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Pharmacognostic evaluation and anthelmintic activity of leaf and stem extracts of *Clinacanthus nutans* Lindau

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Abstract
Clinacanthus nutans Lindau (Vishamooli) is an exotic plant to India belonging to the family Acanthaceae. This plant has diverse potential medicinal uses in traditional herbal medicine and is well studied in South Asian Countries. In this study the physicochemical constants, phytochemical screening, total phenol quantification and anthelmintic property is evaluated. It is observed that the acetone and water extracts of leaf and stem had the same phenolic content. The anthelmintic activity of the aqueous extracts of leaves and stem was evaluated by using *Pheretima posthuma* as test worms. The time of paralysis and time of death were studied and the activity was compared with albendazole as reference standard. The aqueous extract of stem and leaves at 10mg/ml exhibited significant anthelmintic activity as evidenced by decreased paralyzing time and death time. The results thus support the use of *C. nutans* as an anthelmintic agent.

Keywords: *Clinacanthus nutans*, physicochemical constants, phytochemical screening, total phenol quantification, anthelmintic activity

Introduction
Medicinal plants have been used in traditional medicine since prehistoric times. These plants contain numerous phytochemicals with known or unknown activity; however, the effect of using a whole plant as medicine is uncertain [1]. Therapeutic efficacy of medicinal plants depends upon the quality and quantity of its chemical constituents. Wrong identification is one of the reasons for the misuse of herbal medicines or natural products. All these problems can be solved through pharmacognostic studies of medicinal plants. Pharmacognostic studies ensure correct identification of the plants and help in authentication of the plants and reproducible quality of herbal products which will lead to the safety and efficiency of herbal drugs [2].
Clinacanthus nutans Lindau (Vishamooli) is an exotic plant mainly found in Kerala, India [3]. *C. nutans*, which belongs to the Acanthaceae family, is a very well-known traditional herb and vegetable in Southeast Asian countries [4]. This plant has diverse and potential medicinal uses in traditional herbal medicine for treating skin rashes, insects and snake bites, lesions caused by herpes simplex virus, diabetes, and gout in Malaysia, Indonesia, Thailand and China[5]. Phytochemical investigations documented the varied contents of bioactive compounds from this plant namely flavonoids, glycosides, glycoglycerolipids, cerebrosides and monoacyl monogalactosylglycerol [6]. The pharmacological experiment proved that various types of extracts and pure compounds from this species exhibited a broad range of biological properties such as anti-inflammatory, antiviral, antioxidant, and anti-diabetic activities [7, 8]. Even though the plant is studied for its properties in South Asian countries, in India research works on this plant is very less reported. The plant is mostly grown as a garden plant and its leaf paste is used for treating insect bites. In this present study an attempt is made to understand this wonderful exotic shrub *C. nutans* Lindau more closely. The main purpose of the present study is to explore pharmacognostic profile, phytochemistry, total phenol quantification of aqueous, acetone and petroleum ether extract and anthelmintic potential of aqueous extract of *C. nutans*.

Materials and Methods
Pharmacognostic Evaluation
Physio-chemical parameters such as moisture content, total ash, acid-insoluble ash and extractive values were determined as per the Standard Indian Pharmacopoeia methods.

C. nutans was collected and shade dried. The plant material, stem and leaves were separated, shade dried and powdered using a mixer grinder. Dried plant material was successively extracted using soxhlet apparatus with solvents petroleum ether, acetone, distilled water, and extract collected. The extractive values of various solvents (Petroleum Ether, Acetone, and Distilled Water) were determined by standard procedure. The extraction yield is expressed as the percentage of total mass of extracts (*Mext*) with respect to the mass material used (*Mo*)[9] Yield percentage (%) = (*Mext* / *Mo*) x 100. Extract is dissolved in minimum solvent to do the preliminary phytochemical analysis. Preliminary phytochemical screening was carried out by standard procedure [10].

Total phenol quantification: Total phenols were estimated by employing Folin - Ciocalteau reagent [11]. For estimation, Folin - Ciocalteau reagent was diluted to 1N with equal volume of distilled water. 1ml of the same was added to 1ml of the extract in a 25 ml test tube followed by 2 ml of 20% Sodium Carbonate. The mixture was heated in a boiling water bath for exactly one minute. The blue colour was diluted to 25ml with distilled water. The percentage of light transmittance was determined at 725nm using Labtronics NT 290 Spectrophotometer. Total phenol was calculated from Standard curve prepared using Gallic acid and expressed as µg/mg GAE.

Anthelmintic activity: Adult earthworms (*Pheretima posthuma),* were used to evaluate anthelmintic activity *invitro*. The assay [12] was performed *invitro* using adult earthworm (*P. posthuma)* as it is having anatomical and physiological resemblance with the intestinal round worm parasites of human beings for preliminary evaluation of anthelmintic activity. Test samples of the hotwater extract was prepared at the concentrations, 10, 5 and 2.5 mg/ml in dissolved in distilled water and earthworms i.e. *P. posthuma*, of approximately equal size and same type were placed in each nine cm Petri dish containing 15 ml of above test solution of extracts. Albendazole (2.5mg/ml) was used as reference standard as advocated earlier. All the test solution and standard drug solution were prepared freshly before starting the experiments. Observations were made for the time taken for paralysis when no movement of any sort could be observed and time for death of earthworms were recorded after ascertaining that earthworms did not moved when shaken vigorously.

Statistical analysis

All the results were shown in Tables or graphs. Experiments were repeated in triplicates.

Results and Discussion

Pharmacognostic study helps in the standardization of a crude drug as it form an integral part for establishing its correct identity. Microscopic and macroscopical characters are one of the simplest and cheapest methods to start with for establishing the correct identity of the source materials[13]. *C. nutans* is a perennial herb which can grow up to 1 m tall with cylindrical stem and oppositely arranged lanceolate, petiolated leaves. No flowers were observed in the plants found in and around our locality. Here the plant is grown as garden plant knowing its medicinal importance. The plants observed were 3-5 years of age. The morphological features observed are similar as described in earlier studies [14].

The powder characteristics of *C. nutans* leaves exhibited higher values than stem except for acid insoluble ash (Table 1). Both leaf and stem showed same odour, green colouration and bitter taste. Ash values and extractive values can be used as reliable aid for detecting adulteration. Ash values are used to determine quality and purity of crude drug [10]. It indicates presence of various impurities like carbonate, oxalate and silicate. The water soluble ash is used to estimate the amount of inorganic compound present in drugs. The acid insoluble ash consist mainly silica and indicate contamination with earthy material. Moisture content of drugs should be at minimal level to discourage the growth of bacteria, yeast or fungi during storage. Leaves exhibited higher moisture, ash, sugar, protein fat content than stem[15].Our studies also confirmed the presence of high moisture content, total water soluble ash and total ash in leaves than in stem.

Table 1: Powder characteristics and quantitative physicochemical parameters for *C. nutans* stem and leaves.

Parameters	Observations-stem	Observations-Leaves
Colour	Greenish	Greenish
Odour	Characteristic	Characteristic
Taste	Bitter	Bitter
Moisture Content	15 %	17.26 %
Total ash	11.53 %	20.74 %
Water soluble ash	4.35%	7.07%
Acid insoluble ash	18.28%	11.27%

The solvents were removed by evaporation in water bath to obtain the extract. The percentage yields of various extract are presented in Table 2.The extracts obtained by exhausting plant materials with specific solvents are indicative of the approximate measures of their chemical contents[16]. These extractive values are also useful to find out the adulterated drugs [17].

Table 2: Percentage of extractive values for *C. nutans* stem and leaf.

Parameters	Observations -Stem	Observations –Leaf
Water Soluble extractive	22.95	30.5
Acetone Soluble Extractive	4.6	4.05
Petroleum ether Soluble extractives	0.5	3.7

The extracts of the leaf and stem detected the presence of primary and secondary metabolites in (Table 3). Among primary metabolites protein, sugar and carbohydrates were detected in all the three extracts. Secondary metabolites were

detected in the leaf and stem extracts, saponin in acetone and aqueous extract, phenols in petroleum ether and aqueous, steroids in acetone and aqueous extract. Alkaloids were absent in all the three extracts.

Table 3: Phytochemical detection of *C. nutans* leaf and stem extracts

Phytochemical Constituents	Test	Petroleum Ether		Acetone		Water	
		Leaf	Stem	Leaf	Stem	Leaf	Stem
Carbohydrates	Molish	+	+	+	++	+	++
Starch	Iodine	_	_	_	_	+	_
Sugar	Benedicts	++	+	++	++	++	++
Protein	Biuret	+	+	+	+	++	+
Amino acid	Ninhydrin	_	_	_	_	_	_
Saponin	Foam Test	_	+	++	++	++	++
Phenol	Folin Reagent	+++	+	_	_	+	++
Tannin	Ferric Chloride	_	_	+	_	++	+
Alkaloids	Meyers Reagent	_	_	_	_	_	_
Steroids	Salkowski	_	+	+	++	+	+

+ indicates the intensity of occurrence of the compound tested
- Absence of metabolite

In this present study, phenol quantification was done with UV spectrophotometric method. Folin –ciocalteu reagent and hydroxyl groups in the extract reacts to form a Prussian blue colored complex. In this study leaf and stem samples showed almost similar phenol content in acetone and aqueous extract where as the phenol content in petroleum ether extract is very meager. In stem, aqueous extract exhibited maximum phenol content ie 25µgGAE /mg whereas in leaf water extract and acetone extract exhibited almost same phenol content.

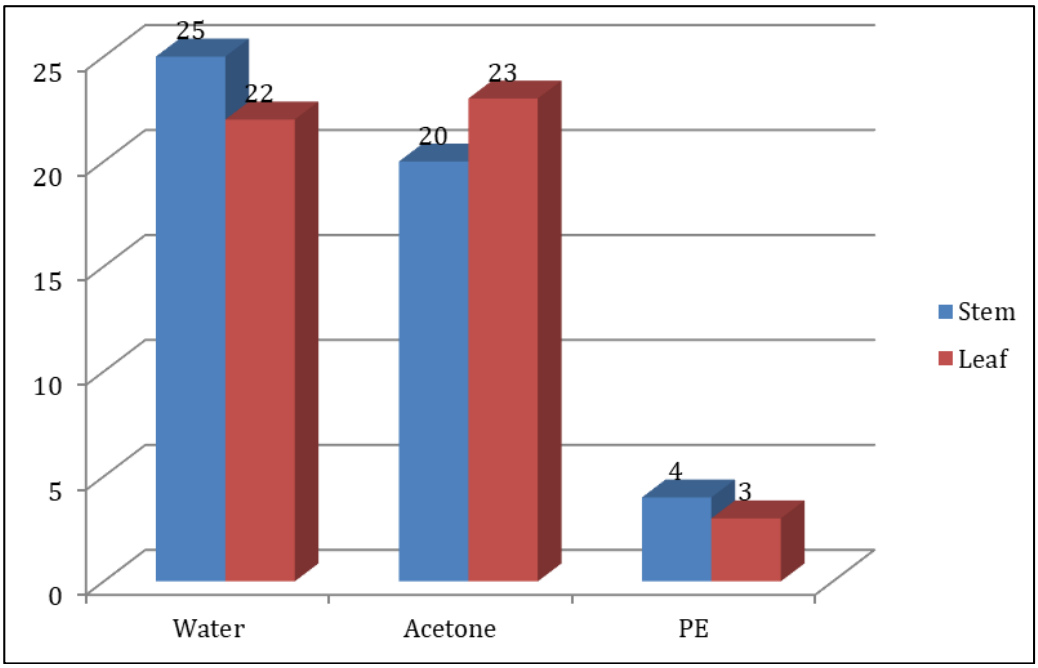

Fig 1: Quantification of Total Phenols of leaf and stem extracts of *C. nutans*

Anthelminthic property of aqueous extract of *C. nutans* was compared with standard albendazole using *Pheretima posthuma*. The paralysis and death of worms indicate its ability to be used as an anthelminthic. Anthelminthic property of aqueous extract is found to be effective at 10 mg/ml concentration (Table 4). Helminthiasis is a serious macro parasite disease of humans and other animals in which a part of body is infected with parasite worm called helminthes [18]. Broad spectrum benzimidazoles are used in its treatment. Development of resistance in helminthes against conventional anthelmintics is the foremost problem in treatment of helminthes disease. Some serious side effects of albendazole is that it causes giddiness, decreased urination, fever, chills, or sore throat tiredness etc. Hence, it is important to look for alternative strategies against gastrointestinal nematodes, which have led to the proposal of screening medicinal plants for their anthelminthic activity. The wormicidal activity of the water extract against earthworms suggests that it is effective against parasitic infections of humans [19]. It would be interesting to identify the active principle responsible for the anthelmintic activity and to study its further pharmacological actions.

Table 4: Anthelmintic activity of aqueous extracts of *C. nutans* leaf and stem

Con of Hot water extracts	Time taken in Minutes			
	Leaf		Stem	
	Paralysis	Death	Paralysis	Death
2.5mg/ml	108±0.5	0	90±.7	0
5 mg/ml	78±0.3	0	68±.5	120±2
10 mg/ml	20±0.5	35±2	15±.5	20±.1
Albendazole 2.5mg/ml	3±0.23	17±1	3±.6	17±.2

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