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Pharmacognostical and preliminary phytochemical evaluation of *Diplocyclos palmatus* linn

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Abstract
Objective: Herbal medicines are in great demand in the developed as well as developing countries for primary healthcare because of their wide biological and medicinal activities, higher safety margins and lesser costs. The objective of present studies deals with the macroscopical and microscopical studies of stem of *Diplocyclos palmatus* Linn. **Method:** This Pharmacognostic study comprises taxonomic details, macro and microscopic characters, physicochemical details and study of phytochemical components of all successive extracts were also carried out.
Results: Seeds of *Diplocyclos palmatus* Linn. powder showed the presence of total ash 12.14% w/w, acid insoluble ash 0.8% w/w, water soluble ash be 3.6% w/w, alcohol soluble extractive 18.85% w/w water soluble extractive 32.48% w/w and moisture content 8.9% w/w.
Conclusion: The present study on Pharmacognostical investigation of stem *of Diplocyclos palmatus* Linn. Whole plant or stem might be useful to supplement information in regard to its identification parameters assumed significantly in the way of acceptability of herbal drugs in present scenario lacking regulatory laws to control quality of herbal drugs.

Keywords: *Diplocyclos palmatus* linn, stem, pharmacognosy, phytochemical

Introduction
Diplocyclos palmatus (L) C. Jeffrey It is belonging to the family Cucurbitaceae plant locally known as 'Shivlingi' is distributed throughout India, an annual climber with bright red fruit and is reported to be highly medicinal [1]. Locally in India its seeds are being used for promoting conception in women. Plant is used against snake-bite. Its leaves are used in inflammation [2]. Roots are used for treatment of asthma. The seeds are used for increasing sperm count also as an aphrodisiac [3]. The main active constituents of the plants are Bryonin, a bitter principle [4] punicic acid, source of seed oil [5] non-ionic glucomannon 3 and goniothalamin [6]. Nonsteroidal anti-inflammatory drugs (NSAIDs), steroidal drugs, and immuno-suppressant drugs, which have been used usually in the relief of inflammatory diseases by the people of the world for a long time. However, these drugs were often associated with severe adverse side effects, such as gastrointestinal bleeding and peptic ulcers [7]. Recently, many natural medicines derived from plants, marine organisms were considered as the effective and safer for the treatment of various diseases including inflammation and pain [8].
The Present work is to frame a standard Pharmacognostic parameters for the stems of *Diplocyclos palmatus* Linn. useful in authentification and standardization of the drug, which give the quality and purity of the drug Figure 1.

Material and Method
Plant material
The plant specimens for the proposed study were collected from Chopda Tehsil (Adawad) MS, India in the month of April 2017 care was taken to select healthy plants and for normal organs. The plant was authenticated by Botanical Survey of India (BSI), Pune, Maharashtra, India. A voucher specimen (No. SSS 01) was deposited at B.S.I., Pune, India.

The required samples of different organs were cut and removed from the plant and microscopical character was studied by using motic microscope. The transverse sections of seed was taken and stained with Phloroglucinol: Conc. HCl (1:1) and Sudan red III. Observed under microscope (Motic) & further photo documentation were reported [9, 10, 11].

Physicochemical Parameters
Physicochemical parameter of whole plant of *Diplocyclos palmatus* Linn. were determined such as Total ash, Acid insoluble ash, Water soluble ash, Sulphated ash, moisture content etc [12, 13, 14, 15].

Preliminary Phytochemical Parameters
Preliminary phytochemical test of whole plant of *Diplocyclos palmatus* Linn. were performed and the chemical constituents were detected [16, 17, 18, 19, 20, 21, 22, 23, 24].

HPTLC Profile of bioactive ethyl alcohol extract of whole over ground part of Diplocyclos palmatus
Sample Preparation: 10 mg of ethyl alcohol extract was dissolved in 10 ml ethyl alcohol.
Stationary Phase: Precoated TLC plates of Silica gel G 60 F254 (E. Merck), 5 x 10 cm in size were used as stationary phase.
Mobile Phase: Chloroform: n-Hexane: Toluene: Ethyl acetate: Glacial acetic acid = 8: 26: 2: 1.8: 0.2 (v/v) was used as mobile phase.
Procedure: 100 µl and 50 µl of the sample solution were applied as band length 5 mm to 8 mm from lower edge of the plate using 100 µl syringe on CAMAG LINOMATE V automatic sample applicator.
Development: Plate was developed in 20 x 20 cm twin trough (CAMAG) chamber. Developing distance was 8 cm

from lower edge of the plate. Plates were then dried by air for 10 min.

Derivatization: The plate was sprayed with anisaldehyde-sulphuric acid reagent and heated at 105^{0}C till the spots appeared. The Rf value and colour of the spots were recorded.

Densitometer Scans: Plates were scanned at 254, 366 nm using scanner-3 (CAMAG)

Documentation: The profile obtained was photo documented at 254, 366 nm before derivatization and after derivatization through camera.

Results and Discussion

Macroscopy of plant

Macroscopically, plant of *Diplocyclos palmatus* (Lingini) was a tuberous, slender annual vascular plant that has hairless stems and forked tendrils. The leaves of the plant were ovate-sub-orbicular in outline and they have a length of 7.5-15 cm and width of up to 12 cm with unpleasant odour when crushed. The base of the deeply 3-5-lobed leaves is cordate, the blade membranous and scabrid above with minute red scales. The leaves were smooth or slightly scabrid beneath, while the lobes are lanceolate, acute to acuminate, with margins coarsely. The lobes were also irregularly serrate or sinuate and distantly denticulate. The small dioecius unisexual flowers of Lingini or Shivalingi are of greenish-yellow in colour. The female flowers of the plant were born in fascicles and the male ones are solitary. The plant`s corolla is about 3-4 mm, with ovate-oblong, acute, pubescent lobes. The fruit or berries of the plant were rounded, with a diameter of 2-3 cm and the bluish-green coloured fruit has eight vertical white streaks. They ripe red and bear a few brown, obovate seeds. The compressed seeds have a length of 4 mm
and width of 3 mm and they are usually encircled by a prominent raised band. The plant generally flowers between the months of August and September and fruits in September and October in central India. Lingini or Shivalingi commonly found throughout India and most common and typically found in village hedges Figure1.

Fig 1: Morphological view of plant

Microscopy study

The transverse section of stem showed an epidermis with thick cuticle. Below the epidermis cortex was present which consist of thin walled parenchyma cells and lignified fibres. The vascular bundle consists of xylem and phloem. The pith region consists of calcium oxalate crystals as well as thin walled lignified polygonal parenchyma with intercellular spaces Figure 2.

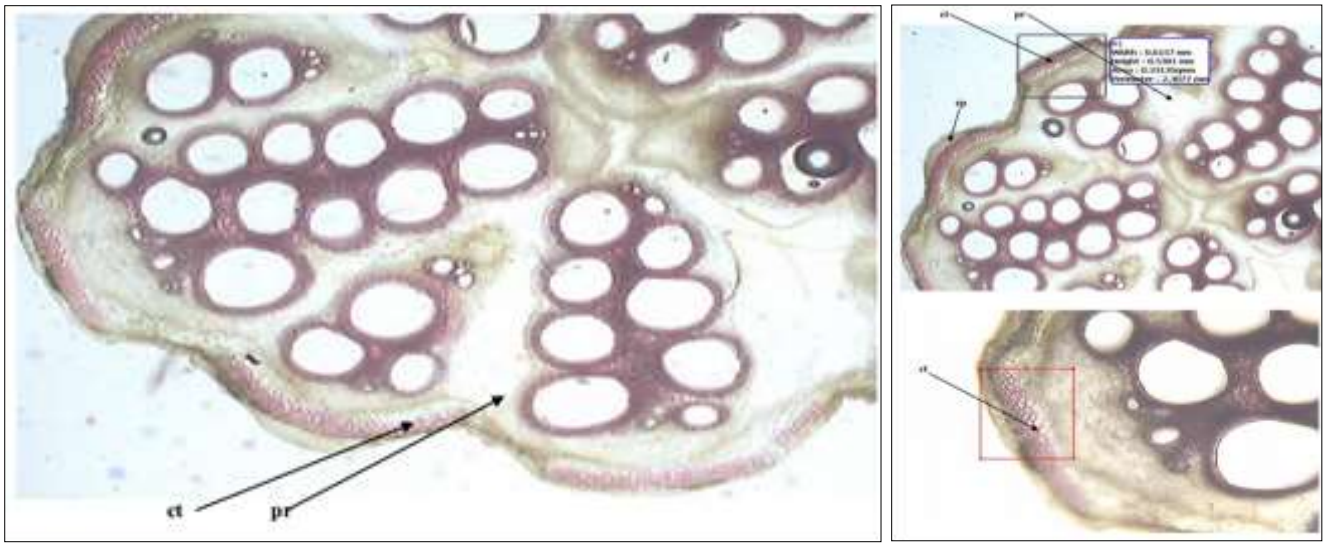

Fig 2: Transverse Section of stem of *Diplocyclos palmatus* by staining with Phloroglucinol and concentrated HCl (1:1) at 10x objective

Physicochemical Parameters

Whole over ground part of *Diplocyclos palmatus* powder showed the presence of total ash 2.05% w/w, acid insoluble ash 1.5% w/w, water soluble ash be 1.0% w/w, alcohol soluble extractive 12.28% w/w water soluble extractive 18.40% w/w and moisture content 2.5% w/w Table 1.

Table 1: Physicochemical analysis of whole over ground part of *Diplocyclos palmatus* Linn.

Sr. No.	Parameters	Values obtained on air dried basis (% w/w)
1	Ash values	
	Total ash	12.14%
	Acid insoluble ash	0.8%
	Water soluble ash	3.6%
2	Extractive value	
	Ethanol soluble extractive	18.85%
	Water soluble extractive	32.48%
3	**Moisture content**	8.9%

Preliminary Phytochemical Studies

Preliminary phytochemical screening was performed for the identification of different group of primary and secondary metabolites

In the crude extracts Extractive value of plant results shown in Table 2.

It revealed the presence of terpenoids, flavonoids, steroids, alkaloids and glycosides etc Table 3.

Table 2: Physical characteristics and Percentage yield of various crude extracts of whole over ground part of *Diplocyclos palmatus*

Sr. No.	Extract	Description			Yield (% w/w)
		Colour	Odour	Nature	
1	Petroleum Ether (60-80ºC)	Dark bright Green	characteristic	Semi solid mass	4.20
2	Chloroform	Fresh Green	characteristic	Semi solid to Solid	8.48
3	Ethyl alcohol (Absolute)	Dark brownish Green	Agreeable	Semi solid	22.58
4	Purified Water	Dark brown	Disagreeable	Semi solid mass	14.10

Table 3: Preliminary Phytochemical screening of the extracts of whole over ground part of *Diplocyclos palmatus*

Sr. No.	Type of Tests	Pet. Ether Extract	Choloroform Extract	Ethanol Extarct	Water Extract
1	Test for Steroids and Terpenoids	+	-	+	-
2	Test for Phytosterols				
A.	Salkowski	-	+	+	-
B.	Liberman-Burchard	-	+	+	_
3	Test for Flavonoids				
A	Shinoda Test	-	-	+	-
4	Test for Alkaloids				
A	Mayer's Test	-	-	+	+
B	Wagner's Test	-	-	+	+
5	Test for Anthraquinones	-	-	+	+
6	Test for Tannins	-	-	+	+
7	Test for Saponins				
A	Foam Test	-	+	+	+
8	Test for Carbohydrates				
A	Molisch's Test	-	-	-	-
B	Iodine Test	-	-	-	-
C	Fehling's Test	-	-	-	-
9	Test for Proteins				
A	Million's Test	-	-	+	-

Remarks: + (Present) and – (Absent).

HPTLC

The HPTLC of the bioactive crude extract (ethyl alcohol extract) at 254 nm (Track of sample 100 µl) shown shows presence of total 9 components with their Rf value and concentration sequentially details as Rf - 0.09 (0.18 µg), 0.12 (0.70 µg), 0.24 (10.08 µg), 0.38 (7.75 µg), 0.47 (15.77 µg), 0.56 (15.24 µg), 0.64 (15.17µg), 0.77 (25.60 µg), 0.85 (9.50 µg). Component number 8 at 0.77 Rf shows maximum concentration of 25.60 µg below to that component 5, 6, 7 are at marginal concentration with their AUC. Results shown in Table 4 and Figure 3.

Table 4: Optimized HPTLC profile of ethanol extract of whole over ground part of *Diplocyclos palmatus* at 254 nm (Track of Sample 100 µl)

Peak	Start Position (Rf)	Start Height (AU)	Max. Position (Rf)	Max. Height (AU)	Max. %	End Position (Rf)	End Height (AU)	Area (AU)	Area %
1	0.06	1.9	0.09	18.0	0.60	0.09	10.7	285.3	0.18
2	0.10	11.0	0.12	49.6	1.66	0.14	34.5	1141.3	0.70
3	0.14	34.5	0.24	434.9	14.53	0.28	32.1	16314.4	10.08
4	0.32	88.1	0.38	304.3	10.16	0.41	57.7	12547.7	7.75

5	0.41	258.0	0.47	456.8	15.26	0.50	14.6	25529.2	15.77
6	0.50	314.7	0.56	409.3	13.67	0.60	50.2	24678.5	15.24
7	0.60	350.4	0.64	440.7	14.72	0.69	29.7	24564.5	15.17
8	0.69	330.6	0.77	493.5	16.48	0.83	32.2	41455.4	25.60
9	0.83	362.7	0.85	386.8	12.92	0.92	16.3	15387.4	9.50

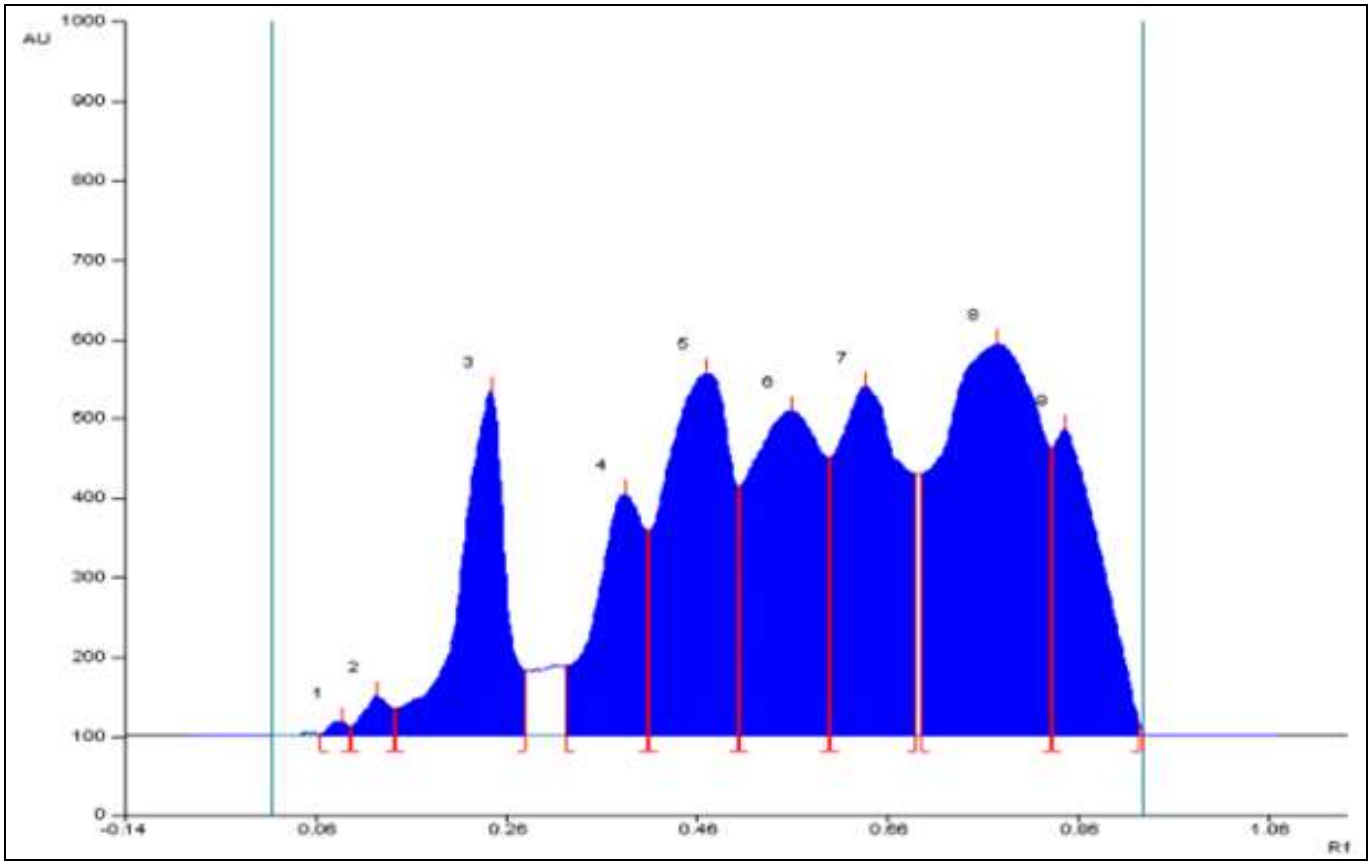

Fig 3: Optimized HPTLC chrmatogram of ethanol extract of whole over ground part of *Diplocyclos palmatus* at 254 nm (Track of Sample 100, 50 µl).

HPTLC chromatogram before derivatization

Mobile Phase: Chloroform: n-Hexane: Toluene: Ethyl acetate: Glacial acetic acid (8: 26: 2: 1.8: 0.2).

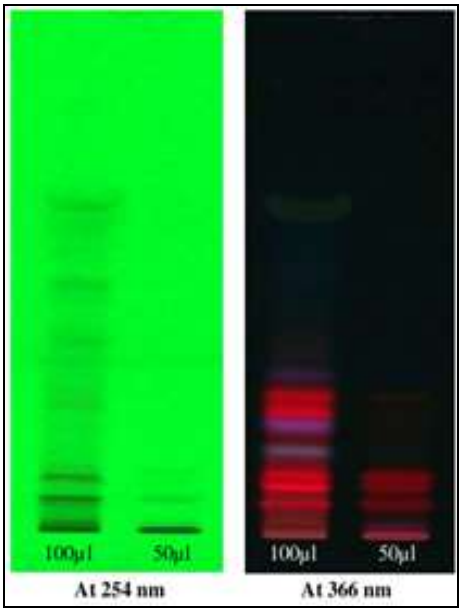

Fig 4: HPTLC chromatogram of ethanol extract of whole over ground part of *Diplocyclos palmatus*

HPTLC chromatogram after derivatization

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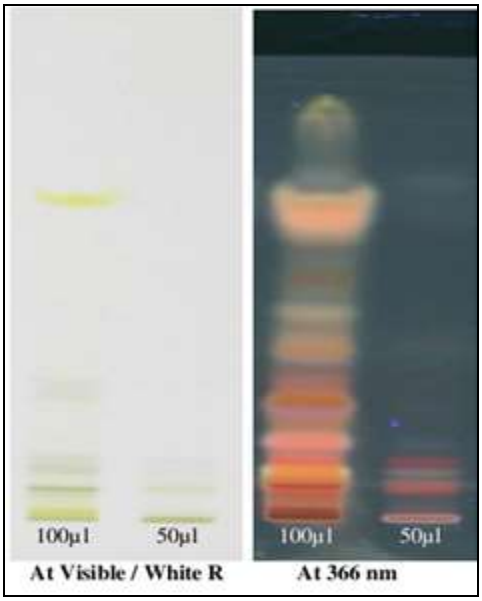

Fig 5: HPTLC chromatogram of ethanol extract of whole over ground part of *Diplocyclos palmatus*

Conclusion

The present Pharmacognostical studies of seed of the plant *Diplocyclos palmatus* might be useful to supplement assumed significantly in the way of acceptability of herbal drugs in present scenario that lacks regulatory laws to control quality of herbal drugs.

Acknowledgement

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Conflict Of Interest

There is no conflict of interest with this research.

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