

Preliminary Phytochemical Investigation on Leaves, Seeds Extract of *Diplocyclos palmatus* (L.) C. Jeffrey Medicinal plant

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Abstract – *Diplocyclos palmatus* (L.) C. Jeffrey commonly known as shivalingi is a lesser heard and perennial climber having diversified medicinal values. It belongs to Cucurbitaceae family. This plant especially the leaves and seeds have immense folkloric usage even today. The leaves of the plant are generally applied as an anti-inflammatory paste. Women take the seeds in combination with other medicinal herbs for helping conception and prevent miscarriage. The seeds of the plant are increased spermatogenesis the present study was undertaken to evaluate the preliminary phytochemical constituents of leaves and seeds extracts. We screened for their phytochemical constituents following hot continuous and successive extraction by Soxhlet apparatus. Qualitative phytochemical screening was undertaken to check for the presence of alkaloids, flavanoids, saponins, tannins, phenols, cardiac glycosides, di and tri terpenoids in various extracts such as petroleum ether, ethyl acetate, chloroform, methanol and water. Among all the five extracts, maximum phytochemicals were found dissolved in methanol followed by water, chloroform, ethyl acetate and then petroleum ether. This provides impetus to conduct advanced research on this leaves and seeds to uncover its vast medicinal potential and further isolated and characterized to establish their potential against ailments.

Keywords - *Diplocyclos palmatus*, Folklore, Phytochemicals, Successive extraction, Soxhlet Extraction.

I. INTRODUCTION

Diplocyclos palmatus (L.) C. Jeffrey It belongs 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 (Kirtikar and Basu, 1987)^[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 (Chopra, *et al.* 1956)^[2]. Roots are used for treatment of asthma. The seeds are used for increasing sperm count also as an aphrodisiac (Singh and Malviya 2006)^[3]. The main active constituents of the plants are Bryonin, a bitter principle (Joshi, 2010)^[4] punicic acid, source of seed oil (Gowrikumar, 1983)^[5] non ionic glucomannon 3 and goniothalamine (Mosaddik, *et al.* 2000)^[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 (Corley, *et al.* 2003)^[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 (Sheir, *et al.* 2001)^[8] and (Bano & Singh, 2016)^[24]. The objective of the study was to evaluate the analgesic and anti-inflammatory activities of extracts of the seeds of *Diplocyclos palmatus* (L.) C. Jeffrey in rodents.

Indian Folklore Use

The leaves of the plant are generally applied as an anti-inflammatory 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 be get a male child (Oudhia, *et al.* 2003)^[9]. Gond and Bharia tribes of Patalkot valley worship this plant and they consider that, this herb is boon for the childless parents. Traditional healers are suggested to take the mixture of Shivalingi seeds with Tulsi (*Ocimum basilicum*) leaves and Jaggery in female infertility (Acharya, *et al.* 2007)^[10].

The seeds of Shivalingi are potentially contraceptive when used in combination with ginger (dry), pepper, Putrajivi Root bark of vata (*Ficus bengalensis*) and milk (Shukla, *et al.* 2008)^[11]. 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 (Patil, *et al.* 2006)^[12].

II. MATERIAL AND METHODS

Procurement of Plant material

Leaves and Seeds *Diplocyclos palmatus* (L.) C. Jeffrey were obtained from Govt. Girls College Rewa in wild condition in the month of September 2015. Then identified and authenticated by Department of Botany. Specimens were submitted to the herbarium.

Extraction Procedure

The leaves and seeds of the plants were dried in shade and powdered. Dried powder (300 g) was subjected to successive extraction in soxhlet extractor as per standard procedure used.

Successive extraction using Soxhlet apparatus

Successive extract of was carried out using Soxhlet apparatus. 20 gr *Diplocyclos palmatus* L. leaves and seed powder was taken in Wattmans No.1 filter paper, placed in Soxhlet thimble and 200ml of solvent was taken in the round bottom flask (still pot). The seed powder was extracted successively with, petroleum ether at 60°C, ethyl acetate at 77°C and chloroform at 61°C, Methanol at 65°C, and water at 80°C. Extraction temperatures were adjusted to boiling points of solvent to allow a faster rate of cycling of fresh solvent. Eight hours of duration was allocated to each solvent for hot continuous and successive extraction. The extracts were cooled, filtered through What man No.1 filter paper and the extraction was done in the order of increasing polarity of the solvents i.e., from hexane to water and proceeded for phytochemical screening.

Screening of Phytochemicals

Detection of alkaloids.

Extracts were dissolved in dilute Hydrochloric acid and filtered

a) Mayer's Test (Evans, 1997)^[14].

To a 2ml ml filtrate two to three drops of Mayer's reagent were added by the side of the test tube a white or creamy precipitate indicated the test as positive.

b) Wagner's Test (Wagner, 1993)^[15].

To a 2ml of filtrate two drops of Wagner's reagent (Iodine in Potassium Iodide) is Added by the side of the test tube, formation of brown/reddish precipitate indicated the Presence of alkaloids

Detection of saponins (Kokate, 1999)^[16].

a) Froth Test: Extracts is diluted with distilled water and made up to 20ml and suspension was shaken in a graduated cylinder for 15 min. Formation of foam layer of about two centimeters indicated the presence of saponins.

Detection of proteins (Fisher, 1968 and Ruthmann, 1970)^[17, 18].

The extract was dissolved in 10ml of distilled water and filtered through Whatman no. 1 filter paper and the filtrate is subjected to tests for proteins.

a) Millon's test (Sasidharan, *et al.* 2011)^[19].

To 2ml of filtrate 0.5ml of million's reagent was added. Formation of a white precipitate indicated the presence of proteins.

Detection of cardiac glycosides (Sasidharan, *et al.* 2011)^[19].

concentrated sulphuric acid were added. Green-blue coloration of solution appeared indicating the presence of cardiac glycosides.

Detection of flavonoids (Sindhu and Uma, 2013)^[20].

In a test tube containing 0.5 ml of the fruit extract, 5-10 drops of dilute HCL and small piece of Zn were added and the solution was boiled for few minutes. Presence of flavonoids resulted in reddish pink or dirty brown.

Detection of triterpenoids

The extract was dissolved in one ml of chloroform; 1ml of acetic anhydride was added followed by the addition of 2ml of concentrated H₂SO₄. Formation of reddish violet color indicated presence of triterpenoids (Sindhu and Uma, 2013)^[20].

Detection of steroids (Yasuma and Ichikawa, 1953)^[21]

Liebermann-Burchardt test: To 1ml of extract, 1ml of chloroform, 2 to 3ml of acetic anhydride, and 1 to 2 drops of concentrated sulfuric acid were added. Appearance of dark green color showed the presence of steroids.

Detection of phenolic compounds

Ferric chloride test (Soni, *et al.* 2013)^[22]

Take to 2ml of extract, treated with 3-4 drops of Ferric chloride solution formation of bluish black color indicates presence of phenols

Detection of tannins (Tiwari, *et al.* 2011)^[23]

1 ml of extract was taken and few drops of 1% lead acetate were added. Formation of yellowish precipitate indicated the presence of tannins.

III. RESULTS AND DISCUSSION

Qualitative assay of the leaves and seeds of *Diplocyclos palmatus* (L.) C.Jeffrey Linn. Revealed the presence of diverse group of phytochemicals. The results are presented in (table 1 and table 2) to enable their comparative study with respect to the solvents used for extraction.

The phytochemical screening in the present study following hot continuous method of extraction using Soxhlet apparatus, revealed the presence of alkaloids, flavonoids, tannins, saponins, glycosides, di and triterpenoids, phenols, steroids, cardiac glycosides. In *Diplocyclos palmatus* (L.) C. Jeffrey Linn. Leaves and seeds. Screening tests for proteins were found to be negative in this method of extraction using different solvents. (Table 1) Alkaloids were identified in methanol, and aqueous extracts; flavonoids were found in only aqueous extracts; qualitative test for tannins was positive in chloroform ethyl and acetate. positive results were obtained for the presence of saponins in methanol and aqueous extracts; cardiac glycosides were detected by modified Borntrager's test in chloroform, methanol, and aqueous extracts; di and triterpenoids were noticed in ethyl acetate and chloroform; phenols were found in only aqueous extracts; steroids were present in ethyl acetate, chloroform, methanol, and aqueous extracts; petroleum ether extracts totally negative results. Extraction process using the solvent, methanol and aqueous could yield most of the phytochemicals, followed by, chloroform, and ethyl acetate. The results differed depending upon the method of extraction. Quite a number of biologically active phytochemicals were identified in the extraction by the method followed for extracts and screening tests done.

Table: 1. screening of Preliminary phytochemical Leaves Extract

S.No.	Name of the phytochemicals	Pet. ether extract	Ethyl acetate extract	Chloroform extract	Methanol extract	Aqueous extract
1.	Alkalioids Mayer's test Wagner test	-ve -ve	-ve -ve	-ve ve	+ve +ve	+ve +ve
2.	Flayanoids	-ve	-ve	-ve	-ve	+ve
3.	Tannins	-ve	+ve	+ve	-ve	-ve
4.	Saponins	-ve	-ve	-ve	-ve	-ve
5.	Proteins	-ve	-ve	-ve	-ve	-ve
6.	Phenols	-ve	-ve	-ve	+ve	-ve
7.	Steroids	-ve	+ve	+ve	+ve	+ve
8.	Cardiac Glycosides	-ve	-ve	+ve	+ve	+ve
9.	Di Terpinoids	-ve	+ve	+ve	-ve	-ve
10.	Tri Terpinoids	-ve	-ve	+ve	+ve	-ve

Qualitative assay of the seeds of *Diplocyclos palmatus* (L.) C.Jeffrey Linn. Revealed the presence of diverse group of phytochemicals. The results are presented in (Table 2) to enable their comparative study with respect to the solvents used for extraction.

Alkaloids were identified in chloroform, methanol, and aqueous extracts; flavonoids were found in chloroform, methanol, and aqueous extracts; qualitative test for tannins was positive in pet ether, chloroform ethyl acetate and methanol; positive results were obtained for the presence of saponins in methanol and aqueous extracts; cardiac glycosides were detected by modified Borntrager's test in pet ether, ethyl acetate, chloroform, methanol, and aqueous extracts; di and triterpenoids were noticed in chloroform and methanol extracts; phenols were found in only methanol extracts; steroids were present in chloroform, methanol, and aqueous extracts; proteins were identified in only methanol extracts; Extraction process using the solvent, methanol and aqueous could yield most of the phytochemicals, followed by, chloroform, and ethyl acetate. The results differed depending upon the method of extraction. Quite a number of biologically active phytochemicals were identified in the extraction by the method followed for extracts and screening tests done.

Table: 2. Screening of Preliminary phytochemical seed extract.

S.No.	Name of the phytochemicals	Pet. ether extract	Ethyl acetate extract	Chloroform extract	Methanol extract	Aqueous extract
1.	Alkalioids Mayer's test Wagner test	-ve -ve	-ve -ve	+ve +ve	+ve +ve	+ve +ve
2.	Flayanoids	-ve	-ve	+ve	+ve	+ve
3.	Tannins	+ve	+ve	+ve	+ve	-ve

4.	Saponins	-ve	-ve	-ve	+ve	+ve
5.	Proteins	-ve	-ve	-ve	+ve	-ve
6.	Phenols	-ve	-ve	-ve	+ve	-ve
7.	Steroids	-ve	-ve	+ve	+ve	+ve
8.	Cardiac Glycosides	+ve	+ve	+ve	+ve	+ve
9.	Di Terpinoids	-ve	-ve	+ve	+ve	-ve
10.	Tri Terpinoids	-ve	-ve	+ve	+ve	-ve

IV. CONCLUSION

The extraction of Phytochemicals from the leaves and seeds of *Diplocyclos palmatus* (L.) C. Jeffrey Linn following hot continuous successive method using eight different solvents yielded variety of Phytochemicals. The chemical constituents of the seeds attests that it can be a potential source of future drugs and vital for good health. The outcome of the analyses will certainly facilitate their quantitative estimation and isolation of pharmacologically active chemical compounds. Future work is ongoing to isolate, identify characterize, and elucidate the structure of the phytoconstituents responsible for the observed pharmacological activities in this study.

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