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COMPARATIVE PHARMACOGNOSTICAL AND PHYTOCHEMICAL EVALUATION OF THE LEAVES OF MESHASHRINGI AND AJASHRINGI USED IN THE MANAGEMENT OF DIABETES MELLITUS

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ABSTRACT

Background: Diabetes mellitus (DM) is a disease caused due to the improper production of insulin from the pancreatic cells. It is reported to be affecting about 10.5% of adult population. It is presently a major health problem many individuals are facing, because of the changes in the life style. It is important at this point of time to treat the patients with safe and cost-effective treatment. Herbal based drugs are being explored for their anti-diabetic activity to produce a safe and effective medication for the management of diabetes. Meshashringi (*Gymnema sylvestre* (Retz.) R.Br. ex Sm.) (GS) is one of the herbal based medicines which is proved as anti-diabetic and is being used in the Ayurvedic treatments also. One of the herbal based medicines Ajashringi (*Pergularia daemia* (Forssk.) Chiov.) (PD), mentioned in Ayurveda is also said to be having similar properties to Meshashringi and is used as an alternative. Hence the present study is aimed to compare the phytochemical and pharmacognostic characters of both the medicines.

Materials and methods: The phytochemical and pharmacognostic characters of both the drugs are identified based on the standard literature.

Results: The result of phytochemical screening of GS showed saponins, phenols and quinones in addition to the constituents present in PD. HPTLC fingerprinting showed flavonoids present in PD. Various bands with colour intensities are seen, different Rf values in both GS and PD.

Conclusion: The study showed there are many similarities between GS and PD with respect to phytochemicals. Whereas there were no similar rf values noted in the HPTLC, indicating that the antidiabetic phytochemicals of both the drugs are different, but may be having similar efficacy when given clinically.

Keywords: *Gymnema sylvestre*, *Pergularia daemia*, Pharmaconosy, Phytochemical, Physico-chemical, HPTLC

INTRODUCTION:

Because of the changes in the lifestyle and adoption to sedentary lifestyle, many people around the world are getting affected with various metabolic disorders like hyperthyroidism, hypothyroidism, diabetes mellitus etc. Of these, Diabetes mellitus (DM) has affected many people with in a span of 20 years and has raise to triple the number of cases since 2000 (estimated 151 million (4.6% of the global population at the time) to 537 million (10.5%) today). It is estimated that it may raise to around 800 million people by 2045. (The IDF Diabetes Atlas (2021)) [1]. Diabetes is caused by the deficiency of the

production of insulin from the beta cells of pancreas. Deficiency of insulin, hinders the metabolism of carbohydrates and makes them to accumulate in the blood leading to various clinical signs and symptoms of diabetes like polyurea, polydipsia, polyphagia, general weakness and infections. Prolonged elevated condition of blood glucose levels damage kidneys (nephropathy), eye (retinopathy) and nervous system (neuropathy) [2]. Hence it is important to control the normal blood glucose level and maintain a healthy life. Ayurveda is a treasure of ancient traditional knowledge of medicine, which has its own unique ways of diagnosis and treatment. It has an elaborative explanation of the various disease conditions and use of plant, animal and mineral based medicines for the treatment of various disease conditions. DM is equated with *Madhumeha* disease mentioned in the Ayurvedic literature, and is a *kapha dosha pradhana vyadhi*, along with vitiation of *vata dosha*. In this condition, the patient excretes urine which is similar in appearance and taste to that of honey (*Madhu*). Hence it is called as *Madhumeha* [3]. Various herbal based remedies are mentioned in Ayurveda for the management of this condition. Many of the herbal based medicines are researched and found to be having anti-diabetic potential. Of these *Curcuma longa* L., *Berberis aristata*, *Salacia roxburghii*, *Gymnema sylvestre* (Retz.) R.Br. ex Sm. etc. are widely used. *Gymnema sylvestre* (Retz.) R.Br. ex Sm. (*GS*) is equated in the ayurvedic literature to *Meshashrungi* [4]. It is popularly known as *gudmar*, as it blocks the sensation for sweet and bitter taste when consumed orally and also reduces the blood glucose levels. Many other herbs are considered as source plants for *Meshashrungi* in various parts of India, based on the local names and the availability. They are *Vallaris Solanaceae* (Roth) kunize, *Cryptolepsis buchanani* Roem. & Schult, *Aristolochia bracteolata* Ham. and *Dolichandrone falcata* (Dc.). Seemann [5]. In many references from the *Ayurveda samhithas* and *Nighantu*, *Ajashrungi* is the term mentioned as synonymous to *Meshashrungi*. This created much more confusion, as the drug *Ajashrungi* is equated with *Pergularia daemia* (Forssk.) Chiov. (*PD*) [6]. Both *GS* and *PD* belong to the same family Apocynaceae (earlier Asclepiadaceae). Both the plants are extensively available in South India as road side plants and they are used in the management of various ailments especially diabetes mellitus in folklore medicine [7, 8, 9]. GS is studied extensively for its anti-diabetic, anti-oxidant and anti-obesity properties[10]. PD is also known to be anti-hyperglycemic acitivty [11]. Hence, it is important to know whether both the plants can be used as an alternative for each other. Comparison of the phytochemicals and the HPTLC profile may give a clue to the similarities and differences of both the drugs.

So, the present study GS and PD were selected to compare the Pharmacognostic and Phytochemical characters of both the plants. This study is aimed at identifying the drugs, macroscopically and microscopically and to explore their phytochemical constituents and HPTLC reports. This study may be useful for further clinical research of the drugs in the management of diabetes.

MATERIALS AND METHODS

Leaves of GS and PD are collected from Zoo-park Road of Tirupati (Lat

13.630954° and Long 79.393123°), Chittoor district. The plants were identified and authenticated by the department of *Dravyaguna* based on the morphological characters and the voucher specimens were deposited in the department of *dravyaguna*[12]. The plant material was shade dried and is used for Phytochemical and Pharmacognostic analysis.

Morphological examination:

The plants were identified in their natural habitat and the morphological characters of the arial parts of the plants were noted and recorded for the authentication of the drugs[12].

Macroscopy

The external features of the plant materials were documented using Canon IXUS digital camera. The Macroscopic features were compared to local flora for authentication.[12] **Microscopy**

The leaf samples of *GS* and *PD* Sample were preserved in fixative solution called FAA (Formalin-5ml + Acetic acid-5ml + 70% Ethyl alcohol-90ml). The materials were left in FAA for more than 48 hours. The preserved specimens were cut into thin transverse section using a sharp blade and the sections were stained with safranine. After ensuring proper staining, the sections were transferred to a neat and clean slide and examined under microscope. Transverse sections were photographed using Zeiss AXIO trinocular microscope attached with Zeiss AXIO Cam camera under bright field light.

Powder Microscopy:

Pinch of *Meshashrungi and Ajashrungi patra curna* previously sieved was put on the slide and mounted in glycerine and powder characters are observed under the Zeiss AXIO trinocular microscope attached with Zeiss Axio Cam camera under bright field light.

Physico-chemical screening:

This was done according to the Standard protocol[13]. Loss on drying, Total ash, Acid-insoluble ash, Water-Soluble ash, Alcohol soluble extractive value, Water soluble extractive value were tested and determined.

Phytochemical screening:

This was done according to the procedure mentioned in Ayurvedic Pharmacopoeia of India. [13]

HPTLC Study:

The study was carried out at Sri Dharmasthala Manjunatheshwara centre for research in Ayurveda and allied sciences, Udupi, India.

***Gymnema sylvestre* (Retz.) R.Br. ex Sm. (*Meshashrungi patra curna*)**

1g of *GS* (Meshashrungi patra curna) sample was soaked in 20ml of ethanol, macerated at room temperature with intermittent shaking. After 24hrs it was filtered through filter paper and filtrate (extract) was further used for HPTLC. 3, 6, 9µl of each of the above extract was applied on a pre-coated silica gel F_{254} on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in **Chloroform: Methanol: water (6.5:3.5:1.0).** The developed plates were visualized in short UV, long UV and then derivatized with vanillin sulphuric acid and scanned under UV 254nm, 366nm and 550nm (Post derivatization). Rf, colour of the spots and densitometric scan were recorded.

***Pergularia daemia* (Forssk.) Chiov. (*Ajashrungi patra curna*)**

PD (*Ajashrungi patra curna*) 1g of sample was kept in 20 ml of ethanol macerated at room temperature with intermittent

shaking. After 24hrs it was filtered through filter paper and filtrate (extract) was further used for HPTLC. 3, 6, 9µl of each of the above extract was applied on a pre-coated silica gel F_{254} on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in **Cyclohexane: Ethyl acetate: Formic acid (4.0: 6.0: 1.0)**. The developed plates were visualized in short UV, long UV and then derivatized with Anisaldehyde sulphuric acid (ASA) and scanned under UV 254nm, 366nm and 540nm (Post derivatization). Rf, colour of the spots and densitometric scan were recorded.

RESULTS AND DISCUSSION:

Taxonomy:

Table 1. Taxonomical classification of *Gymnema sylvestre* (Retz.) R.Br. ex Sm. (*Meshashrungi*) *and Pergularia daemia* (Forssk.) Chiov. (*Ajashrungi*)

Gymnema sylvestre (Retz.) R.Br. ex Sm.	*Pergularia daemia* (Forssk.) Chiov.
Kingdom: Plantae [14] Phylum: Tracheophyta Class: Magnoliopsida Order: Gentianales Family: Apocynaceae Genus: *Gymnema* *Species: Gymnema sylvestre (Retz.) R.Br. ex Sm.*	Kingdom: Plantae [15] Phylum: Tracheophyta Class: Magnoliopsida Order: Gentianales Family: Apocynaceae Genus: *Pergularia* Species: *Pergularia daemia (Forssk.) Chiov.*

Morphological characters:

Table 2. Morphological characters of *Gymnema sylvestre* (Retz.) R.Br. ex Sm. (*Meshashrungi*) *and Pergularia daemia* (Forssk.) Chiov. (*Ajashrungi*)

Morphological characters	*Gymnema sylvestre* (Retz.) R.Br. ex Sm.	*Pergularia daemia* (Forssk.) Chiov.
Whole plant	Woody pubescent climber.	Foetid twinning herb.
Stems	Pubescent, green.	Pubescent, green.
Leaves	Petiolate, Ovate leaf of cordate base, shortly acuminate tip, glabrous above and pubescent beneath with opposite phyllotaxy.	Petiolate, broadly ovate leaf of acuminate tip, deeply Cordate base, opposite phyllotaxy.
Flowers	Pedunculate yellowish lateral umbellate cymes.	Greenish yellow to dull white pedunculate lateral umbellate cymes.
Fruits	Follicles are beaked.	Follicles are beaked, echinate with soft spines.
Latex	Milky latex.	Milky latex.

Images

Fig 1. *Gymnema sylvestre* (Retz.) R.Br. ex Sm.

Fig 2. *Pergularia daemia* (Forssk.) Chiov.

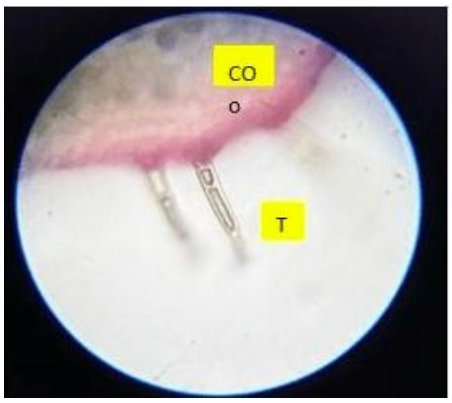
Microscopic characters:

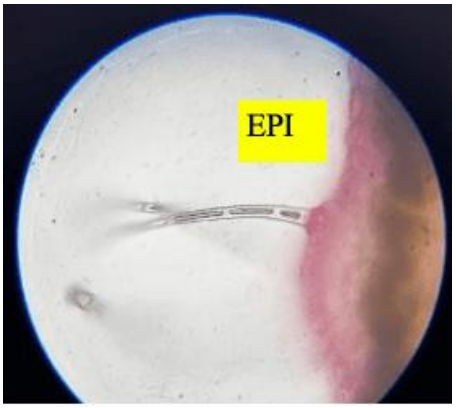
Gymnema sylvestre *(Retz.) R.Br. ex Sm.*:

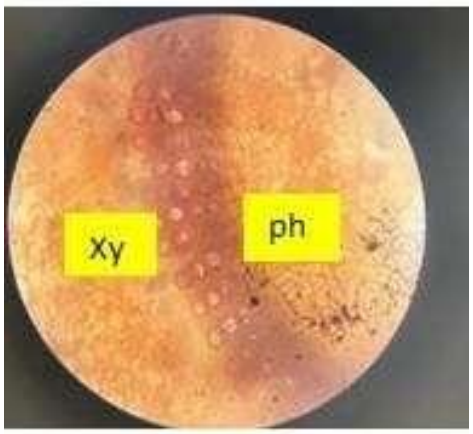
Microscopic examination of *GS* showed upper and lower epidermis with uniseriate, multicellular trichomes. Epidermis is followed by palisade parenchyma and spongy parenchyma. Collenchymatous cells are present near the midrib. Vascular bundles are collateral and closed. Calcium oxalate crystals are present in spongy parenchyma. Paracytic Stomata surrounded by palisade tissue in the lower epidermis.

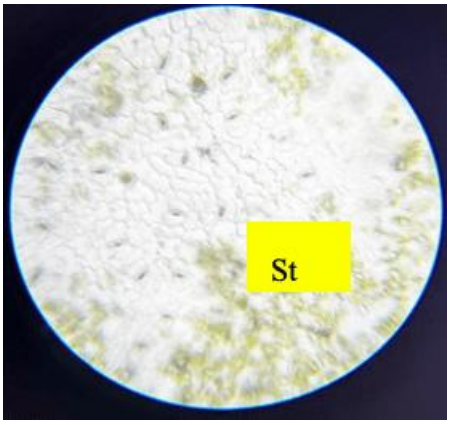
The results are in correlation with the results obtained by Adarsh Kumar Agnihotri et.al,.[16] Indicating that the sample collected was genuine and is having no variation.

Fig 3. Microscopy of *GS Leaf*







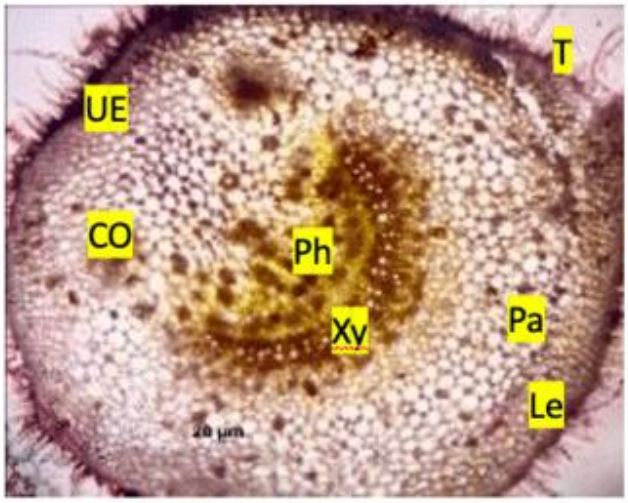


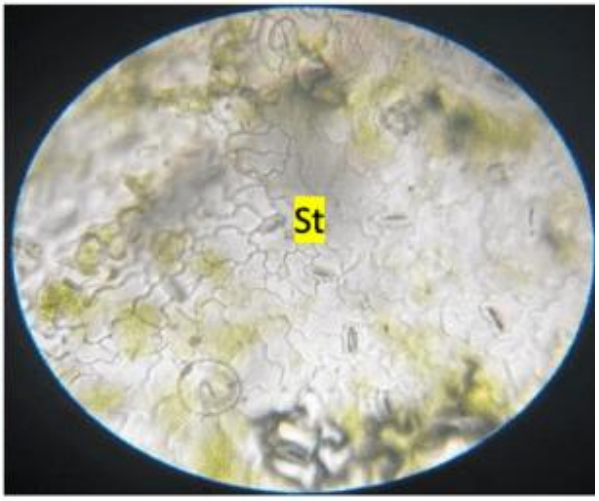
CO– calcium oxalate; St – Stomata; Epi – Epidermis; Ph–phloem; T –trichome; Xy–xylem

***Pergularia daemia (Forssk.) Chiov.*:**

PD transverse section showed the presence of single layered upper and lower epidermis with uniseriate, multicellular trichomes. Mesophyll with palisade and spongy parenchyma, Bicollateral vascular bundles, calcium oxalate crystals, parenchymatous cells and collenchymatous cells, Rubiaceous stomata is present on both upper and lower surface. The results obtained are similar to the pharmacognostic results obtained by Sandhya bhoyar et. al., [17].

***Fig 4.* Microscopy of *PD Leaf*.**





CO– calcium oxalate; LE – lower epidermis; Pa – parenchyma; Ph–phloem; T –trichome; UE– upper epidermis; Xy–xylem; St–Stomata.

Fig 5: Powder microscopy of leaf of *Gymnema sylvestre* (Retz.) R.Br. ex Sm. (*Meshashrungi patra curna*)

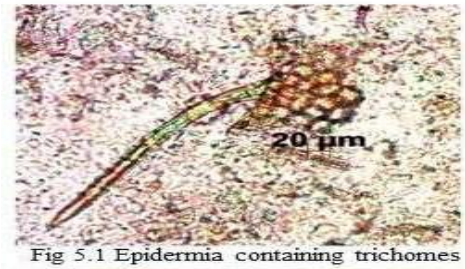


Fig 5.1 Epidermia containing trichomes

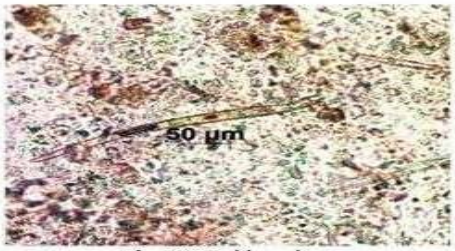


Fig 5.2 Epidermis

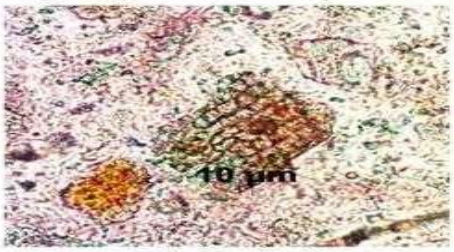


Fig 5.3 sclereid

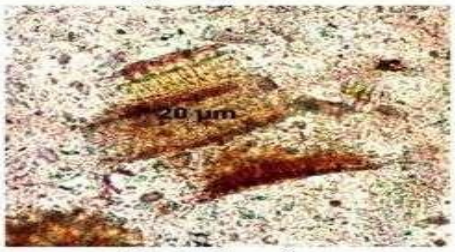


Fig 5.4 Vessels

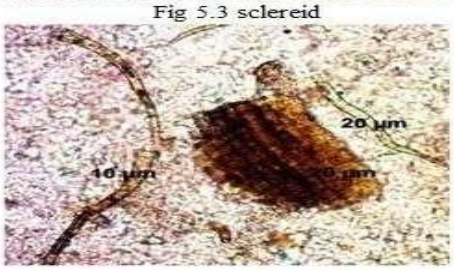


Fig 5.5 Fibres

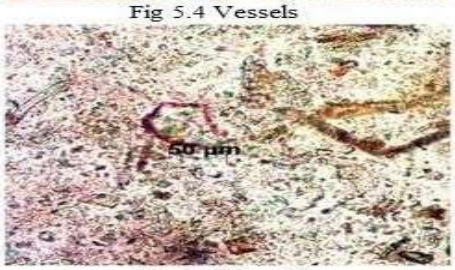


Fig 5.6 Sclereids

Fig 6: Powder microscopy of leaf of *Pergularia daemia (Forssk.) Chiov.*. (Ajashrungi patra curna)

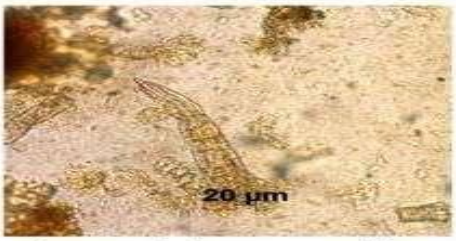


Fig 6.1 Uniseriate , warty trichome

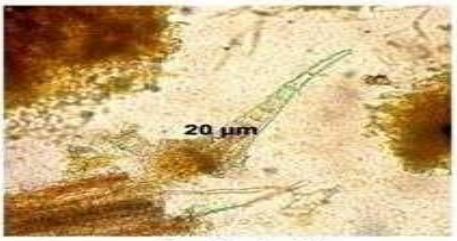


Fig 6.2 Multicelled trichome

Fig 6.3 Trichome

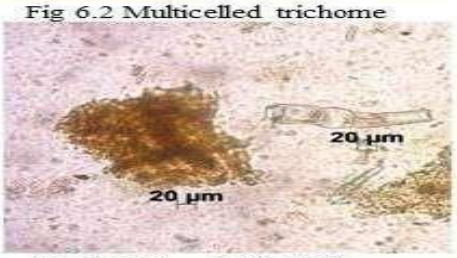


Fig 6.4 Mesophyll, Trichome

Fig 6.5 Epidermis with stomata

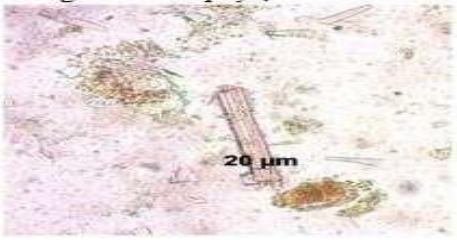


Fig 6.6 Xylem fibres

The Pharmacognostic study, Phytochemical screening, Powder microscopy of GS and PD contains calcium oxalates. The presence of these crystals is mentioned even in the API monographs on GS [18].

Powder microscopy of *GS* and *PD* has the presence of fibers, tracheid's, epidermis, trichomes. In addition, uniseriate, warty and multicelled trichomes, mesophyll, epidermis with stomata is seen in *PD.*

Table 3. Results of standardization parameters of *Gymnema sylvestre (Retz.) R.Br. ex Sm.* (Meshashrungi patra Curna), *Pergularia daemia* (Forssk.) Chiov. (Ajashrungi patra Curna)

Parameter	**Results n = 3 %w/w(Avg±SD)**	
	Gymnema sylvestre (Retz.) R.Br. ex Sm. *(Meshashrungi patra Curna)*	*Pergularia daemia* (Forssk.) Chiov. (*Ajashrungi patra Curna*)
Loss on drying	11.53±0.00	10.66±0.02
Total Ash	11.88±0.17	15.35±0.91
Acid Insoluble Ash	2.01±0.01	3.99±0.01
Water soluble Ash	2.0±0.00	2.94±0.01
Alcohol soluble extractive value	9.40±0.00	5.31±0.02
Water soluble extractive value	22.85±0.00	56.0±0.0

The standardization parameters of the GS are compared with the previous literature obtained by the study done by Adarsh kumar Agnihotri and was found that the results are in correlation with the study[19]. The standardization parameters obtained for PD, when compared with the study done Sandhya bhoyar et. al.,[20], showed that there is a marked difference in the parameters like total ash (24.1%), acid insoluble ash (11.1%) and water soluble ash (12.5%). The results obtained in our experimentation are much less (total ash (15.35%), acid insoluble ash (3.99%) and water soluble ash (2.94%)) indicating the purity of the material and proper drying of the material. The alcohol soluble extractive obtained was much lower (5.31% when compared to above study (23.2%), but the water soluble extractive was much higher (56%) compared to the above study (35.2%). This may be because of the variation in the collecting area and the season of the collection.

Table 4: Results of preliminary phytochemical screening of Gymnema sylvestre (Retz.) R.Br. ex Sm. (*Meshashrungi patra Curna*) and Pergularia daemia (Forssk.) Chiov. (*Ajashrungi patra Curna*)

Test	Inference	
	Gymnema sylvestre (Retz.) R.Br. ex Sm. (*Meshashrungi patra Curna*)	*Pergularia daemia* (Forssk.) Chiov. (*Ajashrungi patra Curna*)
Alkaloid	+	+
Steroid	+	+
Carbohydrate	+	+
Tannin	+	+
Flavonoids	+	+
Saponins	+	-

Terpenoid	+	+
Coumarins	-	-
Phenols	+	-
Carboxylic acid	-	-
Amino acids	-	-
Resin	-	-
Quinone	+	-

Table 5: Tests and Results of preliminary phytochemical screening of *Gymnema sylvestre* (Meshashrungi patra Curna) *and Pergularia daemia* (Forssk.) Chiov. (Ajashrungi patra Curna)

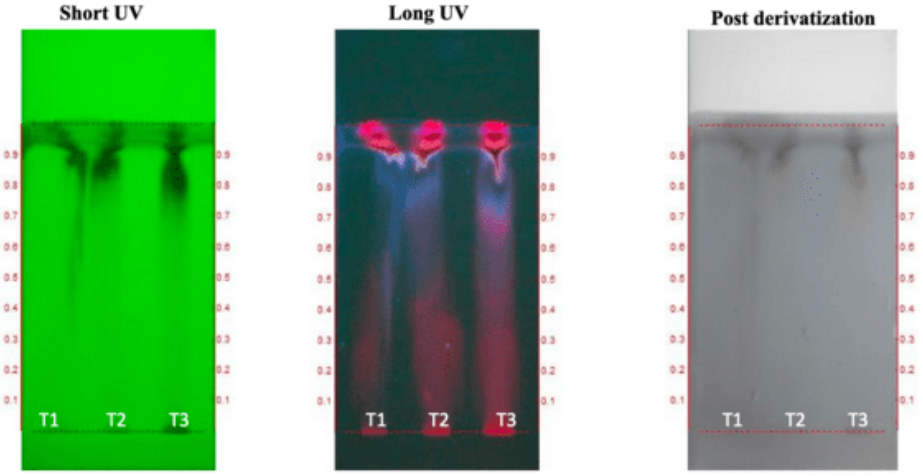
Tests with reference 10	**Colour if positive**	***Gymnema sylvestre* (Retz.) R.Br. ex Sm. *(*Meshashrungi patra Curna*)***	***Pergularia daemia* (Forssk.) Chiov. (Ajashrungi patra Curna)**
Alkaloids			
Dragendorff's test	Orange red precipitate	Orange red precipitate	Orange red precipitate
Wagner's test	Reddish brown precipitate	Reddish brown precipitate	Reddish brown precipitate
Mayer's test	Dull white precipitate	Dull white precipitate	Dull white precipitate
Hager's test	Yellow precipitate	Yellow precipitate	Yellow precipitate
Steroids			
Liebermann-Burchard test	Bluish green colour	Bluish green colour	Bluish green colour
Salkowski's test	Bluish red to cherry red colour in chloroform layer and green fluorescence in acid layer	Bluish red to cherry red colour in chloroform layer and green fluorescence in acid layer	Bluish red to cherry red colour in chloroform layer and green fluorescence in acid layer
Carbohydrate			
Molisch's test	Violet ring	Violet ring	Violet ring
Fehling's test	Brick red precipitate	Brick red precipitate	Brick red precipitate
Benedict's test	Red precipitate	Red precipitate	Red precipitate
Tannin			
With FeCl3	Dark blue or green or brown	Dark brown	Dark brown
Flavonoids			
Shinoda test	Red or pink	Red colour	Red colour
Saponins			

With NaHCO3	Stable froth	Stable froth	No stable froth
Triterpenoids			
Tin and thionyl chloride test	Pink	Pink	Pink
Coumarins			
With 2 N NaOH	Yellow	No yellow colour	No yellow colour
Phenols			
With alcoholic ferric chloride	Blue to blue black	Blue to bluish black	No Blue to bluish black
Carboxylic acid			
With water and NaHCO3	Brisk effervescence	**No** brisk effervescence	**No** brisk effervescence
Amino acid			
With ninhydrin reagent	Purple colour	No purple colour	No purple colour
Resin			
With aqueous acetone	Turbidity	No Turbidity	No Turbidity
Quinone			
Concentrated sulphuric acid	Pink/purple/red	Red color	No red color

The results of preliminary phytochemical screening showed the presence of Alkaloids, steroids, carbohydrate, tannins, flavonoids, terpenoids in both *GS* and *PD* leaves. In addition, *GS* contains saponins, phenols, quinone.

In a study conducted by Syed Sabiha Vajhiyuddin et.al., the presence of flavonoids, tannins, alkaloids, glucosides, terpenoids, steroids and carbohydrates were reported. The same have been identified even in our experimentation on PD, suggesting that the above are the phytoconstituents present in the PD and can be taken as standard [21].

Figure 7. HPTLC photo documentation of ethanol fraction of *Gymnema sylvestre (Retz.) R.Br. ex Sm.* (*Meshashrungi*) leaf

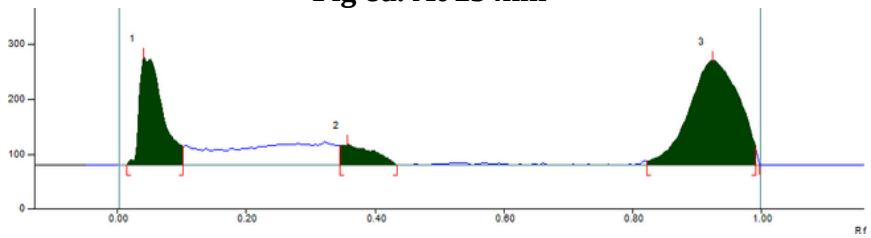


T1 - Track 1 - *Gymnema sylvestre (Retz.) R.Br. ex Sm.* leaf extract (*Meshashrungi* patra curna)– 3µl
T2 - Track 2 - *Gymnema sylvestre (Retz.) R.Br. ex Sm.* leaf extract (*Meshashrungi* patra curna)– 6µl
T3 - Track 3 - *Gymnema sylvestre (Retz.) R.Br. ex Sm.* leaf extract (*Meshashrungi* patra curna)– 9µl
Solvent system – Chloroform: Methanol: water (6.5:3.5:1.0)

Table 6: Rf values of sample of ethanol fraction of *Gymnema sylvestre* (Retz.) R.Br. ex Sm. leaf extract(*Meshashrungi patra curna*)		
Short UV	**Long UV**	**Post derivatization**
-	0.43 (F. red)	-
-	0.67 (F. red)	-
-	0.75 (F. blue)	-
0.94 (Green)	0.94 (F. red)	0.94 (Yellow green)
*F – Fluorescent; L –Light; D – Dark		

Figure 8. Densitometric scan of ethanol fraction of *Gymnema sylvestre (Retz.) R.Br. ex Sm.* leaf extract (*Meshashrungi patra curna*)

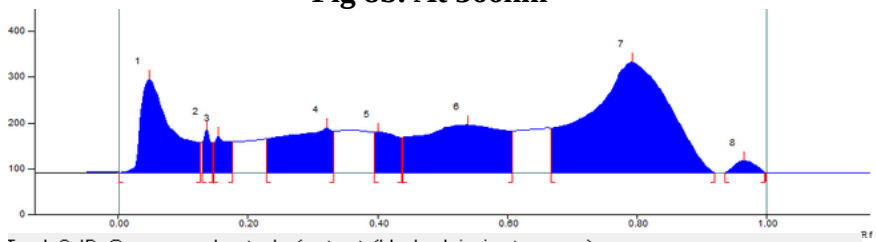
Fig 8a. At 254nm



Track 3, ID: Gymnema sylvestre leaf extract (Meshashringi patra curna)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.5 AU	0.04 Rf	195.0 AU	46.24 %	0.10 Rf	34.2 AU	5053.8 AU	29.83 %
2	0.35 Rf	34.7 AU	0.36 Rf	36.6 AU	8.68 %	0.44 Rf	0.0 AU	1358.5 AU	8.02 %
3	0.82 Rf	7.2 AU	0.93 Rf	190.1 AU	45.08 %	0.99 Rf	32.5 AU	10527.0 AU	62.15 %

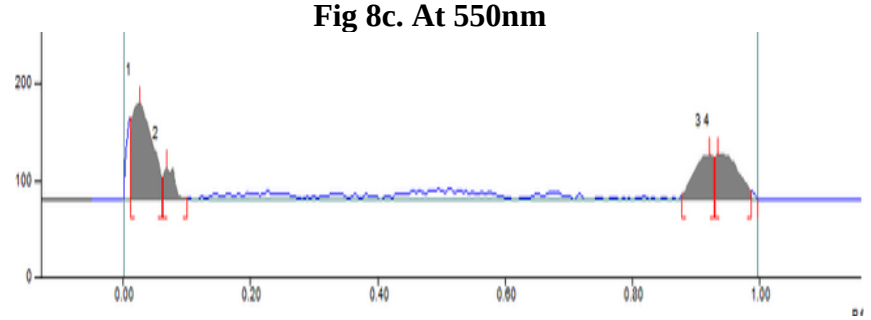
Fig 8b. At 366nm



Track 3, ID: Gymnema sylvestre leaf extract (Meshashringi patra curna)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.1 AU	0.05 Rf	203.7 AU	21.67 %	0.13 Rf	66.1 AU	7135.1 AU	14.50 %
2	0.13 Rf	65.9 AU	0.14 Rf	95.7 AU	10.18 %	0.15 Rf	66.5 AU	853.8 AU	1.74 %
3	0.15 Rf	66.6 AU	0.15 Rf	80.3 AU	8.54 %	0.18 Rf	68.1 AU	1337.9 AU	2.72 %
4	0.23 Rf	73.5 AU	0.32 Rf	98.5 AU	10.48 %	0.33 Rf	90.8 AU	5477.8 AU	11.14 %
5	0.40 Rf	88.7 AU	0.40 Rf	89.6 AU	9.53 %	0.44 Rf	77.3 AU	2281.8 AU	4.64 %
6	0.44 Rf	77.4 AU	0.54 Rf	104.6 AU	11.12 %	0.61 Rf	91.4 AU	9868.5 AU	20.06 %
7	0.67 Rf	97.7 AU	0.79 Rf	240.9 AU	25.63 %	0.92 Rf	0.5 AU	21620.8 AU	43.95 %
8	0.94 Rf	0.2 AU	0.97 Rf	26.8 AU	2.85 %	1.00 Rf	1.5 AU	615.1 AU	1.25 %

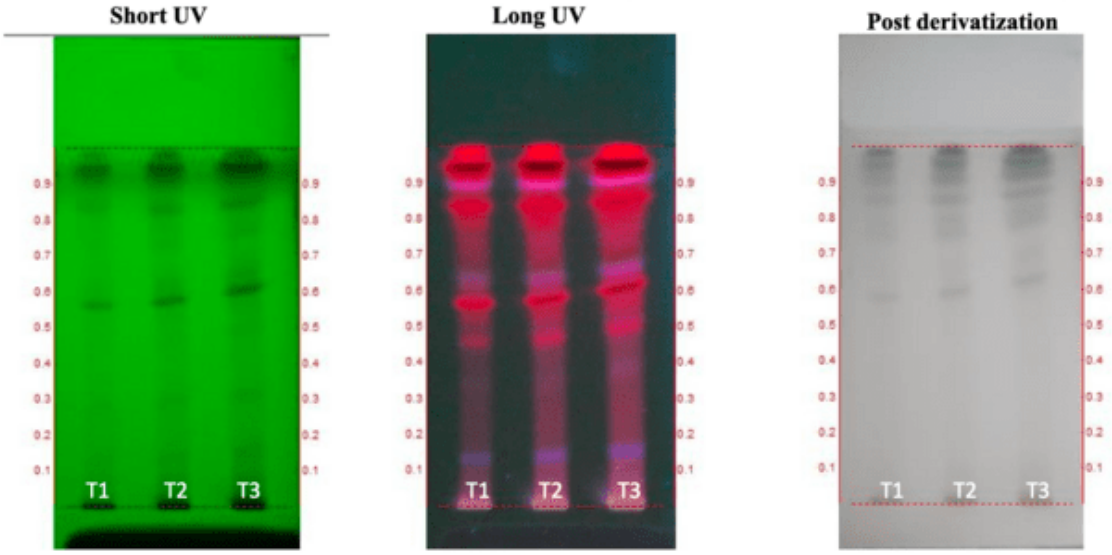
Fig 8c. At 550nm



Track 3, ID: Gymnema sylvestre leaf extract (Meshashringi patra curna)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	83.9 AU	0.03 Rf	99.4 AU	44.28 %	0.06 Rf	22.0 AU	2307.0 AU	46.57 %
2	0.06 Rf	23.2 AU	0.07 Rf	32.6 AU	14.54 %	0.10 Rf	1.2 AU	407.7 AU	8.23 %
3	0.88 Rf	4.8 AU	0.92 Rf	46.0 AU	20.50 %	0.93 Rf	43.3 AU	1007.1 AU	20.33 %
4	0.93 Rf	43.7 AU	0.94 Rf	46.4 AU	20.69 %	0.99 Rf	8.4 AU	1232.5 AU	24.88 %

Figure 9. HPTLC photo documentation of ethanol fraction of *Pergularia daemia (Forssk.) Chiov.* (*Ajashrungi*) leaf



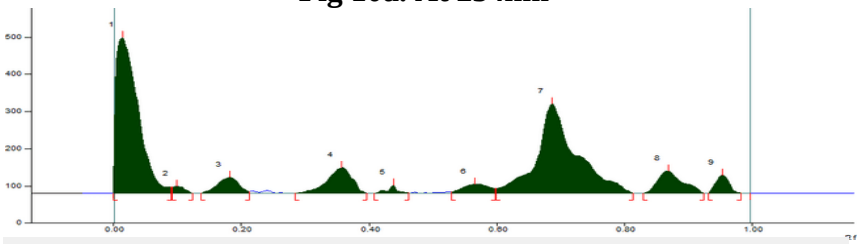
T1 - Track 1 - *Pergularia daemia (Forssk.) Chiov.* leaf extract (*Ajashrungi* patra curna)– 3µl
T2 - Track 2 - *Pergularia daemia (Forssk.) Chiov.* leaf extract (*Ajashrungi* patra curna)– 6µl
T3 - Track 3 - *Pergularia daemia (Forssk.) Chiov.* leaf extract (*Ajashrungi* patra curna)– 9µl
Solvent system – Cyclohexane: Ethyl acetate: Formic acid (4.0: 6.0: 1.0)

Table 7: Rf values of sample of ethanol fraction of *Pergularia daemia* (Forssk.) Chiov. (Ajashrungi) leaf

Short UV	Long UV	Post derivatization
0.16 (Green)	0.15 (F. violet)	-
0.32 (Green)	0.37 (F. red)	-
0.51 (Green)	0.47 (F. red)	-
0.58 (D. Green)	0.53 (F. red)	-
0.74 (Green)	0.58(F. red)	0.60 (Purple)
0.83 (Green)	0.70 (F. red)	0.76 (Purple)
-	0.74 (F. red)	0.79 (Purple)
0.95 (Green)	0.79 (F. red)	-
-	0.84 (F. red)	0.85 (Purple)
-	0.90 (F. red)	0.91 (Purple)
-	0.95 (F. red)	-
*F – Fluorescent; L –Light; D – Dark		

Figure 10. Densitometric scan of ethanol fraction of *Pergularia daemia* (Forssk.) Chiov. (*Ajashrungi*) leaf

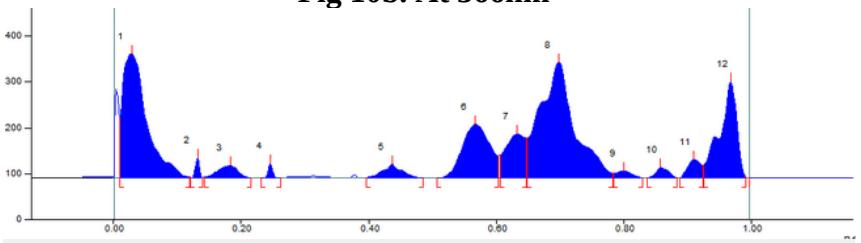
Fig 10a. At 254nm



Track 3, ID: Pergularia daemia leaf extract (Ajashringi patra curna)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.0 AU	0.01 Rf	417.5 AU	44.40 %	0.09 Rf	16.1 AU	10285.5 AU	37.68 %
2	0.09 Rf	16.2 AU	0.10 Rf	18.0 AU	1.92 %	0.12 Rf	0.0 AU	252.0 AU	0.92 %
3	0.14 Rf	0.0 AU	0.18 Rf	42.3 AU	4.50 %	0.21 Rf	4.6 AU	1028.7 AU	3.77 %
4	0.28 Rf	0.3 AU	0.36 Rf	68.2 AU	7.25 %	0.40 Rf	0.5 AU	1919.7 AU	7.03 %
5	0.41 Rf	0.1 AU	0.44 Rf	21.9 AU	2.33 %	0.46 Rf	1.1 AU	220.2 AU	0.81 %
6	0.53 Rf	3.2 AU	0.57 Rf	24.4 AU	2.60 %	0.60 Rf	12.4 AU	734.4 AU	2.69 %
7	0.60 Rf	12.5 AU	0.69 Rf	239.8 AU	25.50 %	0.81 Rf	0.4 AU	10462.6 AU	38.33 %
8	0.83 Rf	0.1 AU	0.87 Rf	59.4 AU	6.32 %	0.93 Rf	0.1 AU	1664.9 AU	6.10 %
9	0.93 Rf	0.8 AU	0.95 Rf	48.8 AU	5.19 %	0.98 Rf	0.8 AU	728.7 AU	2.67 %

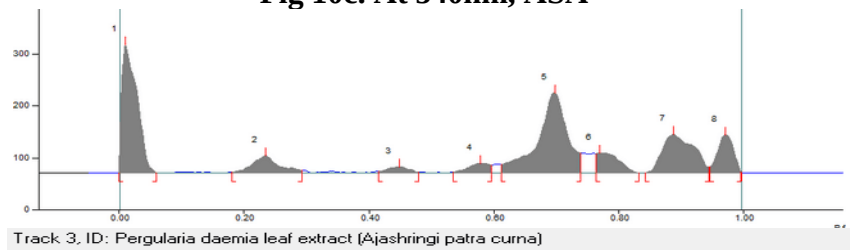
Fig 10b. At 366nm



Track 3, ID: Pergularia daemia leaf extract (Ajashringi patra curna)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	129.2 AU	0.03 Rf	269.1 AU	23.44 %	0.12 Rf	1.8 AU	7378.8 AU	25.12 %
2	0.12 Rf	1.6 AU	0.13 Rf	43.0 AU	3.75 %	0.14 Rf	0.5 AU	188.1 AU	0.64 %
3	0.14 Rf	1.7 AU	0.18 Rf	26.5 AU	2.31 %	0.22 Rf	0.1 AU	594.6 AU	2.02 %
4	0.23 Rf	0.1 AU	0.25 Rf	31.4 AU	2.73 %	0.26 Rf	0.0 AU	145.2 AU	0.49 %
5	0.40 Rf	0.1 AU	0.44 Rf	29.6 AU	2.58 %	0.49 Rf	0.0 AU	596.1 AU	2.03 %
6	0.51 Rf	0.0 AU	0.57 Rf	115.8 AU	10.09 %	0.60 Rf	48.2 AU	3608.6 AU	12.28 %
7	0.61 Rf	48.7 AU	0.63 Rf	95.1 AU	8.28 %	0.65 Rf	85.8 AU	2138.9 AU	7.28 %
8	0.65 Rf	86.3 AU	0.70 Rf	250.8 AU	21.84 %	0.78 Rf	9.9 AU	9604.3 AU	32.69 %
9	0.79 Rf	10.0 AU	0.80 Rf	15.0 AU	1.30 %	0.83 Rf	0.1 AU	255.7 AU	0.87 %
10	0.84 Rf	0.1 AU	0.86 Rf	23.0 AU	2.01 %	0.88 Rf	0.2 AU	308.3 AU	1.05 %
11	0.89 Rf	0.0 AU	0.91 Rf	39.2 AU	3.42 %	0.93 Rf	27.1 AU	587.7 AU	2.00 %
12	0.93 Rf	28.4 AU	0.97 Rf	209.6 AU	18.26 %	0.99 Rf	2.4 AU	3969.3 AU	13.51 %

Fig 10c. At 540nm, ASA



Track 3, ID: Pergularia daemia leaf extract (Ajashringi patra curna)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.0 AU	0.01 Rf	246.4 AU	37.98 %	0.06 Rf	0.4 AU	4273.7 AU	26.30 %
2	0.18 Rf	1.1 AU	0.24 Rf	32.4 AU	4.99 %	0.29 Rf	4.8 AU	994.2 AU	6.12 %
3	0.42 Rf	3.4 AU	0.45 Rf	12.1 AU	1.86 %	0.48 Rf	1.0 AU	303.3 AU	1.87 %
4	0.54 Rf	1.5 AU	0.58 Rf	18.6 AU	2.87 %	0.60 Rf	15.3 AU	459.7 AU	2.83 %
5	0.61 Rf	15.9 AU	0.70 Rf	153.7 AU	23.69 %	0.74 Rf	37.9 AU	5161.5 AU	31.76 %
6	0.76 Rf	37.1 AU	0.77 Rf	38.4 AU	5.92 %	0.83 Rf	0.1 AU	1017.4 AU	6.26 %
7	0.84 Rf	0.0 AU	0.89 Rf	74.1 AU	11.42 %	0.95 Rf	11.2 AU	2684.1 AU	16.52 %
8	0.95 Rf	11.7 AU	0.97 Rf	73.1 AU	11.27 %	1.00 Rf	2.5 AU	1355.3 AU	8.34 %

HPTLC analysis for GS showed one band with Rf value of 0.94 at short UV in Green colour intensity. Long UV showed 4 bands at Rf values 0.43, 0.67, 0.94 with colour intensities of fluorescent red. Except at 0.75, fluorescent blue is observed. After derivatization with vanillin sulphuric acid reagent at 550nm, one band is observed at Rf value of 0.94 with yellow-green colour intensity. Densitometric Scan at 254nm showed 3 peaks at Rf value 0.01 (29.83%), Rf 0.35 (8.02%) and Rf 0.82 (62.15), at 366 nm showed 8 peaks 0.00 (14.50%), Rf 0.13 (1.74%) and Rf 0.15 (2.72%), Rf 0.23 (11.14%), Rf 0.40 (4.64%) and Rf 0.44 (20.06%), Rf 0.67 (43.95%), Rf 0.94 (1.25%) and 4 peaks at 550nm 0.01 (46.57%), Rf 0.06 (8.23%), Rf 0.88 (20.33%), Rf 0.93 (24.88%).

HPTLC for PD showed 7 bands with green colour intensity at Rf values 0.16, 0.32, 0.51, 0.58, 0.74, 0.83, 0.95 in short UV. Ten bands with fluorescent red colour intensity are observed at Rf values of 0.37, 0.47, 0.53, 0.58, 0.70, 0.74, 0.79, 0.84, 0.90, 0.95 and fluorescent violet at 0.15 Rf. Post derivatization with Anisaldehyde sulphuric acid (ASA) showed 5 bands of purple colour intensities at Rf values 0.60, 0.76, 0.79, 0.85, 0.91. Densitometric scan at 254nm showed 9 peaks at Rf 0.00 (37.68%), Rf 0.09 (0.92%), Rf 0.14 (3.77%), Rf 0.28 (7.03%), Rf 0.41 (0.81%), Rf 0.53 (2.69%), Rf 0.60 (38.33%), Rf 0.83(6.10%), Rf 0.93(2.67%) ,12 peaks at 366nm with Rf 0.01(25.12%), Rf 0.12(0.64%), Rf 0.14(2.02%), Rf 0.23(0.49%), Rf 0.40(2.03%), Rf 0.51(12.28), Rf 0.61(7.28%), Rf 0.65(32.69%), Rf 0.79(0.87%),Rf 0.84(1.05%), Rf 0.89(2.00%), Rf 0.93(13.51%) and 8 peaks at 540nm with Rf 0.00(26.03%), Rf 0.18(6.12), Rf 0.42(1.87%), Rf 0.54(2.83%), Rf 0.61(31.76%), Rf 0.76(6.26%), Rf 0.84(16.52%), Rf 0.95(8.34%). These results were comparable to the standards mentioned in some of theother researches.[22,23]

The peaks at 0.56 and 0.60 were identified as the peaks corresponding to flavonoids. The same were observed in the present study at 0.58 (2.83%) and 0.61 (31.76%). These peaks showing the presence of desired flavonoid content in the plant. Thus, from the above Pharmacognostic and phytochemical evaluation it can be understood that the results obtained are standard as compared to the API and also according to the other researches on *PD* and *GS*.[22,23]

CONCLUSION

In the present study, an attempt is made to establish the comparative Pharmacognostic and phytochemical profile of the leaves of *GS* and *PD*. GS and PD were having distinct morphological characters, but belonging to the same family Apocyanaceae. The phytochemicals of both the drugs, show the presence of presence of Alkaloids, steroids, carbohydrate, tannins, flavonoids, terpenoids in both *GS* and *PD* leaves. In addition, *GS* contains saponins, phenols, quinone. No much similarities are observed between the GS and PD, with respect to the HPTLC and rf values. Indicating that both the drugs are having different set of chemical substances. So, an extensive research on individuals chemicals on Anti-diabetic property has to be studied further to prove their clinical efficacy and similarity. The present work will be helpful as the standardization of the two drugs i.e., GS and PD.

Abbreviations used:

GS: *Gymnema sylvestre*, *PD*: *Pergularia daemia*, API: Ayurvedic Pharmacopoeia of India, HPTLC: High Performance Thin Layer Chromatography.

Conflict of Interest:

There is no conflict of interest.

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