



## Research article

### Physicochemical and preliminary phytochemical studies on the fruits of “Shivalingi” [*Diplocyclos palmatus* (Linn.) Jeffrey]

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#### Abstract

Shivalingi (*Diplocyclos palmatus* Linn.) is a lesser heard medicinal plant of Ayurveda with the fruits having important use in the area of reproductive medicine (female infertility, aphrodisiac, tonic, leucorrhoea etc.). The plant especially the fruits have immense folklore usage even today. It has been described in Ayurvedic classical texts like *Rajanighantu* and *Nighantu ratnakara*. So far no study reports are available on chemical analysis on the dried fruits of *shivalingi*. Hence, the present attempt was undertaken with an objective to investigate the physicochemical and preliminary phytochemical studies. The methodology of the study also involved Thin Layer chromatography and fluorescence parameters. The results of the analyses showed the presence of organic constituents like alkaloids, triterpenoids, flavonoids, saponins, steroids and proteins in the dried fruit. This provides impetus to conduct advanced research on this fruit to uncover its vast medicinal potential.

**Keywords:** *Diplocyclos palmatus*, Physicochemical studies, phytochemical constituents, Ayurveda, Shivalingi, TLC

#### 1. Introduction

*Shivalingi* (*Diplocyclos palmatus* (Linn.), syn.*Bryonia laciniosa*) belongs to family Cucurbitaceae. It is an annual scandent herb, a slender much branched tendril climber, from a thick permanent root stock, tendrils bifid; leaves simple, alternate, membranous, 10-15 cm long, green and scabrid above, paler and smooth beneath, 5 lobed, deeply cordate base, lobes oblong lanceolate, margins sinuate

denticulate. Flowers yellow, unisexual, males in small fascicles of 3-6, female flowers solitary or few; Fruits sessile globose, smooth berry, brick-red when ripe with white vertical lines, seeds yellowish brown, similar to baccate, sub sessile, globose, smooth, bluish green, streaked with broad vertical lines (1) Seeds are yellowish brown, and resemble with that of *Shivlinga* (Phallus of Lord Shiva in Hindu mythology).

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The plant is commonly found throughout India, on edges and bushes up to 1200m elevation and is naturally propagated by seeds(2) Locally the fruits of *Diplocyclos palmatus* are known as *Lingatondikai* in Kannada, *Shivalingakkaya* in Malayalam, *Shivalingakkay* in Tamil, *Lingadonda* in



Telugu and *Shivalingi* in Gujarathi & Marathi and in English it is known as *Lollipop climber*(2). The *shivalingi* has got many synonyms like *lingini*, *bahuputra*, *syadeeshwari*, *saivamallika*, *lingi*, *citraphala*, *shivaja*, *sivavalli* (Rajanighantu) and has ascribed the synonyms as mentioned which depict different characteristics of utmost important for facilitating its identification.

The plant has a foetid smell (*durgandha*), acrid(*katu rasa*), thermogenic (*Ushna*), anti inflammatory(*vrana ropana*), alterative, depurative and tonic & rejuvenative (*rasayani*) properties, and is useful in vitiated conditions of *vata* & *pitta doshas*, cough, flatulence, skin diseases, inflammations and general debility and also useful in *sidhma kushta* (psoriasis)(2).

The fruit is bitter, aperient and is considered to have tonic properties (3). The leaves of the plant are used as an ingredient along with Bengal gram flour in a special dietary preparation of the tribal Chhattisgarh as a tonic (4). *Diplocyclos palmatus* is a known *ayurvedic* drug described in *Rajanighantu* and *Nighantu ratnakara*. The fruit is used as an aphrodisiac, tonic and as an antipyretic in Ayurveda whereas in Siddha *system of medicine* entire plant is used as a laxative.

### 1.1. Indian Folklore Use

The leaves of the plant are generally applied as an antiinflammatory paste. Women take the seeds in combination with other medicinal herbs for helping conception and prevent miscarriage. Traditional healers of Gulgul village, Chhattisgarh recommend the use of 3-4 seeds once daily by women, in empty stomach for 1 to 2 months to beget a male child (4). Gond and Bharia tribes of Patalkot valley worship this plant and they consider that, this herb is boon for the childless parents. Traditional healers of Gaildubba suggest a mixture of *Shivalingi*

seeds with Tulsi (*Ocimum basilicum*) leaves and Jaggery in female infertility (5).

The seeds of *Shivalingi* are potentially contraceptive when used in combinaton with ginger (dry), pepper, Putrajivi Root bark of *vata* (*Ficus bengalensis*) and milk (6). Besides, abortifacient action of *shivalingi* seeds has also been reported when it is combined with equal quantity ashwagandha roots and consumed with sugar and milk (7) (Bhawda Janya Sarkya, Amalad).

Increased spermatogenesis and a significant increase in sperm count in epididymis of the male albino rats with concurrent increase in serum testosterone and luteinizing hormone have been reported with the use of *shivalingi seeds*. The above studies clearly reflect androgenic activity and its effects on hypothalamic pituitary gonadal axis (8).

## 2. Materials and methods:

### 2.1. Sample

Fresh fruits of *Shivalingi* were collected from Hassan district forest range of Karnataka (13) and identified as *Diplocyclos palmatus* (Linn.), syn. *Bryonia laciniosa*(L.) (Fig. 1). They were dried and powdered. This powder was used as a sample for analyses.

The total amount of extracts in all solvents is an approximate measure of the constituents of the sample. The amount of sample soluble in a given solvent is an index of its purity. These values indicate the nature of the constituents present in the crude sample. Physicochemical studies and preliminary phytochemical screening was carried out (10). Thin Layer chromatography studies of the petroleum ether at 60<sup>0</sup>-80<sup>0</sup>C, chloroform and ethanol, aqueous extracts were carried out in various solvents at 30<sup>0</sup>C using precoated silica gel GF254 plate as adsorbent(11). The extraction with all solvents except water was carried out by Soxhlet's method, while cold maceration was adopted for hydro-extraction. The process of extraction was performed until the



solvents turned colorless. The behavior of the sample treated with different chemical reagents and fluorescence characters were observed under ordinary and ultraviolet light (12).

## 2.2. Physicochemical analysis (10)

Ash values are helpful in determining the quality and purity of crude drugs in powdered form. The total ash method is designed to measure the total amount of material remaining after ignition. The different ash values like total ash, acid insoluble ash, water soluble ash was carried out. Extractive values are useful for evaluation of crude drugs and give an idea about the nature of chemical constituents present in them. Petroleum ether extract at 60-80°C, 95% ethanol soluble extractive, chloroform soluble extractive and water soluble extractive values were determined. Solubility and pH values were also determined using standard procedures.

## 2.3. Thin Layer Chromatography and Fluorescence Studies (11)

The concentrated petroleum ether chloroform, ethanol and aqueous extract of the fruit were subjected to TLC studies. Equal amounts of these extracts were loaded on the TLC plates. The best separation was achieved using Benzene, Toluene: Ethyl acetate (93:7) and BAW (Butanol: Acetic acid: Water) as a mobile phase. After developing, the plates were viewed under U-V light showed the presence of spots. The R<sub>f</sub> values were calculated and given in the Table 3. Fruit powder was moistened with different solutions to study fluorescence and viewed under ordinary light and U-V light having wave length of 365 nm and 254nm.

## 2.4. Preliminary phytochemical analysis (12)

The dried powder of the material was initially defatted with petroleum ether (60<sup>0</sup>-80<sup>0</sup>C) in a soxhlet apparatus and successively extracted with chloroform, ethanol and water. The extracts were

filtered while hot and solvent removed by distillation. The percentage of yield of the extract was calculated (Table 3).

## 3. Results

It was observed that water soluble ash was found more in comparison to total ash in the crude sample. Solubility in ethanol was more when compared to cold water (Table 1). The sample was alkaline (pH: 8.04). (Table 1). Phytochemical analyses revealed that the percentage yield of petroleum ether extract was found to be more than when compared to other extracts. The qualitative investigation tests were performed in the extracts and revealed the presence of alkaloids, flavonoids, Triterpinoids saponins, steroids and proteins (Table 2). The findings of TLC and fluorescence studies are given in Tables 3 & 4.

## 4. Summary and Conclusion

The different plant parts of the medicinal herb Shivalingi have a long history of traditional usage in various parts of India since times immemorial. The principal usage of the fruits has been in the areas of female infertility, pregnancy facilitation, aphrodisiac and tonic. The medicinal use of the plant has been conspicuously mentioned in Ayurveda also. But, Shivalingi fruit has not been much investigated or used medicinally in mainstream Ayurveda industry. The fruit might play a very important role in the above areas which are extremely challenging in the light of modern lifestyle induced stress and its effects on reproductive system. With this in mind a preliminary study was undertaken to identify and report the physicochemical and phytochemical analyses. The results were highly encouraging with the identification of constituents like alkaloids, flavonoids, Triterpinoids saponins, steroids and proteins which, are known modulators in a biological organism to produce such beneficial effects. There have been very few studies on shivalingi. The seeds have



been reported to contain 12% oil, 40% protein, iodine value of 171.5 (seed oil), saponification value of 208.3, peroxide value of 0.3 and acid value of 2.9 (9) .

This report is therefore the beginning of a series of studies that could be undertaken with advanced parameters like HPLC, pharmacology, animal and clinical trials to put forward a very important contribution from Ayurveda and India in the area of reproductive medicine.

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**Table 1. Physicochemical and Preliminary phytochemical analysis**

| S.No. | Parameters  | Values   |
|-------|---|--|
| 1     | % of Foreign matter   | Less than 2  |
| 2     | % Loss on drying at 105 <sup>0</sup> C<br>(moisture content)  | 3.00   |
| 3     | % Total ash content   | 9.60   |
| 4     | % Water soluble ash   | 10.27  |
| 5     | % Acid insoluble ash  | 0.250  |
| 6     | % Solubility at room temp.:<br>a. Ethanol<br>b. Water (cold)  | 54.00<br>29.60   |
| 7     | % Extractive values:<br>a. Pet. ether extract 60 <sup>0</sup> -80 <sup>0</sup> C<br>b. Chloroform<br>c. Ethanol | 10.80<br>1.08<br>1.0   |
| 8     | pH value  | 8.04   |
| 9     | Phytochemical constituents (Qualitative)  | <ul style="list-style-type: none"> <li>• Alkaloids +</li> <li>• Triterpinoids +</li> <li>• Flavonoids +</li> <li>• Saponins +</li> <li>• Steroids +</li> <li>• Proteins +</li> </ul> |

**Table 2. Preliminary Phytochemical study**

| S.No. | Phytochemical constituents | Aqueous extract | Ethanol extract |
|-------|----------------------------|-----------------|-----------------|
| 1     | Alkaloids                  | + ve            | + ve            |
| 2     | Triterpinoids              | - ve            | + ve            |
| 3     | Flavonoids                 | + ve            | -ve             |
| 4     | Tannins                    | - ve            | - ve            |
| 5     | Saponins                   | + ve            | + ve            |
| 6     | Sugars                     | - ve            | - ve            |
| 7     | Starch                     | - ve            | - ve            |
| 8     | Steroids                   | + ve            | + ve            |
| 9     | Proteins                   | + ve            | +ve             |
| 10    | Resins                     | - ve            | - ve            |

+ve : present, -ve: absent

**Table 3. Thin Layer Chromatography studies**

| S.No. | Extractive                        | Adsorbent                                | Solvent system                        | Rf values<br>(viewed in iodine chamber) |
|-------|-----------------------------------|--|---------------------------------------|---|
| 1     | Pet.ether<br>60-80 <sup>0</sup> C | Silica gel<br>GF254<br>precoated sheets  | Benzene                               | 0.14;0.31;0.42;0.50;0.68;0.86           |
| 2     | Chloroform                        | Silica gel<br>GF254<br>precoated sheets  | Toluene: Ethyl<br>acetate (93:7)      | 0.14;0.25;0.51;0.74;0.91                |
| 3     | Ethanol                           | Silica gel<br>GF254<br>precoated sheets  | Butanol:Acetic<br>acid:Water<br>5:1:4 | 0.13;0.24;0.64;0.72;0.93                |
| 4     | Aq.extarct                        | Silica gel<br>G F254<br>precoated sheets | Butanol:Acetic<br>acid:Water<br>5:1:4 | 0.74;0.84                               |

**Table 4. Fluorescence studies**

| S.No | Sample + Reagent                           | Ordinary<br>light  | U-V Long<br>wave 365 nm       | U-V short<br>wave 254 nm   |
|------|--|--------------------|-------------------------------|----------------------------|
| 1    | Powder as such                             | Brown              | Grey                          | Henna green                |
| 2    | Powder +water                              | C.Brown            | L. Grey                       | Henna green                |
| 3    | Powder+IN.HCL                              | D.Brown            | Dull green                    | Henna green                |
| 4    | Powder+IN. NaOH                            | Brown              | Grayish green                 | Henna green                |
| 5    | Powder+IN. NaOH in<br>MeOH                 | D.Brown            | Grey<br>Fluorescence          | Deep green                 |
| 6    | Powder+50% KOH                             | Brown              | Grayish green                 | Henna                      |
| 7    | Powder+50% H <sub>2</sub> SO <sub>4</sub>  | Deep Brown         | Greenish grey<br>Fluorescence | Deep green                 |
| 8    | Powder+Conc.H <sub>2</sub> SO <sub>4</sub> | R. Brown           | Dull green<br>Fluorescence    | Henna green                |
| 9    | Powder+50% HNO <sub>3</sub>                | Y. Brown           | Dull brown                    | Dark green                 |
| 10   | Powder+ Conc.HNO <sub>3</sub>              | D Brown            | Dull brown                    | Henna green                |
| 11   | Powder + Acetic acid                       | D Brown            | Grayish white<br>Fluorescence | Henna green                |
| 12   | Powder + Iodine water                      | D.Brown            | Dull brown                    | Deep green<br>Fluorescence |
| 13   | Pet.ether extract                          | Golden yellow      | ---                           | Deep green<br>Fluorescence |
| 14   | Chloroform extract                         | Orange brown       | ---                           | ----                       |
| 15   | Ethyl alcohol extract                      | Greenish<br>yellow | ---                           | ----                       |



**Fig.1. Shivalingi fruits and seeds [*Diplocyclos palmatus* (L.) C. Jeffrey]**



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