattern of phloem fibers. *D. gangeticum* shows 4-6 discontinuous bands of phloem fibers while in other species, the fibers are scattered and lesser in number. The transverse section of the root of *P. vescida* is altogether different concerning the cork, cortex and phloem region. The physicochemical parameters and TLC fingerprint profile showed much variation in all the samples used as Salparni except for two common spots at R_f 0.48 and 0.77 in the chemoprofile of all the samples of Salparni. These parameters can be used as standardized parameters to identify the commercial samples used or sold with the name of Salparni.

1. Introduction

Quality control evaluation of ethnomedicine helps in their regulation or authentication purpose. Due to morphological or organoleptic characteristic similarity, authentication of traditionally used botanicals or ethnomedicines has been concerning at the global scale. In contrast, morphological, microscopical and phytochemical profiling of the ethnomedicine using advanced analytical tools has been exponentially developed for authentication purposes (Gaurav *et al*., 2022). Salparni means leaves like those of Sal tree (*Shorea robusta*). It is botanically equated to *D. gangeticum*. The roots of *D. gangeticum* are the official drug used as Salparni (Rastogi *et al*., 2011). But, aerial parts (chiefly stem) are being used or sold as Salparni in different herbal drug markets of the country. Besides, other species of Desmodium, *viz*., *D. velutinum*, *D. triflorum* often mixed with the roots and stem of *D. gangeticum* and in the southern part of India mainly the roots of *Pseudarthria vescida* being used as Salparni (Dev *et al*., 2021). It is used as an essential herbal drug in Ayurveda, Siddha as well as Unani systems of medicine. These drugs are being used either as single constituents or in the polyherbal combination. Salparni is also acknowledged in the Samhitas as well as in Chikitsagranthas for the treatment of various deleterious diseases. It is characterized by ushna veerya, tikta-kashaya rasa, madhura vipaka as well as guru guna. It is exponentially used in the treatment of Shwasa, Chardi, Jwara, Atisara, Shwasa, *etc.* (Joshi *et al*., 2012; Dev *et al*., 2021). It is bitter in taste, smooth digestive, febrifuge, antiemetic anticatarrhal. In most cases, it is used as a prominent anti-inflammatory, agent. Traditionally, its root is widely used as an expectorant, antidot for snake bites as well as scorpion stings (Mohan *et al*., 2021; Dev *et al*., 2021). Modern literature showed that *D. gangeticum* exhibits a potential role in ischemic heart disease in form of a single drug as well as in polyherbal combination (Kirtikar and Basu, 1987). Most of the Ayurvedic preparations possess *D. gangeticum* as an essential and major ingredient, namely; 'Dashmoolarishta' and 'Dashmoola kwaath' are endorsed to avoid secondary biological complications such as puerperal fever (Dev *et al*., 2021; Kurian *et al*., 2005).

D. gangeticum is reported to contain different chemical constituents like amino acid, phenols, glycoside, alkaloids, flavonoids, and coumarins (Mohan *et al*., 2021). There are numerous therapeutic activities such as smooth muscle relaxant, antiasthmatic, antileishmanial, immunomodulatory, anti-inflammatory, antiulcer, antidiabetic, cardioprotective, antiviral, antiamnesia, antioxidant as well as hepatoprotective activities (Rastogi *et al*., 2011).

During the survey of herbal drugs based on the markets of major Indian crude, it was observed that maximum samples of Salparni were the mixture of two or three species may be because of almost the same morphology and regional names, which affect the originality of Salparni. Considering the facts, the present study is associated to standardize the Salparni based on the macro-microscopic description, physicochemical parameters and TLC fingerprint profiling and validating the typical parameters for authentication and quality control of Salparni.

Corresponding author: Dr. Dinesh Kumar Yadav

Associate Professor, Department of Pharmacognosy, SGT College of Pharmacy, SGT University, Gurugram-122505, Haryana, India

E-mail: dineshnbri08@gmail.com

Tel.: +91-7042348251

2. Materials and Methods

2.1 Chemicals

Each chemical used was of analytical grade from MERCK and SD Fine chemicals Ltd.

2.2 Plant material

The roots of different species of Salparni were procured from the Chitrakoot (Madhya Pradesh) and Lucknow (Uttar Pradesh), India. Each sample was authenticated based on taxonomic classification at National Botanical Research Institute, City Lucknow, India. The voucher specimen of each sample such as *D. gangeticum*, *D. velutinaum*, *D. triflorum* and *P. vescida* with voucher numbers 92476, 216569, 216585 and 216735, respectively were submitted to the Institutional herbarium center for future reference. The crude plant materials were shade dried, crushed using a grinder and stored for further analysis. Fresh plant material was used for macroscopical and microscopical analysis.

2.3 Macroscopic and microscopic analysis

Macroscopy and microscopic studies were conducted as per the reference protocol with some modifications. Briefly, transverse sections (TS) for root bark and stem of the bark of Salparni and root of other samples were prepared for the microscopical analysis. The thickness of each section was approx. 10 µm. Each sample was mounted in glycerin and stained with safranin on a glass slide and dried for 1 min at 50°C through an air drier followed by washing with water to remove the content of sugar. A saturated solution of chloral hydrate was used to further mount the prepared specimen. Each sample was cooled at room temperature, mounted with glycerin to moisturize the specimen and proceed for the microscopic observation (Akbar *et al*., 2014).

2.4 Physicochemical analysis

The complete dried material of each sample was used for the quantitative determination of total ash, acid insoluble ash, water as well as alcohol-soluble extractives. Each parameter was determined according to the protocol mentioned in the Indian Pharmacopoeia. The sugar and starch content (%, w/w) were also determined, spectrophotometrically (Akbar *et al*., 2014; Alam and Us Saqib, 2015).

2.5 TLC fingerprinting

One-gram (1 g) powder of each sample was properly weighed and refluxed for 5 min for extraction on a water bath using methanol (5 ml) as an extracting solvent. After the extraction process, the extracts were filtered using a Whatman filter paper. The filtrates were used as a test solution. Each sample was then applied on TLC plate (5 x 10 cm) pre-coated with silica gel G60 F254 with the help of a Camag Linomat-IV applicator. Toluene and ethyl acetate (7: 3, v/v) were used as the solvent system to develop the TLC plate. The plate was developed in a pre-saturated TLC development chamber to a distance of 8.0 cm at room temperature (25°C). After the development of the plate, it was derivatized with anisaldehyde-sulphuric acid followed by heating at 105°C for 5-10 min for visualization of spots. The Rf values and color of each resolved band were recorded (Khan *et al*., 2021).

Table 1: Comparative distinguishing characters of possible adulterants/substitutes of Salparni

Characters	*D. gangeticum* (root)	*D. gangeticum* (stem)	*D. velutinum* (root)	*D. triflorum* (root)	*P. vescida* (root)
Macroscopy					
Size	0.2-0.6 cm diameter	0.5-1 cm in diameter	0.1-0.8 in diameter	1-5mm in diameter	0.5-0.9 cm in diameter
Outer surface	Brown woody with fine wrinkles and bacterial nodules	Pubescent with prominent leaf scars	Rough due to presence of short vertical slits with bacterial nodules	Rough due to presence of small lenticels	Dark brown, woody, rough due to presence of lenticels
Fracture	Hard and fibrous	Hard and fibrous	Hard	Soft	Hard
Odour	Pleasant smell	No characteristic odour	No characteristic odour	No characteristic odour	No Characteristic odour
Taste	Astringent	Slightly bitter	Astringent	Astringent, mucilagonous	Astringent
Microscopy					
Cuticle	Absent	Present	Absent	Absent	Absent
Cork	Stratified, 3-8 layered of thick walled, tangentially elongated cork cells, having prismatic crystals of calcium oxalate	Absent	Stratified, 3-6 layered of thick walled, tangentially elongated cork cells, having prismatic crystals of calcium oxalate	Stratified, 3-6 layered	Stratified, 10-15 layered with thick walled rectangular cork cells
Cork cambium	Present	Absent	Present	Present	Present
Cortex	4-12 layered sclarenchymatous	4-6 layered parenchymatous followed by endodermis	2-3 layered, collenchymatous	3-8 layered sclarenchymatous	Parenchymatous cells with filled with brown content
Fiber	Groups of 4-6 forming prominent discontinuous bands	Absent	Scattered	Scattered	Present
Phloem	Secondary phloem 4-10 layered with sieve tubes, companion cells, fibres, parenchyma traversed by 1 to 4 celled thick phloem rays	6-16 layered, consisting of sieve tubes companion cells, phloem parenchyma with small groups of phloem fibres	Secondary phloem 4-8 layered with sieve tubes, companion cells, fibres, and phloem parenchyma	Secondary phloem 4-6 layered with sieve tubes, companion cells, fibres, and phloem parenchyma	Presence of sieve tubes, companion cells, phloem fibres and phloem parenchyma, cells crushed to form 2-4 layered ceretenchyma
Xylem	4-5 Protoxylum groups, sclerenchymatous cells broad,	Xylem which consist of vessels, tracheids and xylem parenchyma	4-5 Protoxylum groups, sclerenchymatous cells small,	Sclerenchymatous in scattered groups, cells filled with dark contents, parenchyma cells filled with starch.	Large number of wood fibres, with small amount of xylem vessels filled with starch grains
Medullary rays	1-2 seriate filled with starch grains	1-2 seriate	1-2 seriate filled with starch grains	2-3 seriate filled with starch grains	2-3 seriate filled with starch grains

3. Results

The pharmacognostic characteristic of different samples of Salparni was performed successfully. *D. gangeticum* was used as the standard drug as compared to the pharmacognostic characteristic of another most adulterated Salparni samples such as *D. velutinum*. *D. triflorum* and *P. vescida*.

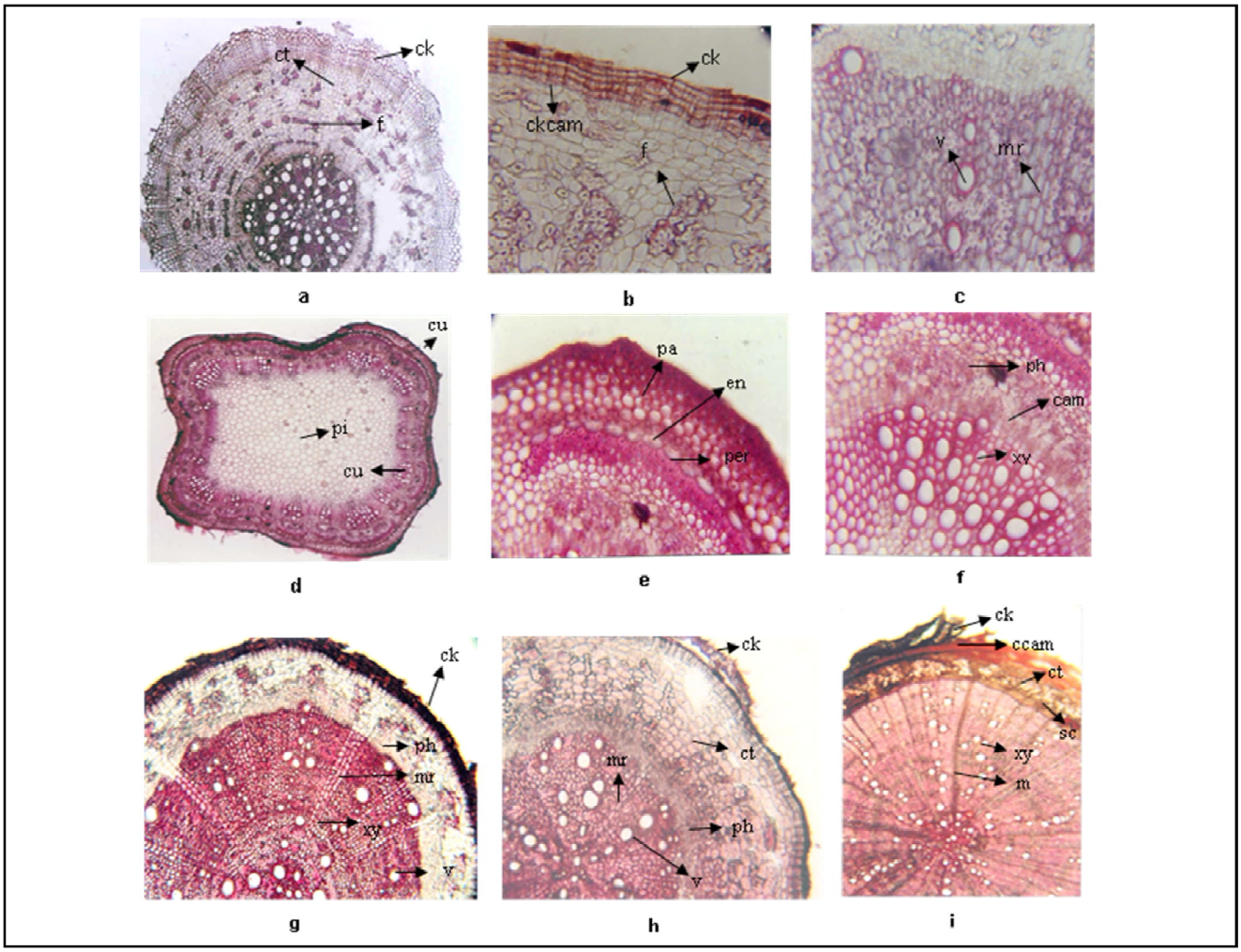
3.1 Macroscopy and microscopy analysis

Macroscopic and microscopic characterization of the medicinal plants represents their originality even authenticity. Based on these characteristics, medicinal plants can be evaded through spurious or adulterated drugs (Rastogi *et al*., 2011). Considering the facts, macroscopy and microscopy analysis for each collected sample of Salparni were done, successfully. *D. gangeticum* (root), *D. gangeticum* (stem), *D. velutinum* (root), *D. triflorum* (root) and *P. vescida* (root) were attributed for the macroscopic characteristic. The parameters such as size, outer surface, fracture, odor and taste. The size of each selected species was found 0.2-0.6 cm diameter, 0.5 -1 cm in diameter, 0.1-0.8 cm in diameter, 1-5 mm in diameter and 0.5-0.9 cm in diameter, respectively. The outer surface of each was found like brown woody with fine wrinkles, pubescent with prominent leaf scars, rough due to presence of short vertical slits with bacterial nodules, rough due to presence of small lenticels, and dark brown, woody, rough due to the occurrence of lenticels, respectively. The astringent or slightly bitter test was found of each sample. The observations outcome of each sample has been summarized in Table 1 and Figure 1.

Figure 1: Macroscopic details of Salparni, Figure (a) *D. gangetcum*, Figure (b) *D. velutinum*, Figure (c) *D. triflorum*, Figure (d) *P. vescida*, Figure (e) dried root of *D. gangeticum*, Figure (f) dried stem of *D. gangeticum*, Figure (g) dried root of *D. velutinum* and Figure (h) dried root of *P. vescida*.

Table 2: Value and colour of R_f in TLC profile of Salparni

Sr. No	*D. gangeticum* (root)		*D. gangeticum* (stem)		*D. velutinum* (root)		*D. triflorum* (root)		*Pseudarthria vescida* (root)	
	R_f Values	Colour	R_f Values	Colour	R_f Values	Colour	R_f Values	Colour	R_f Values	Colour
	0.26	Blue	0.26	Blue	0.25	Blue	0.25	Grey	0.21	Blue
	0.33	Brown	0.33	Brown	0.34	Blue	0.48	Blue	0.39	Grey
	0.48	Blue	0.48	Blue	0.43	Blue	0.77	Blue	0.41	Brown
	0.50	Blue	0.50	Blue	0.48	Blue	0.83	Blue	0.54	Blue
	0.55	Blue	0.55	Blue	0.77	Blue	0.87	Blue	0.62	Blue
	0.70	Blue	0.70	Blue	0.83	Blue	0.93	Brown	0.70	Blue
	0.77	Blue	0.77	Blue	0.87	Brown	-	-	0.79	Blue
	0.87	Grey	0.83	Grey	0.93	Brown	-	-	0.85	Brown
	0.93	Blue	0.93	Blue	-	-	-	-	0.90	Blue
Total metabolites	**09**		**09**		**08**		**06**		**09**	



Figures 2: Microscopic details of Salparni; Figures (a,b,c) represent the transverse section of root of *D. gangeticum*, Figures (d,e,f) represent the transverse section of stem of *D. gangeticum*, Figure (g) represents the transverse section of root of *D. velutinum*, Figure (h) represents the transverse section root of *D. triflorum*, Figure (i) transverse section root of *P. vescida* [Abbreviations: ckcam, cork cambium; ck cork; ct, cortex; cu, cuticle; en, endodermic; ep, epidermis; f, fibers; pa, parenchyma; ph, phloem; p, pith scl, sclarenchyma; xy, xylem; v, vessel].

In microscopic analysis, the parameters such as cuticle, cork, cork cambium, cortex, fiber, phloem and xylem were identified in each sample. The analysis was performed to determine the variability or differentiation of the cellular architect for the three species of Desmodium. The analysis can also be differentiated based on the distribution pattern of phloem fibers. *D. gangeticum* shows 4-6 discontinuous bands of phloem fibers while in other species the fibers are scattered and lesser in number. The transverse section of the root of *P. vescida* is altogether different with respect to cork, cortex and phloem region where 10-15 layered cork is presently filled with brown content and the presence of 2-4 layered ceretenchyma in phloem region, the number of prismatic crystals lesser in number in comparison to that of *Desmodium species*. The outcome of the study has been summarized in Table 1 and Figure 2.

3.2 Physicochemical analysis

The physicochemical analysis was performed to determine total ash, acid insoluble ash, water as well as alcohol-soluble extractives, sugar and starch in each selected sample. The outcome of the study showed that total ash, water extractives and starch content were found higher in *D. velutinum* while these parameters were found significantly higher in *D. gangeticum* and *D. triflorum*, also. Moreover, showed not much difference in Desmodium species while the values for *P. vescida* were significantly lower as compared to the other samples of Salparni. The outcomes of the phytochemical analysis have been summarized in Figure 3.

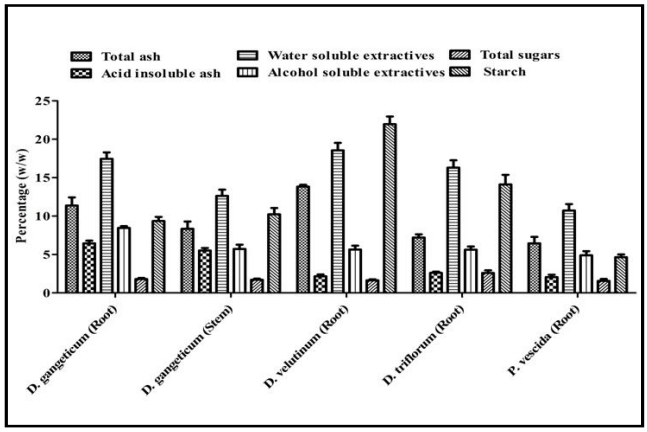


Figure 3: Physicochemical parameters of Salparni.

3.3 TLC fingerprinting

TLC fingerprinting analysis was conducted by using a suitable solvent system and stationary phase. The chromatographic condition and the application volume remained the same as per the protocol. The observations were made based on the visualized spots on the TLC plate in form of the different colors. The maximum number of the band (represents the compounds) was of the violet color. The outcome of the study showed that each sample of Salparni in the thin layer chromatography showed almost the same profiles in the case of root and stem of *D. gangeticum* except two bands at Rf 0.33 and 0.87 which were absent in the stem of *D. gangeticum* while *D. velutinum* (root), *D. triflorum* (root) and *P. vescida* (root) shows entirely different profiles. The outcome of the study has been summarized in Table 2 and Figure 4.

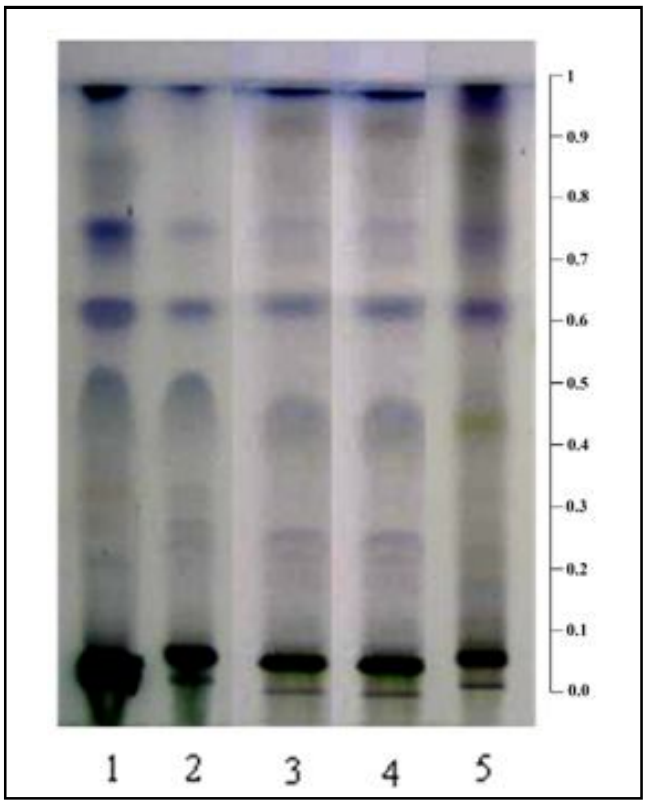


Figure 4: TLC fingerprint profile of methanolic extract of Salparni: Figure (1). *D. gangeticum* (root), Figure (2). *D. gangeticum* (Stem), Figure (3). *D. velutinum* (root), Figure (4). *D. triflorum* (root), Figure (5). *P. vescida* (root).

4. Discussion

Herbal drug adulteration is one of the concerning matters for the quality-based assessment of the herbal drugs used for therapeutic purposes. Due to adulteration or spurious crude drugs or botanicals, it is difficult to reach the significant therapeutic action even causing unintended biological action. For the regulatory purpose of the herbal drug, herbal drug standardization of the medicinal plants is one of the critical needs to validate the ethnomedicine or botanicals for their regulatory purpose. Based on the facts, the study was designed to investigate and generate the scientific evidence for the most known Indian traditional medicinal plant *D. gangeticum* for its quality-based standardization with respect to the most adulterated drugs such as *D. velutinum*, *D. triflorum* and *P. vescida*, *etc.* In this study, macroscopy and microscopic characteristics of each sample followed by TLC fingerprinting analysis for phytochemical evaluation were done. The outcome of the study showed that the average size of each selected species was found approximately 0.2-0.1.5 with an outer surface like brown woody and fine wrinkles. *D. triflorum* was found with the pubescent with prominent leaf scars while short vertical slits with bacterial nodules were found in *P. vescida*. The outer surface of each specimen was found as Dark brown, woody, rough due to the presence of lenticels with astringent or slightly bitter. The previous study supports the findings of the present study (Pathak *et al.*, 2021; Mohan *et al.*, 2021). In phytochemical analysis, total ash, water extractives and starch content were found higher in

D. velutinum while these parameters were found significantly higher in *D. gangeticum* and *D. triflorum* also. Moreover, showed not much difference in Desmodium species while the values for P. vescida were significantly lower as compared to the other samples of Salparni. The findings were matched the previous study. A study conducted by Mohan and his team showed that *D. gangeticum* physicochemical parameters like percent moisture content, total ash as well as acid-insoluble ash were found to be 11.85%, 7.38%, and 1.78%, in areal respectively. The same contents were evaluated for the root art which was found as 11.34%, 4.80%, and 0.76%, respectively (Mohan *et al*., 2021).

TLC fingerprinting is one of the easiest, economic and robust methods to determine the phytochemical profiling of the herbal drugs, and based on the separated phytochemicals library, band intensity and pigmentation pattern, the variability among the different samples can be identified easily. In our fingerprinting profiling, same profiles in the case of root and stem of *D. gangeticum* except for two bands at Rf 0.33 and 0.87 which were absent in the stem of *D. gangeticum* while *D. velutinum* (root), *D. triflorum* (root) and *P. vescida* (root) show entirely different profile (Yadav and Gupta, 2014; Dev *et al*., 2021). The findings represent the method as a standardized scientifically validated note in the regulation of *D. gangeticum*.

5. Conclusion

The present study concludes that all four species of Salparni can easily be differentiated based on morphologically, microscopically as well as through chromatographic profile. Cork, cork cambium, cortex, fiber, phloem and xylem were found varied as compared to the *D. gangeticum* while TLC fingerprinting represents the phytochemicals variability among different samples.

Acknowledgments

Authors are thankful to the Director, National Botanical Research Institute (NBRI) - Council of Scientific and Industrial Research (CSIR) for providing the facilities and grant for research and also thankful to the Director, Joint Director, Higher Education Uttar Pradesh, Prayagraj for their encouragement.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

References

Akbar, S.; Hanif, U.; Ali, J. and Ishtiaq, S. (2014). Pharmacognostic studies of stem, roots and leaves of *Malva parviflora* L. Asian Pac. J. Trop. Biomed., **4**:410-415.

Alam, F. and Us Saqib, Q. (2015). Pharmacognostic study and development of quality control parameters for fruit, bark and leaf of *Zanthoxylum armatum* (Rutaceae). Anc. Sci. Life, **34**:147.

Dev, S.A.; Unnikrishnan, R.; Jayaraj, R.; Sujanapal, P. and Anitha, V. (2021). Quantification of adulteration in traded ayurvedic raw drugs employing machine learning approaches with DNA barcode database. Biotech., **11**:1-6.

Gaurav; Khan, M.U.; Basist, P.; Zahiruddin, S.; Ibrahim, M.; Parveen, R.; Krishnan, A. and Ahmad, S. (2022). Nephroprotective potential of *Boerhaavia diffusa* and *Tinospora cordifolia* herbal combination against diclofenac induced nephrotoxicity. South African J. Bot., 000. https://doi.org/10.1016/j.sajb.2022.01.038

Joshi, P.; Patel, B. and Shukla, V. (2012). An overview of the causes of current practices in Pratinidhi Dravyas (substitution of drugs) in Ayurveda including newer techniques for their evaluation. AYU (An Int. Q. J. Res. Ayurveda), **33**:481.

Khan, A.; Zahiruddin, S.; Ibrahim, M.; Basist, P.; Gaurav; Parveen, R.; Umar, S. and Ahmad, S. (2021). Thin layer chromatography-mass spectrometry bioautographic identification of free radical scavenging compounds and metabolomic profile of *Carica papaya* Linn. fruit and seeds using high-performance thin-layer chromatography, gas chromato graphy-mass spectro. Pharmacogn. Mag., **17**:21.

Kurian, G.A.; Philip, S. and Varghese, T. (2005). Effect of aqueous extract of the *Desmodium gangeticum* DC root in the severity of myocardial infarction. J. Ethnopharmacol., **97**:457-61.

Mohan, P.K.; Adarsh Krishna, T.P.; Senthil Kumar, T. and Ranjitha Kumari, B.D. (2021). Pharmacochemical profiling of *Desmodium gangeticum* (L.) DC. with special reference to soil chemistry. Futur. J. Pharm. Sci., **7**:1-1.

Pathak, J.; Aswathi, M.P.; Patel, B.R.; Harisha, C.R. and Shukla Vinay, J. (2021). Microscopic and phytochemical analysis of *Desmodium velutinum* (Willd) DC and *Desmodium gangeticum* (L.) DC. panchanga powder. Res. J. Pharm. Technol., **14**:2950-2956.

Rastogi, S.; Pandey, M.M. and Rawat, A.K.S. (2011). An ethnomedicinal, phytochemical and pharmacological profile of *Desmodium gangeticum* (L.) DC. and *Desmodium adscendens* (Sw.) DC. J. Ethnopharmacol., **136**:283-96.

Yadav, A.K. and Gupta, M.M. (2014). Validated RP-HPLC and HPTLC methods for determination of anti-inflammatory bis-indole alkaloid in *Desmodium gangeticum*. Nat. Prod. Res., **28**:275-7.

Citation **Santosh Kumar, Dinesh Kumar Yadav and Richa Gautam (2022). Authentication and quality evaluation of an Indian traditional medicinal plant: Salparni. Ann. Phytomed., 11(1):613-618. http://dx.doi.org/10.54085/ap.2022.11.1.72.**