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Development of *Cleistanthus collinus* (Roxb.) (Benth) based botanical formulations against *Spodoptera litura*

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Abstract
Glycosides such as Cleistanthin A and B are the principle toxins isolated from this poisonous plant, as all parts of this plant is reported as highly toxic. It is also used as cattle and fish poison. The leaves are considered as an abortifacient. There is a significant amount of arylnaphthalide lignans such as diphyllin, collinusin and many other related compounds present in the plant *Cleistanthus collinus*. Development of formulation is the one way to popularize and commercialize the *Cleistanthus collinus* plant. In this present study, Formulations development by *Cleistanthus collinus* (Roxb.) (Benth) extracts. Finally found 6 number of formulations respectively Water Soluble Granules, Water Soluble tablet, Soap, Syrup, Soluble tablet (Preformed), Soluble globules and there insecticidal activity. The average weight of soluble tablet, Water soluble granule, soluble globule, and soluble tablet (Preformed) were found as 0.12, 0.02, 0.05 and 0.15 respectively. The 'F' value worked out for the final weight of the formulations was 8, 600, 100 and 7.

Keywords: *Cleistanthus collinus*, water soluble granules, water soluble tablet, soap, syrup, soluble globules

Introduction
Cleistanthus collinus (Roxb.) (Euphorbiaceae) is a medium size toxic tree and it grows in the dry forests of Southern and Central parts of India, Malaysia and Africa, commonly known as karra or garari in Hindi 'oduvan' in Tamil, 'Vadise' in Telugu and 'Nilapala' in Malayalam. *Cleistanthus collinus* (CC) is an extremely poisonous due to the presence of diphylline (lignan) lactone and its glycoside cleistanthin A and B. The leaf, root and specially fruit act as a violent gastrointestinal irritant. Anticancer activity has been reported against epidermal carcinoma from the alcoholic extract of CC. Water decoction of the leaves is used for suicidal purpose in many parts of Southern India. The plant also possesses insecticidal properties against the red flour beetle, *Tribolium castaneum* [10] and used as insecticides in rice fields. The active ingredient responsible for variety of anti-insect properties like antifeedant, insecticidal and insect growth regulatory activity was structurally elucidated as "Lactone glycoside" and was found to impart very significant antifeedant activity [3], emergence inhibition larval mortality against third, fourth and fifth in star *Spodoptera litura* larvae [15,16]. [4] Observed that hyper excitation, ataxia, tremors and unbalanced walking in *C. collinus* extract treated larvae of *S. litura* as in organophosphorus and organochlorine poisoned insects. Further, the crude leaf extracts of *Cleistanthus collinus* was reported for larvicidal activity against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* [2].Traditionally the farmers of Maharashtra used this plant leaves solution as an effective pesticide or insecticide, when insects attack on rice crop [9]. The usage of these botanical insecticides has increased at local level; their quality and efficiency are serious concerns. Thus accurate scientific assessment has become a prerequisite for acceptance. By providing scientific information and converting the insecticidal plants into good formulations can influence its market and competitiveness [5].The correct design of a formulation is one of the critical steps in the development of the botanical pesticide. If things go wrong, it could lead to various field problems and adversely affect the performance [17]. In early days of formulation technology, the choice of formulation types was limited to a few standard formulations. But today a plant product can be formulated into various types depends on market needs, the physical limitation of the active ingredient and the states of technology at the time the formulation development.

In this research work, an attempt was made to formulate the leaf extract of *Cleistanthus collinus*.

Materials and Methods

Culture of *Spodoptera litura*

Spodoptera litura was reared on bengal gram flour based semi synthetic diet (PDBC, 1998) under 25 ± 2°C and 70 to 80 % relative humidity continuously throughout the study period in the laboratory and utilized in the bioassays as test insect.

Plant collection and extraction

C. collinus leaves collected in and around Kolli hills of Tamil Nadu, India were brought to the laboratory; shade dried under room temperature and powdered using an electric blender. Then extraction was made using acetone (HPLC grade) in soxhlet apparatus, extractives obtained was used for the preparation of preformed tablets and globules. Hot water extract obtained by adding 100gm of chopped fresh leaves in 1L of distilled water and boiled until they reached 500ml. then the contents were filtered through kada cloth and used in the preparation of soap, soluble tablet, soluble granules and syrup.

Bioassay

2 cm diameter castor leaf discs were treated on either side with kept individually in plastic containers (200 ml capacity). Three third instar larvae pre starved for 4 hours were released per disc and the container was closed with muslin cloth. Absolute control, hot water and solvent extracts and control were also maintained. All treatments were replicated five times.

Formulations techniques of *C. collinus* extract

Water Soluble Granules (WSG)

10ml of hot water extract of *C. collinus* leaf at 10% was added with 100g of lactose powder (made out of cane sugar). Then this was mixed thoroughly, passed through 16 mesh sieve and granules formed were shade dried for 6h [11].

Soluble Tablets

100g of water soluble granules prepared using hot water extract of *C. collinus* leaf were fed into tablet punching machine and tablets were formed [11].

Soluble Tablets (using preformed tablets)

1g of extractive was mixed with 10ml acetone (HPLC grade) and dipped readymade tablets (100g) (Bakson Drugs & Pharmaceuticals Pvt. Ltd.) were into solvent and then removed after 30 seconds. Then tablets were shade dried.

Soluble Globules (using preformed globules)

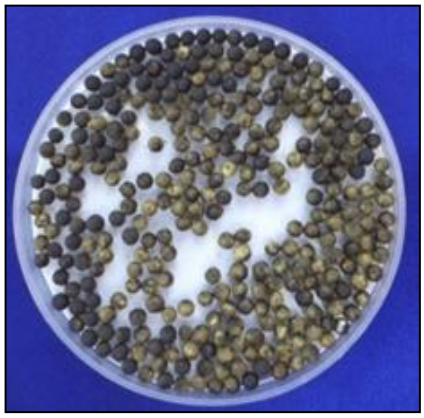
Globules (100g) (Bakson Drugs & pharmaceuticals Pvt. Ltd.) were placed in a test tube and 10ml of solvent extract of *C. collinus* leaf was added drop by drop then shaken well and shade dried.

Syrup

One liter of distilled water was added with 850g of sucrose in a conical flask and mixed well. Then 100ml of *C. collinus* hot water extract was added to the solution and stirred continuously. The mouth was fitted with stopper and covered using a thick layer of cotton and aluminum foil and kept at room temperature. After 24h, a slimy growth of bacterial and fungal was observed on the syrup. Then it was filtered using kada cloth double the times and stored at room temperature.

Soap

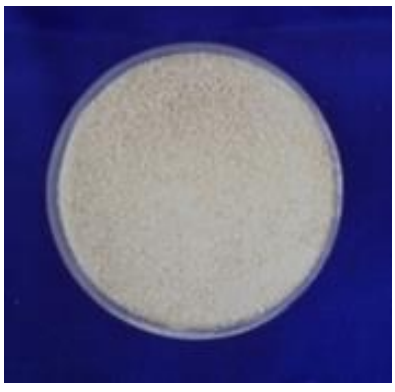
10ml of *C. collinus* leaf hot water extract was added with 100 ml of sesame oil. Then ley solution (50 g. of sodium hydroxide + 50 ml of water) was added and thoroughly mixed by using blender. Then the mixture was poured into suitable plastic container to get desired shape. After 24 hours soaps were recovered.



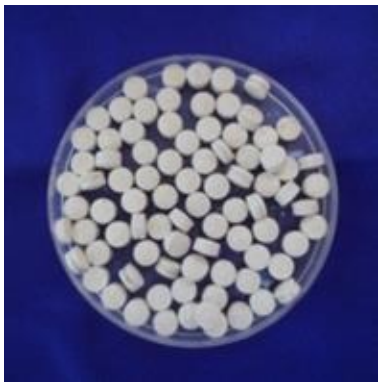
Soluble globule

Syrup

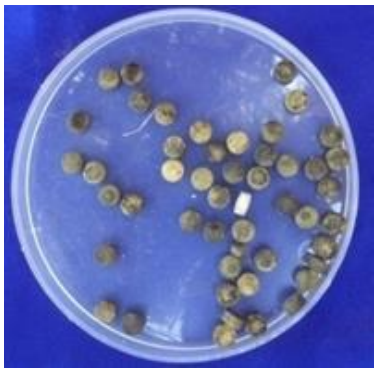
Soap



Water soluble granule



Soluble tablet



Soluble tablet (preformed)

Quality and stability of the formulations

Weight uniformity test

This is a satisfactory method for determining drug content uniformity/distribution. Twenty tablets, granules and globules selected at random were weighed individually and the average weight was determined. Then two individual tablets, granules and globules from the lot were weighed and noted. Then it was compared with the average weight. The following yardstick was used to determine acceptability [11].

$$\% \text{ Deviation} = \frac{\text{Average weight} - \text{individual weight}}{\text{Average weight}} \text{x } 100$$

Average weight	Per cent deviation
130mg or less	10
130mg to 324mg	7.5
More than 324mg	5

Friability test

It is a measure of strength of a product. Twenty tablets, granules and globules selected at random were weighed individually, placed in friabilator (Rimek model) and subjected to tumbling motion. The chamber was revolved at 25 rpm and dropping of tablets were from a distance of 6 inches at each revolution. It was operated for 100 revolutions. Then the tablets, granules and globules were dusted and reweighed individually [11].

The friability is given by f (Per cent weight loss)

$= 100 (1 - w_2 / w_1)$ (or) (W_1-W_2) x100/W_1

Where

W_1= initial weight

W_2= final weight

Value of 'f' from 0.5 – 1.0 % are regarded as the upper limit of acceptability.

Hardness test

Twenty tablets, granules and globules were placed between two anvils individually one by one and force was applied to the anvils. The crushing strength that just caused the tablets, granules and globules to break was recorded. Crushing strength is otherwise referred as hardness. Normally hardness of 3 to kg is considered as good one [11].

Crushing strength = Force of fracture – Zero force reading

Solubility test

Water was collected from three different water sources (canal water, pond water, tap water) at Annamalainagar and the pH (8, 9, and 7) was noted. 10ml of water was measured and transferred to test tubes of 100ml capacity separately and two grams of tablets, granules, globules and soap were placed in water. Then the contents were passed through filter papers (weighed previously) each separately after 10 minutes and shade dried. Then the weight was recorded and compared [11].

Solubility = $(w_2 – w_1)$

Where,

W_1 = initial filter paper weight

W_2 = final filter paper weight

Purity test for syrup

The purity of the syrup was tested by analyzing the microbial growth spread on the syrup after 48h and identified.

Studies on the laboratory efficacy of the formulations

All the above formulations were tested at 10% concentrations against *S. litura* for their efficacy by following the leaf disc bioassay techniques.

Results and discussion

Quality and stability of the formulations

The average weight of soluble tablet, Water soluble granule, soluble globule, and soluble tablet (Preformed) were found as 0.12, 0.02, 0.05 and 0.15 respectively. Per cent deviations worked out for the weight uniformity test were zero and 8.3, 8.3 and 8.3, zero and zero and zero and 6.66 respectively. The average final weight after the friability test for soluble tablet, water soluble granule, soluble globule and soluble tablet (Preformed) were 0.11, 0.14, 0.05 and 0.14 respectively. The 'F' value worked out for the final weight of the formulations was 8, 600, 100 and 7 respectively. The average crushing strength of the formulations *viz.*, soluble tablet, water soluble granule, soluble globule and soluble tablet (Preformed) were 1, 0.25, 0.50 and 1 kg. The average crushing strength of the formulations *viz.*, soluble tablet, water soluble granule, soluble globule and soluble tablet (Preformed) were 1, 0.25, 0.50 and 1 kg. The differences between initial weight and final weight in the solubility test for the formulations such as soluble tablet, water soluble granule, soluble globule, soluble tablet (Preformed) and soap were 0.55, 0.66, 17.95, 26.55 and 16.55. Recording the purity of syrup formulation, it was found that the microorganism like *Penicillium notatium*, *Rhizopus* sp, *Aspergillus flavas* and *Aspergillus niger* contaminated the

syrup (Table 1). Weight uniformity test for soluble tablets, water soluble granules and soluble globules are found satisfactory and falls within the acceptable limits. Regarding the friability, all the formulations were not in the acceptable limits. It indicates that the formulations may breakdown during transport. Crushing strengths of the formulations are also not in the acceptable limits. They bared the maximum of 1kg presser, but the acceptable limit is 3 – 4kg.Solubility of soluble tablets, water soluble granules and soluble globules are good but preformed tablets and soaps were not better. A layer of microbial growth was observed within 24h over the syrup. But after filtration, using kada cloth (double layered), there was no growth even up to one month. Our findings are partially in accordance with the findings of [8] who reported that, the papaya and bay leaves tablet was light brown and had flat top and bottom, a specific odor and a bitter taste. The physical properties of the tablet were in accordance with pharmaceutical standards. The total flavonoid content in the papaya and bay leaves extract was 1.562% and 2.240%, respectively. Water content 4.16%, flow rate g/s 10.80, friability 26.00%, and compressibility 13.47% of the granules. The uniformity in the weight of the papaya and bay leaves tablets met the quality requirement deviation value tablets ranged from 5% to 10% for the tablets that weighed 300 mg and Diameter of the tablets was not >3 times their thickness and not smaller than 11/3 times their thickness, the hardness of tablets range between 4 and 8 kp. Average friability ranged between 0.8% and 1%. The disintegration time of a good tablet should be <15 minutes [1]. reported that, effervescent tablets were formulated using the senna extract and the results obtained were: 7.4 kg / cm2, 10%, 59.01 s, 0.74 %, 97.30 % and 5.4 for resistance to crushing, weight variation, disintegration time, friability test, content uniformity test and pH respectively.

Table 1: Quality and stability of the formulations

Test			Weight of Soluble tablet (mg)	Weight of Water soluble granule (mg)	Weight of soluble globule (mg)	Weight of Soluble tablet (Preformed) (mg)	Soap
Weight Uniformity	Average Weight		0.12	0.02	0.05	0.15	
	Per cent Deviation		Sample 1- 0.0 Sample 2- 8.3	Sample 1- 8.3 Sample 2-8.3	Sample 1-0.0 Sample 2-0.0	Sample 1- 0.0 Sample 2- 6.66	
Friability	Average Weight	Initial wt. (mg)	0.12	0.02	0.05	0.15	
		Final wt. (mg)	0.11	0.14	0.05	0.14	
	'F' value		8	600	100	7	
Hardness	Average Crushing strength		1	0.25	0.50	1	
Solubility	Average	Initial wt. (mg)	100	100	100	100	100
		Final wt. (mg)	100.55	100.66	117.95	126.55	116.55
	Solubility		0.55	0.66	17.95	26.55	116.55
Purity of syrup	Sample 1		Sample 2	Sample 3	Sample 4	Sample 5	
	Rhizopus sp. & Aspergillus flavas		*Aspergillus niger & A. flavas*	*Penicillium notatium,Rhizopus sp. & A.flavas*	*Penicillium notatium,Rhizopus sp. & A.flavas*	*Penicillium notatium,Rhizopus sp., A.flavas & A. niger*	

Studies on the laboratory efficacy of formulations

While assessing the data related to insecticidal value of the extracts, against *S. litura* leaf powder (solvent) and fresh leaf (Hot water) were found to cause above 90% mortality of exposed larvae at higher (10%) concentration. Regarding insecticidal action our results are partially in accordance with the findings of [3] recorded cent per cent mortality of the larvae at 10 and 100 ppm concentrations after 96 and 48 hrs of exposure respectively when poison food technique was followed. Our findings are partially in accordance with the findings of [6] who reported that the leaf extract (methanol as solvent) was effective against *P. xylostella* and our findings are in corroboration with the results of [7] who reported that the fresh leaf juice, decoction and alcoholic extract of *C. collinus* at different concentrations were possessing insecticidal activity. Leaf decoction (50%) was found to be most effective, where single application was able to kill all the insects within 12 hr and control the insects till harvesting especially rice caseworm (*Nymphula depunctalis*) a common paddy pest of Chhattisgarh region of India. Fresh leaf juice and alcoholic extract at 50% also prevented insect attack on rice crop but with some extent to adverse effect. In general the biocidal properties of *C. collinus* reported by many authors *viz.*, [14, 12, 4, 13] supports our findings. As well as 9% concentration of formulations like Soluble tablet, water soluble granule, soap, syrup, soluble tablet (Preformed) and soluble globules caused 77.66, 77.00, 85.66, 85.00,80.00 and 80.00% larval mortality over control. (Table 2). In general the insecticidal actions exhibited by formulations were slightly lower than the data recorded in the primary screening using fresh extracts. Insecticidal action was well established by soap, syrup, preformed tablets and soluble globules than the soluble tablets and water soluble granules. Based on the above analysis it can be concluded that *Cleistanthus collinus* (Roxb.) (Benth) is a potential insecticidal plant and the formulations brought out by using the extract was also effective. Tailoring of some hiccups in the formulation should be undertaken. Further field studies are needed to confirm the efficacy. Thus the future work will be on the quality improvement and field assessment.

Table 2: Comparative efficacy of various formulations and extracts insecticidal action against *S. litura*

Treatment no.	Per cent larval mortality*
Fresh leaf –Hot water extract	99.5 (86.71)^{g}
Leaf powder – solvent extract	95.4 (77.59)^{f}
Soluble tablet	77.43 (61.65)^{ab}
Water soluble granule	77.1(61.38)^{a}
Soap	85.23 (67.39)^{a}
Syrup	80.1 (63.48)^{bc}
Soluble tablet (Preformed)	80.1 (63.50)^{bcd}
Soluble globules	80.00 (62.99)^{a}
control	-

CD = 2.022

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