



Detailed comparative pharmacocognostical and preliminary physicochemical investigation of two source plant of *Rasna*: *Pluchea lanceolata* CB Clerk and *Blepharispermum subsessile* DC.

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Abstract :

Background: India has rich floristic and ethnic diversity. *Rasna* is a very important medicinal plant having many therapeutic uses. *Pluchea lanceolata* CB Clerk is an API recommended source plant of *rasna* found in upper gangiatic plain and Rajasthan and *Blepharispermum subsessile* DC is a folklore medicinal herb, locally known as *Rasnajhadi* in Odisha, found in Odisha, Karnataka, Madhya Pradesh, and Maharashtra. These two sources are being used as *Rasna* in treating rheumatic, gynecological, nervous disorders etc. **Aim:** Comparative review on pharmacocognostical and preliminary physicochemical profiles of *Pluchea lanceolata* CB Clerk root (PLR) and *Blepharispermum subsessile* DC root (BSR). **Materials and Methods:** Roots of *Pluchea lanceolata* CB Clerk (PLR) were collected from Jamnagar, Gujarat and *Blepharispermum subsessile* DC roots (BSR) were collected from Odisha. Its macroscopic, microscopic, powder characters and preliminary physicochemical characters, Comparative HPTLC were studied following standard procedures. **Results and conclusion:** the findings of the study will be useful in the identification and standardization of roots of *Pluchea lanceolata* CB Clerk and *Blepharispermum subsessile* DC.

KEY WORDS: *Pluchea lanceolata* CB Clerk (PLR) and *Blepharispermum subsessile* DC (BSR), Root, Asteraceae, *Rasna*, ethnomedicine, physicochemical, HPTLC.

1. INTRODUCTION:

Rasna is very popular and widely used drug for diseases caused due to vitiation of vata.¹ But its botanical source is highly controversial, different plant species are being used in the name of *Rasna*. Such as, *Rasna* of U.P. (*Pluchea lanceolata* Oliver & Hiren), *Rasna* of Bengal or east (*Vanda roxburghii* R.Br.), *Rasna* of Madras

(*Alpinia galanga* Willd.) etc. In the present study attempt has been made for comparative study on two botanical sources of *Rasna* i.e. *Pluchea lanceolata* CB Clerk and *Blepharispermum subsessile* DC for their pharmacognostical and preliminary physicochemical profiles.

Pluchea lanceolata CB Clerk (Asteraceae), an erect, stiff herb or undershrub, distributed in Punjab, Upper Gangetic Plain, Rajasthan, Maharashtra, Gujarat and Bengal. It is the API recommended botanical source plant of *Rasna*.² The roots are bitter, thermogenic, alexiteric, antipyretic, laxative and used for allaying the pain caused by the sting of scorpions. Externally it is used in rheumatism and also in diseases of the nervous system. The plant is used for the inflammations and bronchitis, cough, psoriasis, piles. It is also used as laxative, analgesic, antipyretic, nervine tonic. The decoction of plant is used to prevent the swelling of joints in arthritis, inflammations, rheumatism, bronchitis, cough, psoriasis, piles and neurological diseases.³

While *Blepharispermum subsessile* DC (Asteraceae) is a glabrous shrub with small close globose cluster head, distributed in Odisha, Karnataka, Madhya Pradesh and Maharashtra. It's a less explored folklore medicinal plant and is marketed with high price in the name of *Rasna*, known as *Rasnajhadi*.⁴ An ethno botanical study reports the uses of its root in the management of various diseases. Whole plant is used as an Anti-inflammatory,⁵ Paste made of *Shunthi* and *Rasna* (*Blepharispermum subsessile*), some juice of *Palandu* (*Allium cepa*) and Honey are mixed and given to children in *Atisara* (Diarrhoea),⁶ Decoction of *Ashoka* root, *Arjuna* bark, *B. subsessile* root and *Guduchi* stem is used in Irregular Menstruation,⁷ Decoction as well as powder of root is used in Nervous disorders,⁸ Socio-religious customs of Bastar region i.e. Raj gonds, Murias, Bhatra, uses decoction of *Rasnajhadi* on 3rd day of menstruation and after delivery,⁹ Root juice is given in Rheumatoid Arthritis. Dose- 10 ml once a day,¹⁰ also used in Skin diseases^{11,12} and in Krimiroga as Wormicidal.¹³

No previous work is on comparative study on *Blepharispermum subsessile* DC and *Pluchea lanceolata* CB Clerk has been done yet. Hence in the present study pharmacognostical and preliminary physicochemical characters of these two sources of *Rasna* has been studied.

2. MATERIALS AND METHODS

Materials

Sample of root of *Blepharispermum subsessile* DC and root of *Pluchea lanceolata* C B Clerk were used as material for the pharmacognostical and preliminary physicochemical study. The study was conducted as per the guidelines of Ayurvedic Pharmacopia of India.¹⁴

2.1 Plant Identification

Rasnajhadi, growing in Gurudongar medicinal plants conservation area of Nuapada district of Odisha, India, was identified by local traditional practitioners and authenticated by expert taxonomist as *Blepharispermum subsessile* DC. (Asteraceae), on the basis of characters given in flora of Orissa.¹⁵

Pluchea lanceolata C B Clerk growing in peripheral area of Jamnagar town, was identified in the field by observing its taxonomical and morphological characters as mentioned in different Materia medica and Floras and published research papers^{16,17,18,19} and authenticated in the Pharmacognosy Department of G.A.U., Jamnagar.

2.2 Collection of the sample

The fresh plant sample of *Blepharispermum subsessile* DC was collected from natural habitat, Odisha, in the month of October 2011 and voucher specimen has been preserved in the pharmacognosy laboratory of ITRA,GAU, Jamnagar, vide no36561. *Pluchea lanceolata* CB Clerk were collected from the Jamnagar railway station area in the month of December 2011 and voucher specimen has been preserved in the pharmacognosy laboratory of ITRA,GAU, Jamnagar,vide no 37325.

2.3 Preservation and processing:

The collected plant samples were shaken to remove adherent soil and dirt. Root of *Blepharispermum subsessile* DC and root of *Pluchea lanceolata* CB Clerk were washed separately with running fresh water and few pieces stored in solution of AAF (Alcohol: Acetic acid: Formalin) in the ratio of (90:5:5),²⁰ to utilize them for microscopic studies. The remaining samples were shade dried and then powdered with mechanical grinder and passed through mesh no.85# and preserved in an air-tight glass container.

2.4 Pharmacognostical studies:

2.4.1 Organoleptic study: The fresh and dry samples of root, of *Blepharispermum subsessile* DC and root of *Pluchea lanceolata* CB Clerk were evaluated for their organoleptic characters including taste, odour, colour, and touch.²¹

2.4.2 Macroscopic study: The macroscopic characters of root of *Blepharispermum subsessile* DC and root of *Pluchea lanceolata* CB Clerk were studied systematically as per the standard textbook of Botany²² as well as with the help of floras.²³

2.4.3 Microscopic study

Transverse section microscopy: For detailed microscopical observation, free hand thin transverse section of root, of *Blepharispermum subsessile* DC and root of *Pluchea lanceolata* CB Clerk were taken, and cleared with chloral hydrate and observed as such for the presence of any crystals, then were stained with Phloroglucinol and Hydrochloric acid to notice the lignified elements like fibers, vessels etc.²⁴ Photographs of the section were taken with the help of Canon digital camera attached to Carl Zeiss trinocular microscope. Powder characters were observed and histochemical tests carried out, as per guidelines of Ayurvedic Pharmacopoeia of India.²⁵

2.5 Phytochemical Evaluation: preliminary physicochemical investigations were carried out.

2.6. HPTLC Analysis : High performance Thin Layer Chromatography(HPTLC) was done for active ingredient analysis for both the plants at I.T.R.A. Jamnagar, Gujarat Ayurved University following standard procedures recommended by API.²⁶

3. RESULTS AND DISCUSSION:

3.1 Pharmacognostic study

3.1.1 Macroscopic & Microscopic evaluation (Figure:1,2)

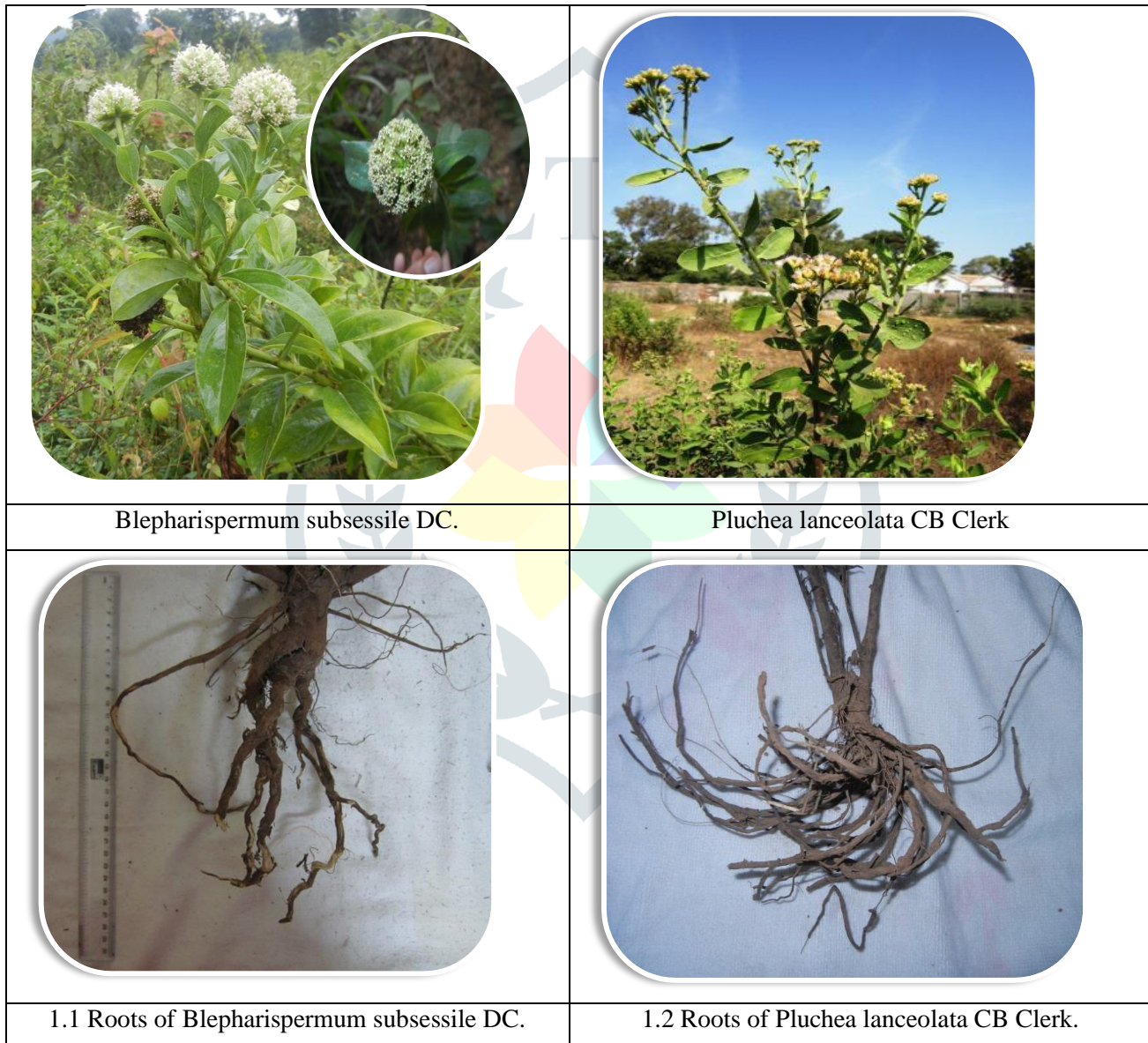
Morphology: (Figure:1)

Root of *Blepharispermum subsessile* DC consist of cylindrical unbranched straight or slightly bent or tortuous pieces of roots, 2.5 - 5.5cm in length and 0.3 to 0.5 cm in diameter, longitudinally ridged, wrinkled and fissured,

show few transversely running lenticels, transversely cracked at places exfoliated exhibiting the inner narrow yellow wood. Fracture is short, externally earthy brown in color, internally yellowish. Some pieces show the crown with cluster of aerial stem arising from its upper surface and roots from the lower surface. **(Figure 1.1)** Roots of *Pluchea lanceolata* C B Clerk are about 10 to 20 inches in length, 3 to 20 mm in diameter, somewhat twisted and gradually tapering. The external surface is white when young while it is light to dark brown in mature one and the wood is brownish. External surface showed longitudinal rough striations. **(Figure 1.2)**

Both the plants are shrub, roots of *Blepharispermum subsessile* DC are 2.5 - 5.5cm in length and 0.3 to 0.5 cm in diameter while Roots of *Pluchea lanceolata* C B Clerk are about 10 to 20 inches in length, 3 to 20 mm in diameter,

Figure 1: External Morphology



Microscopic study:

The pharmacognostical characters of Roots of *Blepharispermum subsessile* DC have already been reported.²⁷

Roots of *Pluchea lanceolata* C B Clerk -In mature root, the cortex is composed of 3-5 layers of tangentially elongated cells, which are radially arranged but in most of the cases the cork is usually ruptured. Phallogen is composed of 1-2 layers of thin-walled and tangentially elongated cells. The phallogen is composed of 6-10 layers of thin-walled and tangentially elongated cells. Schizogenous type of resin canals is formed in the phellogen region. In the cortex zone parenchyma cells are loosely arranged along with air spaces, some of the parenchyma cells contain yellow brownish contents and starch grains. The secondary phloem is a narrow zone and is composed of sieve tubes, companion cells, phloem parenchyma and phloem fibres traversed by phloem rays. The phloem fibres are lignified, thick walled and pointed. The innermost zone of phloem is devoid of fibres. Fibres mostly confined to the outer and middle zone of phloem and appear as covering on the pyramid shaped secondary phloem region.(Figure 2.2)

The secondary xylem is wide region consisting of vessels, tracheids, parenchyma and fibres traversed by xylem rays. In a macerated preparation, the vessels are cylindrical with transverse to oblique articulation and well-marked perforation rims. The walls are lignified, showing reticulate and pitted type of thickening. The pits are simple. Xylem parenchyma is square to rectangular in shape with lignified walls and simple pits. The medullary rays are broad and funnel shaped. Some of the ray cells, both in the xylem as well as in the phloem region. The rays are multiseriate, 4-9 cells wide in the phloem region and 3-6cells wide in the xylem region. In tangential section through the phloem, they are oval in shape with prominent intercellular spaces. Resin canals which appear as sub-spherical or elliptical cavities in transverse section.

Table 1 : Characters of Transverse sections of *Blepharispermum subsessile* DC and *Pluchea lanceolata* CB Clerk root.(Figure 2.1 and 2.2)

	BSR	PLR
Differentiating characters	<ol style="list-style-type: none"> 1. Patches of interaxylary phloem and intervascular pits were found within xylem. 2. Inner towards the center, band of xylem forming ring leads into anomalous growth. 3. xylem abruptly interperited with phloem and makes second layer of xylem towards the centre. 	<ol style="list-style-type: none"> 1. Sufficient intercellular spaces within cortex and pith. 2. Resin canals which appear as sub-spherical or elliptical cavities in tranverse section. 3. Bundles of phloem fibres, thick walled, lignified surrounded by parenchymatous sheath.
Similar characters	<ol style="list-style-type: none"> 1. Unicellular trichomes 2. Cortex full of oil cells, starch grains and lignified cells. 3. Multiseriate medullary rays. 	

(BSR- Roots of *Blepharispermum subsessile* DC and PLR- Roots of *Pluchea lanceolata* CB Clerk)



Figure 2.1: T.S. of *B. subsessile* DC. Root

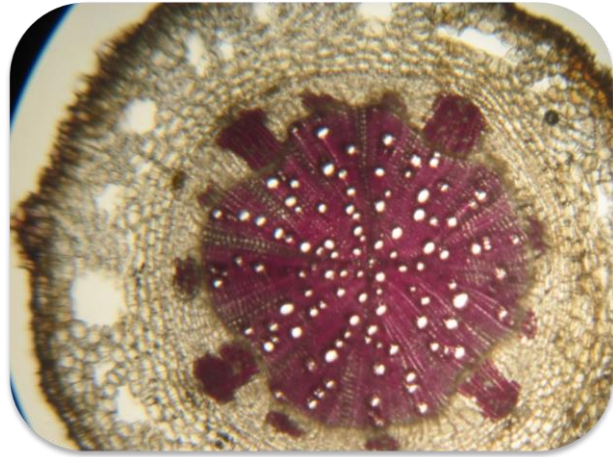
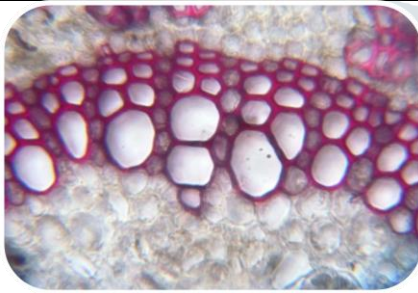
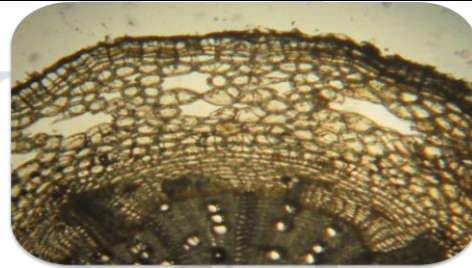


Figure 2.2: T.S. of *P. lanceolata* C B Clerk Root

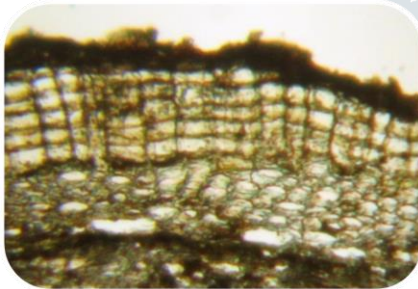
Various parts of T.S. in enlarged view



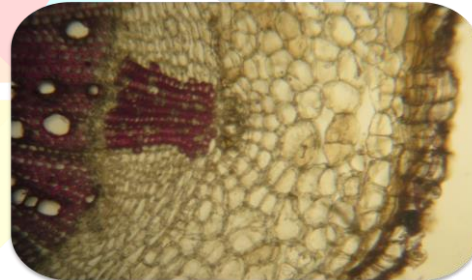
Centrally xylem, Xylem fibres



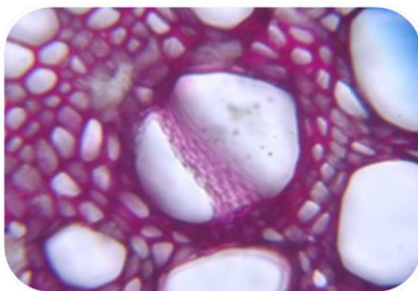
Secretory cavity



Cork, Cortex



Bundle of phloem fibre



Xylem with parenchyma and fibres



Xylem, phloem, Medullary rays

***Blepharispermum subsessile* DC root**

***Pluchea lanceolata* C B Clerk Root**

3.1.2 Powder microscopy (Figure 3)**Table 2.1: Organoleptic characters of BSR and PLR**

Sr. No.	Organoleptic characters	Blepharispermum subsessile DC Root (BSR)	Pluchea lanceolata C B Clerk Root (PLR)
1.	Color	Brownish-black	yellowish brown
2.	Odour	aromatic odour	slightly aromatic odour
3.	Taste	bitter taste	slightly bitter and astringent taste
4.	Touch	Slightly rough and oily	very rough texture

(BSR- Roots of *Blepharispermum subsessile* DC and PLR- Roots of *Pluchea lanceolata* CB Clerk)

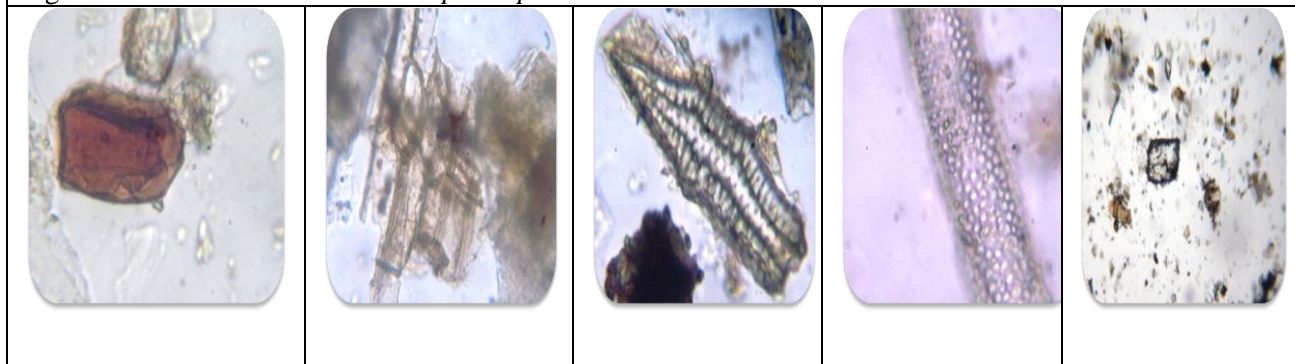
Organoleptic characters BSR shows Brownish-black colour while PLR is yellowish brown colour, BSR is highly aromatic while PLR shows slightly aromatic odour. BSR is bitter in taste while PLR possesses slightly bitter and astringent taste, BSR is slightly rough and oily while PLR very rough texture.

Table 2.2: Powder microscopy of BSR and PLR(Figure 3.1 and 3.2)

	BSR	PLR
Differentiating characters	Prismatic crystals	Acicular crystals, rhomboidal crystals, stone cells with wide lumen
Similar characters	1. Dark yellowish brown tannin containing material 2. Border pitted xylem vessels and trachieds 3. Oil globules, simple and compound starch grains 4. Pitted scleraids with wide lumen 5. Annular and spiral vessels.	

(BSR- Roots of *Blepharispermum subsessile* DC and PLR- Roots of *Pluchea lanceolata* CB Clerk)

Powder microscopy- The diagnostic characters of powder of *Blepharispermum subsessile* DC have already been reported.²⁸ The diagnostic characters of powder of *Pluchea lanceolata* C B Clerk shows cork in tangential view, fragments of border pitted vessels, acicular crystals, rhomboidal crystals, oil globules, simple and compound starch grains, dark yellowish brown tannin containing material, stone cells with wide lumen and sclereids.(figure3.2)

Figure 3: Powder microscopyFigure 3.1 Powder characters of *Blepharispermum subsessile* DC root

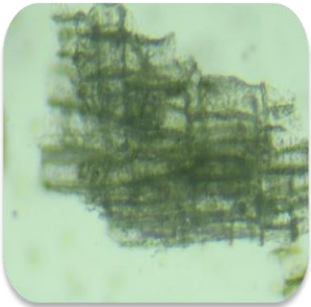

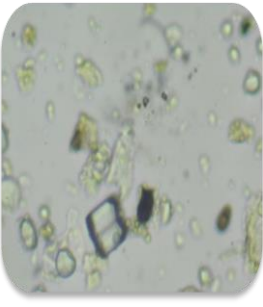
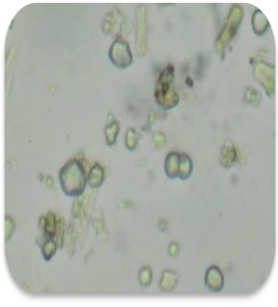
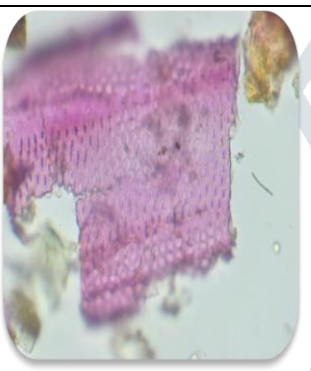

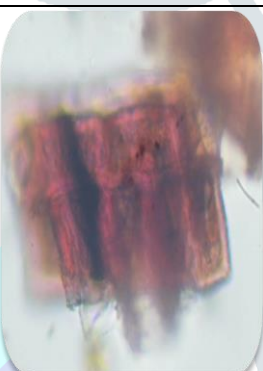

Tannin containing cell	Cork in surface view of the	Pitted scleride	Border vessels	pitted	Prismatic crystal
Figure 3.2 : Powder characters of <i>Pluchea lanceolata</i> C B Clerk					
					
Cork in surface view	Border Border pitted vessels	Prismatic crystals	Oil globules		
					
Fragment of border pitted vessels	Fibres	Tannin containing cell	Pitted scleride		

Table 2.3 : Histochemical Characters of of *Blepharispermum subsessile* DC and *Pluchea lanceolata* CB Clerk

Sr. No.	Reagent	Observation	Characteristics	Results	
				BSR	PLR
1	Phloroglucinol+Conc. HCl	Red	Lignified cells	++	++
2	Iodine	Blue	Starch grains	++	++
3	Phloroglucinol+Conc. HCl	Dissolved	Ca Ox – crystals	++	++
4	FeCl ₃ solution	Dark blue	Tannin cells	++	++
5	Ruthenium red	Red	Mucilage	++	++
6	Sudan III	Red	Oil globule	++	++

(BSR- Roots of *Blepharispermum subsessile* DC and PLR- Roots of *Pluchea lanceolata* CB Clerk)

Preliminary Physicochemical Analysis

Powder of *BSR* and *PLR* were tested for various physico-chemical parameters such as loss on drying, ash value, water, methanol soluble extract as well as petroleum ether extractive value.

Table 3.1–Physico-chemical Analysis of BSR and PLR

Sr No.	Parameter	<i>Blepharispermum subsessile</i> DC root. (BSR)	<i>Pluchea lanceolata</i> CB Clerk root.(PLR)
1	Foreign matter	Nil	Nil
2	Loss on drying	6.65	8.00
3	Ash value (% w/w)	4.05	9.75
4	Acid insoluble ash (% w/w)	0.5	2.52
5	Water soluble extract [WSE] (% w/w)	31.3	7.13
	Alcohol soluble extract [ASE] (% w/w)	23.2	1.83
	Petroleum ether soluble extractive [PEE]	6.11	0.52
6	Ph	5.5	5.5

Physico-chemical Analysis of BSR and PLR showed no foreign matter were present in both BSR and PLR. The ash value was more (9.75%) in PLR than BSR(4.05%) the acid insoluble ash was also more in PLR (2.52%) than BSR(0.5%) whereas the pH value was same in both the drugs (5.5)

Preliminary qualitative chemical test:

Preliminary qualitative chemical test for both the samples were done following standard procedure. The observed results are shown in table 3.2

Table 3.2: Qualitative analysis of root powder of BSR and PLR.

Phytoconstituents	Test (water extract)	BSR	PLR
Tannin	Lead acetate	+	+
	FeCl ₃	+	+
Flavonoids	Shinoda test	+	+
Cyanogenetic glycoside	Sodium picrate	+	-
	3% aq. HgNO ₃	+	-
Saponin	Foam test	+	+
Protiens	Biuret test	-	+
	Ninhydrin test	-	+
Alkaloids	Dragendorff's test	-	-
Carbohydrate	Molish's test	-	+
Reducing sugar	Fehling's test	-	+

(BSR- Roots of *Blepharispermum subsessile* DC and PLR- Roots of *Pluchea lanceolata* CB Clerk, “+”: Positive, “-”: Negative)

Qualitative analysis of root powder of *BSR* and *PLR* showed Tannin flavonoids and saponin were found in both *BSR* and *PLR* whereas cynogenetic glycoside was found in *BSR* only. Proteins, carbohydrates, reducing sugars were found in *PLR* sample only. Both the samples were found devoid of alkaloids.

3. HPTLC (figure 4)

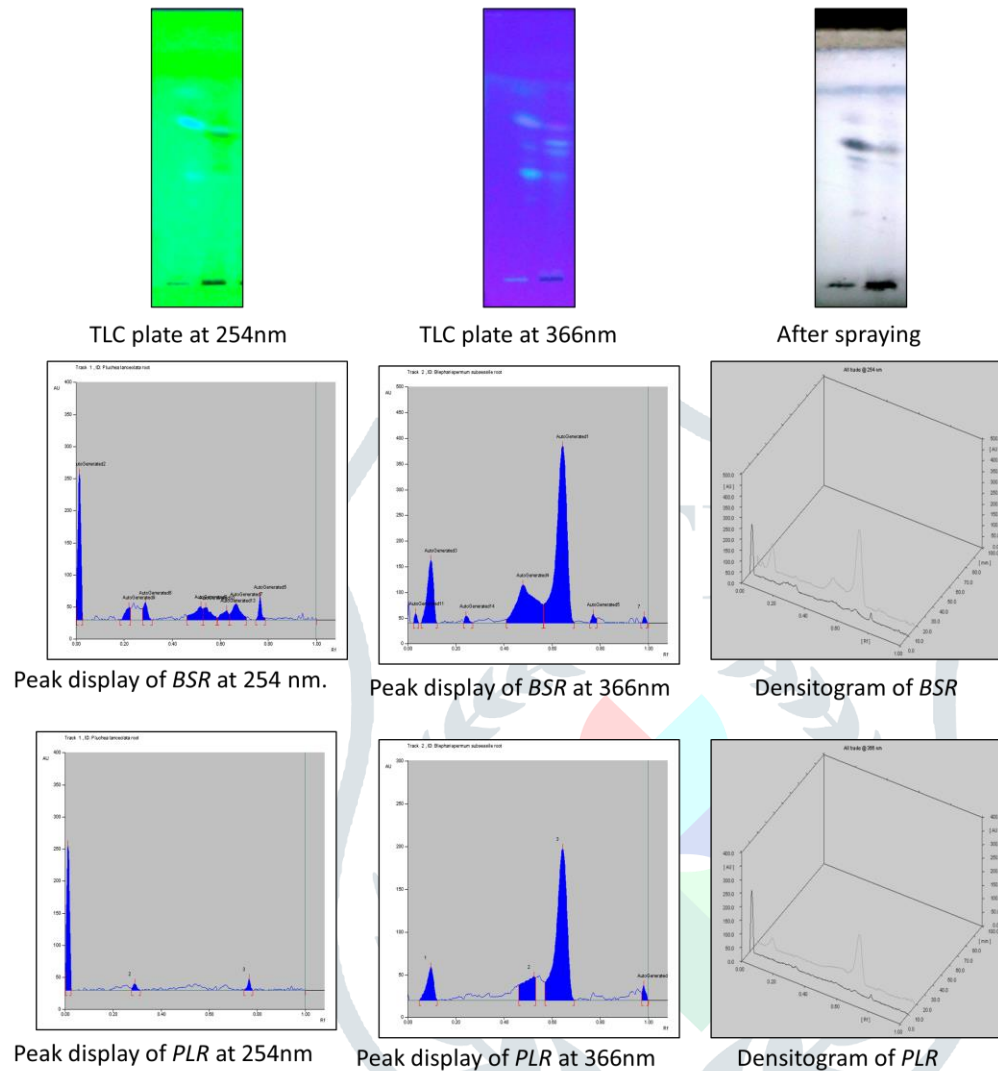
Results of the HPTLC study of the root of *Pluchea lanceolata* CB Clerk and *Blepharispermum subsessile* DC shown in track 1 and track 2 respectively were scanned under 254 nm & 366 nm. The observed results are shown in table 4

Table 4: Showing HPTLC profile for *BSR* and *PLR*.

Solvent system	Track No	Under UV light			
		254nm (Short UV)		366nm (Long UV)	
		Number of spots	R _f value	Number of spots	R _f value
Toluene: Ethyl acetate: Formic acid 7.5:2:0.5 V/V	1(PL)	8	0.01, 0.22, 0.29, 0.52, 0.54, 0.63 , 0.67, 0.77	3	0.01, 0.29, 0.77
	2(BS)	7	0.03, 0.10, 0.24, 0.48, 0.64, 0.77 , 0.98	4	0.10, 0.52, 0.64, 0.98

In HPTLC

- *Planceolata* root methanolic extract showed 8 spots at 254 nm and 3 spots at 366 nm.
- *B subsessile* root methanolic extract showed 7 spots at 254 nm and 4 spots at 366 nm.
- Both the drugs have one common R_f values (0.77.) and two nearer R_f value(0.63,0.64)
- The Common R_f values for track *Pluchea* root and track *Blepharispermum* root is 0.77.
- R_f value 0.63 and 0.64 found nearly the same under 254nm.

Figure 4: HPTLC**CONCLUSION:**

Rasna is best vata pacifying drug. Both the source plant of *Rasna* are anti-inflammatory, showing the similar indications in applied aspects being used in alleviation of vata. The comparative pharmacognostical and preliminary physicochemical study of these two sources of *Rasna* is reported in this article, Similar and differentiating characters are studied in detail, which will be useful for correct identification and authentication of these two sources of *Rasna*.

In HPTLC The distribution of component (migration distance of component) on plate is more in BSR than PLR. It is observed that low Rf at between 0.2-0.3, 0.5-0.6 and 0.6-0.7 in PLR. Rf is very high in both the samples when observed in long UV. So it can be interpreted that, the chemical profile of both the plants are different within studied protocol.

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AUTHORS CONTRIBUTION STATEMENT: Amruta Jadhav and Rabinarayan Acharya conceptualized and designed the study. Dr Ajinkya Acharekar provided valuable inputs towards the designing of manuscript. Harisha CR contributed for Pharmacognostical study. All authors discussed the methodology and results and contributed to final manuscript.

CONFLICT OF INTEREST : Conflict of Interest declared none.

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