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Research Article

Exploring the Anthelmintic Activity of Polyherbal Chocolates Formulated with *Dolichos Biflorus* and *Lepidium Sativum* Seed Extracts: An In-Vitro Evaluation

Gautam Gundecha*, Puja Bhangare, Om Mane, Vaishnavi Tomar, Aditya Shimple, Gourav Rokade

Department of Pharmacology, All India Shri Shivaji Memorial Society's, College of Pharmacy, Kennedy Road, Near RTO, Pune- 411001, Maharashtra, India.

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ABSTRACT

Helminthes infections are among the most common infections in human beings in which intestinal parasitic worms cause infection and physiological damage. Specially in children, intestinal parasite infections frequently result in morbidity and death. In the present study, we have investigated the anthelmintic activity of the Lepidium sativum and Dolichos biflorus, medicinal plant used for treatment of number of ailments. In addition, emphasis is given on formulation of polyherbal chocholate to increase palatability and compliance. Anthelmintic activity was evaluated on mature Pheretima Posthuma (Earthworms) by adult motility assay. Anthelmintic activity was determined by keeping earthworms with different concentration of plant extract and then paralysis and death time was recorded. Albendazole was used as a standard. Results shows that the Dolichos biflorus seed extract at 15 gm % concentration shows greater i.e. 1.6 ± 0.18 min paralysis time and 23.2 ± 0.18 min death time as compare to Lepidium sativum extract. The combinations of Dolichos biflorus and Lepidium sativum were also done, however the results of combination were not found to be synergistic. The polyherbal chocolate at 15 gm % concentration shows 4.3 ± 0.26 min paralysis time and 30.1 ± 0.18 min death time. These findings indicate the anthelmintic potential of a polyherbal chocolate formulation containing Lepidium sativum and Dolichos biflorus against a single species of helminth (Pheretima Posthuma), indicating its promise as a novel, palatable and natural alternative to conventional remedies. By improving drug stability and compliance, chocolate-based formulations could significantly contribute to more effective management of parasitic worm infections.

***Corresponding Author:** Gautam Gundecha
Address: *Department of Pharmacology, All India Shri Shivaji Memorial Society's, College of Pharmacy, Kennedy Road, Near RTO, Pune- 411001, Maharashtra, India.*
Email ✉: gautam_gundecha@aissmscop.com
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INTRODUCTION

Helminthiasis has been a serious health burden globally from ancient times, posing a variety of health risk not only in humans but also in animals. Helminths are also called as parasitic worms which are responsible for causing parasitic infections.[1] Particularly in children, intestinal parasite infections frequently result in morbidity and death. Helminthiasis is caused due to various risk factors which includes poor sanitation, rural areas, malnutrition, lack of knowledge, poor hygienic practices, malnutrition, poor availability of clean water, lack of healthcare service access and overcrowded population. [2, 3] One of the most prevalent illnesses in the globe is intestinal worms, also known as soil-transmitted helminths, or STHs. According to WHO estimates, 241 million children between the ages of 1 and 14 are estimated to be at risk of acquiring STH, making it a serious public health concern for India. This corresponds to over 68% of all children in this age group worldwide and roughly 28 percent of all children thought to be at risk of acquiring STH infections.[4] Frequent complications arising from helminth infections include anaemia, diarrhoea, malnutrition, hindered mental and physical growth, decreased appetite, weight loss, and lower participation in school activities.[5] The emergence of resistance to anthelmintic medications, coupled with their limited availability and high costs, particularly affects low-income farmers in developing nations, highlighting the necessity for non-expensive alternative strategies to control helminths. Anthelmintic medicinal plants offer an opportunity to improve conventional medications and aid in discovering bioactive components for new pharmaceutical advances. Because of their sustainability and environmental friendliness, as well as the growing problem of anthelmintic resistance worldwide, research on plant-derived anthelmintics is becoming increasingly vital. [5, 6]

In this study, we have selected *Dolichos biflorus* and *Lepidium sativum* as they are widely used in traditional medicine for treatment of number of ailments, In the present study, we examined anthelmintic activity of the aqueous extract of the *Dolichos biflorus* and *Lepidium sativum*. In addition, emphasis is also given on formulation of polyherbal chocholate to increase palatability and enhance compliance.

MATERIALS AND METHODS:

Plant materials:

I. Dolichos biflorus:

Fig 1: *Dolichos Biflorus* Seeds

Organism Name: *Dolichos Biflorus*

Genus: Dolichos

Species: Biflorus

Family: Fabaceae (also known as Leguminosae)

Kingdom: Plantae

Common name: Horse gram, Catjang

It is widespread in Asia and Africa.

Macroscopic Characters of the seeds: The drug comprises of compressed, shiny, finely polished, reddish-brown, or brownish-brown seeds. These are shrubs and herbs that grow upright, occasionally with climbing stems. The leaves are

either pinnately split into three leaflets or have solitary blades. Sometimes, after flowering, the plants begin growing their leaves. The flowers are either purple, white, or sometimes yellow. A flattened legume pod is the fruit.[7] The seed of *D. biflorus* is used in Ayurveda to cure a variety of conditions, including piles, discomfort, constipation, wounds, urinary stones, cough, fluid retention, asthma, and more. Seed-based soup is also good for enlarged spleen and liver. The seeds of *D. biflorus* are known to exhibit antilithiatic, antihepatotoxic, and hypolipidemic effects, and they play a role in reducing blood sugar and total cholesterol levels. Two Ayurvedic formulations that contain *D. biflorus* have demonstrated antinephrotoxic properties and the ability to scavenge free radicals.[8]

II. Lepidium sativum

Fig 2: *Lepidium Sativum* Seeds

Organism Name: *Lepidium sativum*

Genus: Lepidium

Species: Sativum

Family: Brassicaceae/Cruciferae

Kingdom: Plantae

Common name: Cress, Common Cress, Garden cress It is native to Asia and Africa.[9] L. sativum is an annual edible plant that is straight, branching out, glabrous, and occasionally pilose. Its height ranges from 15 to 50 cm. Its cauline leaves are linear and sessile with a full edge, while its pinnatisect basal leaves are 5–10 cm long by 2.5–3.5 cm wide and stalked too subsessile. Racemes are ebracteate, have several branches, and have 20–40 flowers each. Fruit ranges in size from 5 to 6.4 mm and is appressed to the rachis on a suberect to ascending pedicel. Flowers are tiny, around 3 mm in size, and can be white or pink. It is emarginated, obovate or broadly elliptic, apically broadly winged, and has an apical notch that is between 0.2 and 0.8 mm deep. The brownish-red, ovate-oblong, three-lobed seeds are 3 mm in length and 1 mm in width.[10] Lung diseases including asthma, bronchitis, and cough, as well as liver problems, bleeding piles, and scorbutic diseases, all are treated with seeds. *L. sativum* is also well-known for a variety of pharmacological properties, including laxative, hypolipidemic, diuretic, gastro-protective, stomachic, gastrointestinal stimulant, anti-inflammatory, cardioprotective, anti-carcinogenic, and antioxidant properties. [10, 11]

Plant Extract Collection: *Dolichos biflorus* and *Lepidium sativum* seed extract was purchased and authenticated from Shamantak Enterprises, Bhor, Pune, Maharashtra.

Experimental model: Adult earthworm (Pheretima Posthuma) was selected due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human being and collected from moist soil, obtained from Gandul Khat Mahiti Vibhaag, Aundh, Pune. In order to remove dirt and prepare them for the anthelmintic investigation, earthworms were cleaned using regular saline (0.9% NaCl). Earthworms having 7(±1) cm length and 0.3-0.4 cm width were selected for study.[12]

Standard Drug

Albendazole is taken as standard drug and dissolved in a distilled water to obtain the concentration of 0.05, 0.075 and 0.1 gm %.

Test Drug

The aqueous extracts of *Dolichos Biflorus* and *Lepidium sativum* seed extract were diluted with distilled water separately to obtain the concentration of 5, 10 and 15 gm %. The combination of *Dolichos Biflorus* and *Lepidium sativum* seed extract were prepared by taking equal amount of both of them to make a concentration of 5, 10 and 15 gm %.

Formulation of Polyherbal chocolate:

Table 1: Formulation table of Polyherbal chocolate

Sr. No.	Name of Ingredients	Intended use	Quantity (gm) or (ml)
1	*Dolichos biflorus* seed extract	Anthelmintic agent	3.5 gm
2	*Lepidium sativum* seed extract	Anthelmintic agent	0.5 gm
3	Vegetable oil	Flavour and anti-oxidant activity	q.s.
4	Chocolate base	Principle ingredient or base	9 gm
5	Honey	Sweetening agent	q.s.

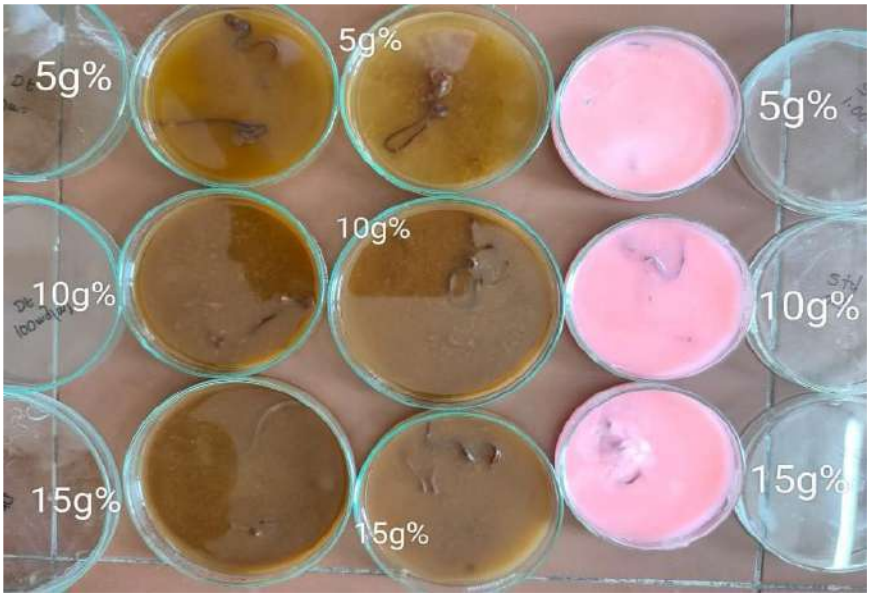
The water in the bath was intentionally heated up to about 50°C in temperature. After that, the chocolate base was cooked until it became liquid in a porcelain dish. Subsequently mix the melted chocolate base with the required quantity of honey. After that add the proper quantity of medicine extract, namely extracts of *Dolichos biflorous* (3.5 gm), *Lepidium sativum* (0.5 gm), vegetable oil and vanilla essence for flavour to the chocholate base mixture and continually stirred to ensure proper mixing. The formed mixture is then poured into a silicon chocolate mould and then kept in a refrigerator for 5-6 hr to solidify. Store the chocolate in an airtight container in a dry, cool location after it has hardened.

Fig 3: Herbal Chocolate Preparation Using Molds

Anthelmintic Activity: An in-vitro assay was performed to evaluate the anthelmintic efficacy of both *Dolichos biflorus* and *Lepidium sativum* seed extract using the earthworm *Pheretima Posthuma* as an experimental model using Adult Motility Assay (AMA). AMA was conducted on mature *Pheretima Posthuma* worms following the technique of Sharma et al. [13] Six groups of approximately equal size earthworms consisting of nine earthworms in each group were used for the

present study. Group I serves as control, receive only normal saline; Group II serve as Test-1, receive three different concentration of aqueous extract of *Dolichos Biflorus* seed extract (5, 10 and 15 gm %); Group III serve as Test-2, receive three different concentration of aqueous extract of *Lepidium sativum* seed extract (5, 10 and 15 gm %); Group IV: Test Drug Combinations, receive three different mixtures of equal quantity of both *Dolichos biflorus* and *Lepidium sativum* seed extract (5, 10 and 15 gm %), Group V serves as standard group, receive three different concentration of albendazole (0.05, 0.075 and 0.1 gm %); and Group VI: Polyerbal chocholate, receive three different concentrations of polyherbal chocolate (5, 10 and 15 gm %). Inhibitions of worm motility were utilized to detect paralysis or worm mortality. The amount of time it took for each worm to become paralyzed and death was measured. When the worms do not resuscitate even in regular saline, it was claimed that paralysis has taken place. When the worms lost their mortality and their body color faded, death was confirmed. [12, 06]

Fig 4: Earthworms



Dolichos biflorus ***Lepidium sativum*** **Albendazole**

Fig 5: Different concentrations of *Dolichos biflorus* extract, *Lepidium sativum* extract and Albendazole

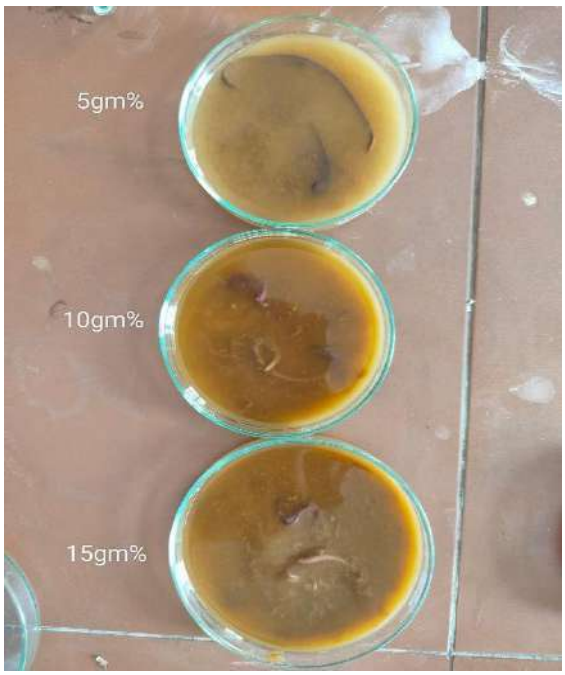


Fig 6: Different concentrations of Combinations of *Dolichos biflorus* extract and *Lepidium sativum* extract

Fig 7: Polyherbal Chocolate

Evaluation and Characterization of polyherbal chocholate:

1. **Organoleptic evaluation:** The colour, odour, taste and appearance of chocholate was evaluated by using sense organs.

2. **Dimensions:** A Vernier calliper was used to measure the chocolate's dimensions.

3. **Moisture content determination:** This test helped to measure moisture content in chocholate. After carefully weighing the chocolate, it was put in a desiccator with anhydrous silica gel in order to absorb any kind of moisture. After 24 hours, the samples were taken out and weighed again. The amount of moisture they had absorbed was calculated using the following formula:

% Moisture = (Initial Weight − Final Weight) / Final Weight × 100

This formula shows how much weight was lost due to moisture removal, giving a clear idea of how much water was originally present in the chocolate.

4. **Viscosity determination of chocolate:** To measure the viscosity of the prepared chocolate, a Brookfield viscometer is used. Before taking any readings, the chocolate samples are gently heated to 50°C. During the test, the spindle is set to rotate at a constant speed of 20 rpm. This helps determine how thick or fluid the chocolate is.

5. **Weight Variation:** To check consistency, five chocolates are weighed individually and together. The average weight of the samples is then calculated and compared against each

individual sample to see how much each one deviates from the average. The percentage variation is calculated using this formula:

$$\%\ \text{Deviation} = \frac{\text{Individual Weight} - \text{Average Weight}}{\text{Average Weight}} \times 100$$

This percentage should stay within acceptable limits to ensure quality and consistency.[14]

6. **Bloom Test (Sugar Bloom Test):** This is rough and irregular layer on top of chocolate formulation. When chocolate is removed from the refrigerator, condensation occurs, which is the reason of this. The chocolate's sugar will be dissolved by this moisture. Sugar recrystallizes form rough, asymmetrical crystals on the surface after the water evaporates. This creates an undesirable appearance. Treatment cycles for each sample included: (1) 11 hours at 30°C, (2) 1 hour of temperature shifting, (3) 11 hours at 18°C, and (4) 1 hour of temperature shifting. Following step 3 at 18°C for 11 hours, a chocolate formulation was examined to see whether blooming had occurred.

7. **Stability**: Generally, a medicine is still considered acceptable as long as it keeps at least 90% of the strength stated on its label. Drug degradation can occur in a number of ways as a result of modifications to its physical, chemical, and microbiological characteristics. The changes may increase the toxicity of the medicine or decrease its therapeutic efficacy.[15]

8. **Ash content determination:**

Total Ash:

After weighing three grams of the chocolate, it was heated up in a China dish at a temperature of not more than 450°C until the carbon was gone. It was then cooled and weighed once again until the weight was the same for three readings. The air-dried chocolate was used to calculate the percentage of ash.

$$\%\ \text{Total ash} = \frac{\text{Weight of ash}}{\text{Weight of chocolate}} \times 100$$

Acid Insoluble Ash:

The entire amount of ash was produced after boiling it for five minutes with 25 milliliters of diluted hydrochloric acid. Subsequently, the insoluble material was gathered in a Gooch crucible, cleaned with hot water, and burned until its weight stabilized. The proportion of acid-insoluble ash was calculated in relation to the air-dried chocolate.

$$\%\ \text{Acid insoluble ash} = \frac{\text{Acid insoluble ash weight}}{\text{Weight of chocolate}} \times 100$$

9. **pH determination-**

pH of polyherbal chocholate was determined by digital pH meter.[16]

Statistical analysis:

The data was statistically analyzed using Graph Pad Prism 8.4.3. All parameters were analyzed by Two-way analysis of variance (ANOVA), which was followed by the Dunnett's multiple comparison test. The data are reported as the mean ± SD. It was also considered statistically significant at $P<0.05$.

RESULT AND DISCUSSION:

Result of evaluation parameters of Polyherbal chocolate:

Table 2: Evaluation parameters of Polyherbal chocolate

Sr. No.	Parameter	Result
1.	**Organoleptic character:**	
	1. Colour	Brown
	2. Odour	Pleasant with no burnt smell
	3. Taste	Sweet
	4. Appearance	Smooth and even
2.	Dimensions	Height- 2.40 ± 0.7 Diameter-1.85 ± 0.4
3.	Moisture content	1.88 %
4.	Viscosity	0.55 kg/ms
5.	Average weight	4.15 g
6.	% Total ash	15 %
7.	% Acid insoluble ash	12.5 %
8.	pH	6.24

Sugar Bloom Test:

A rough and irregular layer on top of the chocolate formulation characterizes sugar bloom. When chocolate is removed from the refrigerator, condensation causes this to happen. The chocolate's sugar is dissolved by the moisture, and when the water evaporates, the sugar recrystallizes into uneven, rough crystals on the surface, giving the chocolate a hideous appearance. The chocholate with sugar bloom are presented in the Figure 8.

Fig 8: Sugar bloom

Stability Testing-

The final formulation was stored at room temperature for 24 hours in a foil container lined with shiny butter paper on the outside to check its stability.

Fig 9: Stability after 24 hrs.

Anthelmintic assay:

The anthelmintic efficacy of *Dolichos biflorus*, *Lepidium sativum*, their mixture and polyherbal chocolate was evaluated using *Pheretima posthuma* earthworms. Effect of those extracts and formulated chocholate was checked by determining the paralysis and death time of earthworm after keeping with respective treatment regimen. Table 3 shows the effect of treatment on paralysis time and death time. Fig 10 and Fig 11 shows the effect of treatments on paralysis time and death time, respectively.

Table 3: Effect Of Treatments on Paralysis Time and Death Time

Group	Name of Group/Treatment	Concentration (gm %)	Paralysis time (min)	Death time (min)
1	Control Group (Normal saline)	-	-	-

2	Test Group-1 (*Dolichos biflorus*)	5 10 15	13.2 ± 0.63 5.55 ± 0.17 1.6 ± 0.18	27.5 ± 0.35 26.2 ± 0.27 23.2 ± 0.18
3	Test Group-2 (*Lepidium sativum*)	5 10 15	31.55 ± 0.4 14.2 ± 0.18 10.2 ± 0.3	42.45 ± 0.22 28.35 ± 0.43 24.5 ± 0.35
4	Test drug combination (*Dolichos biflorus* and *Lepidium sativum*)	5 10 15	22.2 ± 0.18 12.6 ± 0.44 6 ± 0.52	28.3 ± 0.22 22.2 ± 0.22 14.45 ± 0.18
5	Standard Group (Albendazole)	0.05 0.075 0.1	37.1 ± 0.08 30.15 ± 0.21 17.4 ± 0.13	48.14 ± 0.15 44.3 ± 0.35 41.2 ± 0.13
6	Polyherbal chocolate	5 10 15	17.5 ± 0.39 10.15 ± 0.1 4.3 ± 0.26	50.75 ± 0.21 38.45 ± 0.36 30.1 ± 0.18

Results are expressed as mean ± SD, N=3, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ as compared to standard. Statistically analysed by Two-way ANOVA followed by Dunnett's Multiple Comparisons Test

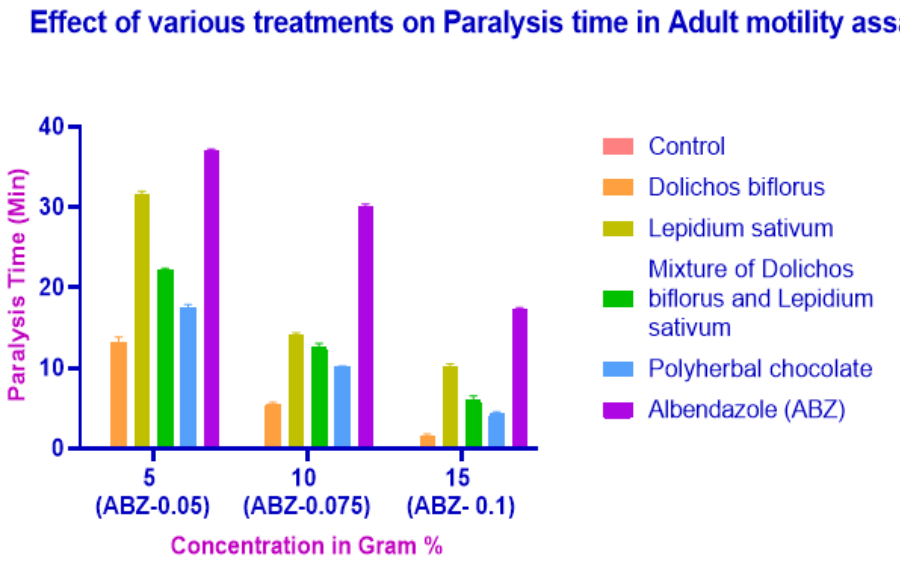


Fig 10: Effect Of Various Treatments on Paralysis Time in Adult Motility Assay

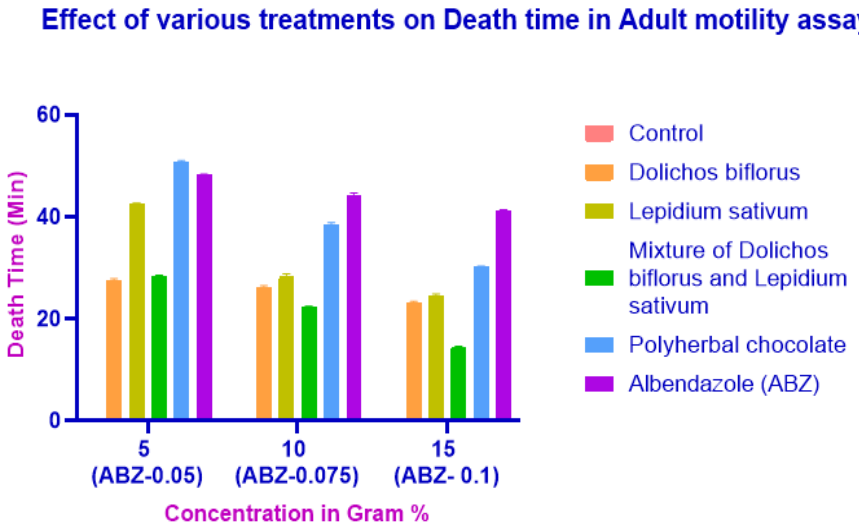


Fig 11: Effect Of Various Treatments on Death Time in Adult Motility Assay

For Control (Normal saline) group no effect noted (—), as predicted. This group serves as a negative control, establishing that paralysis and death are a result of the drugs. The *Dolichos biflorus* seed extract at 15 gm % concentration shows greater i.e. 1.6 ± 0.18 min paralysis time and 23.2 ± 0.18 min death time. The *Lepidium sativum* seed extract at 15 gm % concentration shows 10.2 ± 0.3 min paralysis time and 24.5 ± 0.35 min death time. Therefore, *Dolichos biflorus* was found to be more effective anthelmintic agent. For Test group 1 (*Dolichos biflorus*), Test group 2 (*Lepidium sativum*) the dose dependent effect was reported, with the increase in concentration the paralysis time and death time is reduced. However, as compare to *Lepidium sativum* the *Dolichos biflorus* was found to be more effective anthelmintic agent. Test drug combinations of *Dolichos biflorus* and *Lepidium sativum* shows 6 ± 0.52 min paralysis time and 14.45 ± 0.18 min death time, however the results of combination are not found to be synergistic. The standard drug albendazole shows maximum efficacy at concentration of 0.1 gm % viz. 17.4 ± 0.13 min paralysis time and 41.2 ± 0.13 min death time. Hence, less effective as compare *Dolichos biflorus* and *Lepidium sativum.* The polyherbal chocolate at 15 gm % concentration shows 4.3 ± 0.26 min paralysis time and 30.1 ± 0.18 min death time. Therefore, the polyherbal chocolate shows promising results as anthelmintic agent in comparison to albendazole.

CONCLUSION:

This study demonstrates that *Dolichos biflorus* and *Lepidium sativum* possess significant anthelmintic properties. Among the two, the *Dolichos biflorus* seed extract demonstrated stronger and more potent anthelmintic activity compared to the *Lepidium sativum* seed extract. The combination of *Dolichos biflorus* and *Lepidium sativum* was also done to check for synergistic activity but the results found were not satisfactory. The extracts effectively paralyze and kills earthworms indicating anthelmintic potential, while also shows maximum efficacy in comparision to albendazole. The anthelmintic activity of polyherbal chocolate was found to be efficacious in comparision to albendazole.The current research successfully the anthelmintic potential of a polyherbal chocolate formulation containing *Lepidium sativum* and *Dolichos biflorus* seed extract against a single species of helminth (*Pheretima Posthuma*), indicating its promise as a novel, palatable and natural alternative to conventional anthelmintic

agents. However, to establish its broader efficacy and therapeutic relevance, future research should focus on evaluating its activity against a wider spectrum of helminth species, including both intestinal and tissue-dwelling parasites. Additionally, in vivo studies are essential to assess pharmacodynamics, pharmacokinetics, and safety profiles in biological systems. Additionally, progressing to clinical trials will further help to validate its efficacy and tolerability in human populations. These investigations will be essential for standardizing the formulation, ensuring dosage accuracy and possibly opening the door for its commercialization as a new, patient-friendly anthelmintic product.

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