Formulation & Evaluation of Anthelmintic Potential of Clitoria Ternatea Flower Against Pheretima Postuma

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Abstract: Objective: This article deals with the antihelminthic activity of Clitoria ternatea flower syrup on Pheretima posthuma, which is a model widely accepted system to test anthelmintic activity. The traditional medicinal use of Clitoria ternatea was processed as a syrup through standardized extraction procedures. Its effectiveness was determined by conducting in vitro assays by analyzing paralysis and death rates of Pheretima posthuma over different time intervals.

Results showed a dose-dependent effect with the high syrup concentration posing a potential for causing severe paralysis that could kill the worms. The findings of this current study suggest that anthelmintic activity is apparently marked in Clitoria ternatea flower syrup, justifying the traditional use in herbal medicine. This study opens up a potential pathway in the development of natural anthelmintic therapy and hence, the need for further research into active compounds and mechanisms of action involved.

Keyword: Anthelmintic, Clitoria Ternatea, Earthworm, Syrup

INTRODUCTION

Helminth infections are a significant global health concern, affecting millions of people worldwide, particularly in tropical regions. These infections can lead to anemia, imbalance and pneumonia. Helminthiasis, caused by worm infections, primarily affects the digestive system, liver, and other organs[4]. The gastrointestinal helminths have a potential resistance to currently available anthelmintic drugs. Therefore, there is a leading problem in treatment of helminths infections. This causes a lot of resistance due to chemical use for management. It led to screening medicinal plants for their anthelmintic activity[5].

Helminthic diseases are prevalent in tropical and subtropical regions, where poor sanitation, inadequate hygiene, and contaminated food and water facilitate transmission. According to the World Health Organization (WHO), over 1 billion people globally suffer from helminthic infections, resulting in substantial morbidity, mortality, and economic burden. In general, Anthelmintic activity of a drug is their ability to expel parasitic worms from the body. The active constituents and secretions present in the plant are potent against nematodes.

Oral route of drug administration is the most important to obtain systemic effects. Except for a few cases, the parenteral route is not used routinely for self-administration of medicine. So, oral suspensions, solutions, syrups etc are used for producing special effects of the medicinal agents[10].

Syrup are concentrated aqueous preparation of 85% of sugar & sugar substitute with or without flavouring agents & medicinal substance.

Due to the inability of some children & elderly people to swallon solid dosage forms. It is fairly common today for a pharmacist to be asked to prepare an oral liquid dosage form of a medication available in the pharmacy[3].

Clitoria ternatea Linn (Fabaceae), commonly known as Aparajitha in India, is a creeping herbaceous perennial legume. It is Native to south-east Asia and widely distributed throughout the world, mainly in tropical countries. The roots, Seeds, and leaves of this plant have medicinal Importance. The plant has a reputation for its folkloric uses in Various diseases. The roots are bitter, ophthalmic, Laxative, intellect promoting, diuretic, depurative, Aphrodisiac and is used as a tonic. It is used in ophthalmology, helminthiasis, leprosy, leucoderma, elephantiasis, bronchitis, asthma, ascites, ulcers and fever. The seeds are cathartic and are useful in visceralgia. Leaves are

useful in otalgia, hepatopathy and eruptions. The plant has been scientifically studied for various pharmacological activities including antioxidant, anthelmintic, analgesic, anxiolytic, antidepressant, anticonvulsant, sedative, hypoglycemic, and anticancer activities[2].

Since ancient times, "Shankhpushpi" has been regarded as a reputable Ayurvedic medication and used as a laxative, nervine tonic, and brain tonic. It is referred to in Ayurvedic writings as a "Medhya-Rasayana." Clitoria ternatea (Papilionaceae), Evolvulus alsinoides (Convolvulaceae), Conscora decusata (Gentianaceae), and Convolvulus pluricaulis (Convolvulaceae) make up the complete herb. This Ayurvedic medication works on the central nervous system (CNS), particularly to increase memory and enhance intellect. In India, the blossoms of the Clitoria plant are used to heal scorpion stings and snakebite[1].
Phytochemical survey reveals the existence of polyphenols, tannins, flavonoids, alkaloids, saponins, and steroids in the flower of Clitoria ternatea. The current study was thus undertaken to assess the in vitro anthelmintic activity of crude extract of Clitoria ternatea flower (70% alcoholic extract) and its Difference fractions against Pheretima posthuma.

MATERIAL & METHOD

Chemical & reagent
Clitoria ternatea, Sucrose, orange syrup, methyl parabean

Plant collection:
Clitoria ternatea flowers were collected from the local market of Parbhani, Maharashtra, India.

Plant extraction:
The process of extracting the flowers of Clitoria ternatea in a Soxhlet apparatus is done using a ratio of 1:10 of plant material and solvent. More precisely, 50 grams of dried flowers are utilized with 500 mL of methanol (95%) at 78°C for a total duration of 6-8 hours. For this, the dried flowers are first ground into a fine powder, after which they are allowed to place inside the Soxhlet thimble. The bioactive compounds are then efficiently extracted through circulation of the solvent in the device. Then, through rotary evaporation, the solvent is removed after extraction process and, after drying, stored in airtight containers.

Formulation study :
Clitoria ternatea flower was used to prepare the simple syrup IP, i.e., 66.67% (w/v) by following the procedure given in IP. 20 ml of this simple syrup was prepared. 10-12 ml distilled water was taken in a beaker and 13.2g sugar was added in it. The mixture was heated to dissolve the sugar completely by continuous stirring. This resulted in a super saturated solution of sugar in water. After the sugar had dissolved completely, the extracts were poured into the solution. The extracts were put into the hot state of the sugar solution and dissolved completely by proper stirring. After the complete dissolution of the extracts, the volume of the syrup was topped up to 20ml by adding the requisite amount of distilled water. Lastly, the syrup was made[12].

TABLE 1: FORMULATION TABLE

INGREDIENT	Formulation 1 (50 mg/ml)	Formulation 2 (100 mg/ml)
Clitoria ternatea extract	1 mg	2 mg
Sucrose	13.2g	13.2g
Methyl parabean	16g	16g
Orange syrup	2 drop	2 drop
Distilled water	q.s	q.s

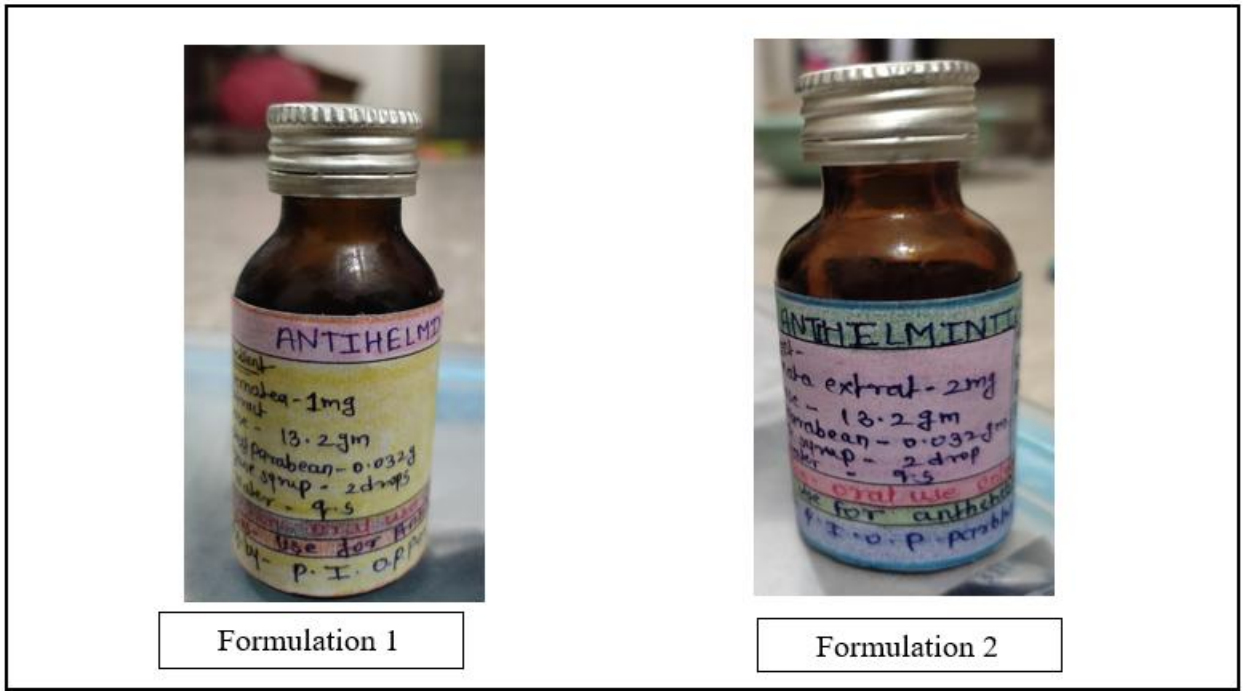

Fig 1: Formulation Batch

QUALITATIVE ANALYSIS:

Physical characteristics of extract:

i. Colour: Deep blue-purple to purple amorphous powder
ii. Odour: Characteristic, slightly sweet and earthy
iii. Texture: Fine to moderate powder
iv. Appearance: Dry, free-flowing
v. pH (10% solution): 5.5-6.5
vi. Clarity (10% solution): Clear to slightly opaque
vii. Solubility: Soluble in methanol, ethanol, and water
viii. Stability: Stable at room temperature (25°C ± 2°C)

Physical charateristics of syrup

(i) Organoleptic Properties: The taste, color and odor of the syrup were examined directly after preparation[12].

(ii) pH: The pH of the syrup was checked by using the pH meter[12].

(iii) Estimation of Crystal Growth: The crystal growth was measured after 24 hr[12].

(iv) Consistency or test of clarity: The developed Polyherbal Anthelmintic Syrup was kept at room temperature for three weeks. Then, the formulation was prepared and tested for clarity. Clarity testing was done by Light box illumination of containers against a black and white background. It was found out that the solution was clear, and it did not contain any solid particles or Fibre[13].

EXPERIMENTAL DESIGN

Test organism for anthelmic activity

Indian adult earthworms (Pheretima posthuma) were collected from a local farmer who practices organic vermicomposting. Earthworm was selected as model for Anthelmintic activity due to its Anatomical and Physiological similarity with the intestinal roundworms parasites of human beings. The Earthworms were maintained under normal vermin-composting medium and there was an adequate supply of nutrients and water, for about two weeks. Before the start of experiment, the earthworms were washed with distilled water. Adult earthworms of approximately 4 cm. In terms of dimensions, they were 10 cm in length and 0.2-0.3 cm wide for the experiment[11].

Procedure:

The anthelmintic activity was evaluated at the concentrations of 50 and 100 mg/mL against Indian earthworms P. posthuma four groups of Indian

earthworms, each with six earthworms approximately of equal size were used for the experiment. Two groups of earthworms were tested with the extract of different concentrations (50 and 100 mg/mL) and one group was treated with 20 mg/mL with reference standard as albendazole and one group was used as control which is treated with normal saline. The anthelmintic activity on earthworm was observed and time required for paralysis and death recorded[14].

EVALUATION STUDY

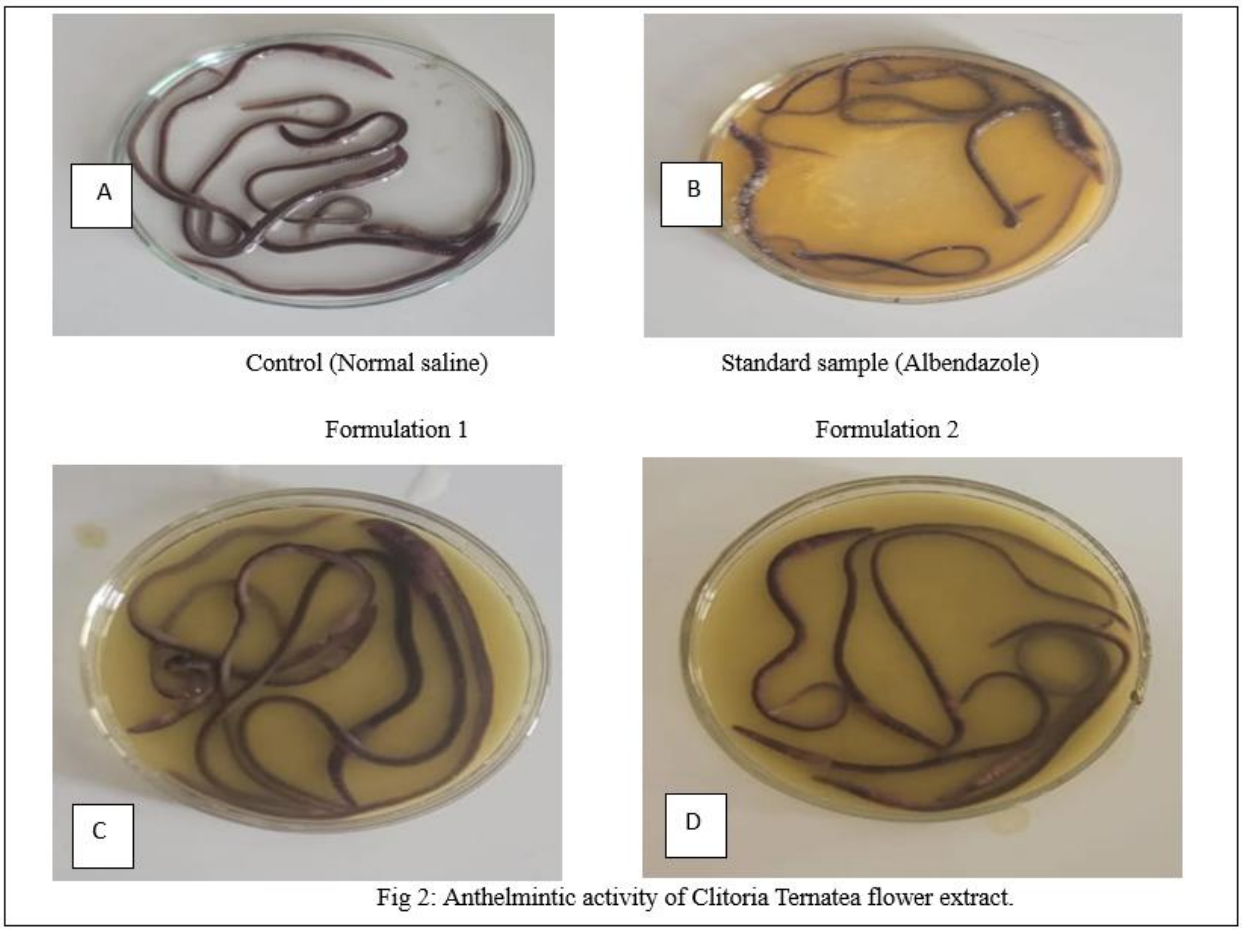

Fig 2: Anthelmintic activity of Clitoria Ternatea flower extract.

RESULT & DISCUSSION

Preliminary phytochemical screening of the extract revealed the presence of alkaloids, phenols, tannins, flavonoids, carotenoids, terpenoids, and steroids. The extract induced a dose-dependent paralysis in earthworms, ranging from loss of motility to unresponsiveness to external stimuli, ultimately leading to their death. presents the anthelmintic activity of clitoria ternatea flower syrup at different concentrations, compared with the reference standard albendazole. At concentrations of 50 and 100 mg/mL, Clitoria ternatea flower syrup produced paralysis times of 35 & 28 min, and death time of 46 & 36 min, respectively.

TABLE 2: Anthelmintic activity of Clitoria Ternatea flower extract.

Test substance	Paralysis time (in min)	Death time (in min)
Normal saline	-	-

Albendazole (20 mg/ml)	26	34
Formulation 1 (50 mg/ml)	35	46
Formulation 2 (100 mg/ml)	28	36

The formulated herbal syrup (100mg/ml) containing clitoria ternatea flower extract exhibited the paralytic effects over the earthworms within 28 min 50 sec & death after 36 min, while the marketed suspension containing albendazole caused paralysis within 26min 36 sec and death after 34 min and 36 sec. These results are compiled.

Conversely, the highest concentration showed very rapid paralysis and death which were not too dissimilar to the reference standard albendazole. In comparison, albendazole treated at 20 mg/mL had a paralysis time of 26 minutes and death time of 34 minutes. The worms in the control group (normal saline) were examined for 24 hours and no signs of paralysis or death were reported.

TABLE 3: Result

Sr.no	Parameter	Observation
1	Appearance	Viscous, syrupy liquid
2	Colour	Slight orange
3	Smell	Moderate
4	Flavour	Sweet, Slightly bitter
5	Clarity test	Clear solution, Absence of solid particle
6	Crystal growth	None
7	PH	5.5 to 7.0
8	Density	1.231g/cm^3

DISCUSSION

When tested against adult Indian earthworms (P. posthuma), Clitoria ternatea's anthelmintic activity showed a notable, dose-dependent effect. As a reference standard, albendazole mostly paralyzed the worms in a flaccid manner, which caused them to be expelled via peristalsis. Albendazole disrupts the worms' microtubule-dependent glucose absorption by attaching itself to free β-tubulin and preventing its polymerization. Being 300–400 times more effective against helminths than against mammalian tissues, it shows a specific inhibitory effect on helminth microtubular function[15]. Several secondary metabolites found in Clitoria ternatea extract support its anthelmintic properties.

Alkaloids, phenols, tannins, flavonoids, carotenoids, terpenoids, and steroids were found in Clitoria ternatea based on preliminary phytochemical screening. Notably, anthelmintic effects have been reported for tannins, which are polyphenolic substances[16]. By disabling oxidative phosphorylation, some synthetic phenolic anthelmintics, including niclosamide, oxyclozanide, and bithionol, are known to impair helminth parasites ability to produce energy[17]. The tannins in the clitoria ternatea extract might have comparable effects by preventing helminths from producing energy. Additionally, by attaching to free proteins in the host animal's digestive tract and perhaps killing the parasites, tannins may support anthelmintic activity. Alkaloids are known to affect the central nervous system and cause paralysis[18-19].

Thus, the presence of phytochemicals like tannins and alkaloids may be the cause of Clitoria ternatea's anthelmintic activity[20]. Clitoria ternatea's historic use for its anthelmintic qualities is supported by the results of this bioassay. To identify and describe the precise bioactive ingredients and clarify their modes of action, more investigation is required.

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