Evaluation of Anthelmintic activity of *Quillaja Saponaria bark* of Saponins on Indian Earthworms

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Abstract:
Quillaja saponaria /fam: *rosaceae*) known as soap bark tree is a plant native to central Chile. Prior to anti-helminthic activity, it also shows other biological activities such as anti-inflammatory, Anti-viral, immuno stimulant. The current study Examines *Quillaja Saponaria bark's* anthelmintic activity against Indian Earthworms (pheretima posthuma). which is renowned for having a high saponin content. The phytochemical constituents of *Quillaja bark* are triterpenoids saponins, polyphenols, tannins, ethanol was used to extract *Quillaja Saponaria bark.* Earthworms Paralysis and Death times at Different concentrations (150,100,200mg/ml) were utilized to evaluate the extract's anthelmintic effects. The current study's findings support the theory that *Quillaja saponaria's* anthelmintic activity could be caused by the presence of saponins.

Keywords: *Quillaja bark*, anthelmintic activity, albendazole induced in Indian earthworm

Introduction

Helminthiasis

"Helminths" is a Greek word that means "worms or caterpillar." Human-infecting parasites are classed as either inheritances or monuments. The parasites found in African ancestors are called Heirlooms, and those found in animals during our interactions with evolution, mi
gration, and Monuments are agricultural techniques. Helminthic infections are the most prevalent infectious diseases in underdeveloped nations. Approximately two billion people, or more than 25% of the world's population, are afflicted with the helminthic parasite, which is a severe burden on developing nations, particularly on children[1].
The two main groups of helminths are called platyhelminths and nematodes. Nematodes, which include filarial worms that cause onchocerciasis and lymphatic filariasis (LF), and ground-carrying helminths, are also referred to as roundworms.
Some Flatworms, also known as flukes, schistosomes, and worms like tapeworms that cause cysticercosis, are members of the phylum platyhelminths. The terms trematode and cestode refer to worms and tapeworms, respectively. Hookworms (Ancylostoma duodenale and Necator americanus), whipworms (Trichuris trichiura), and roundworms (Ascaris lumbricoides) are the vectors of helminthiasis[2].

MATERIAL AND METHODS

Collection of Drug

Our experiment used a commercial saponin mixture that was derived from *quillaja bark* (Sigma-Aldrich) and that has quillaic acid, a triterpene, as its primary sapogenin[3].

Chemicals and Reagents

Albendazole, *quillaja bark*, saline, water

Identification test for Saponins

Standard foam test: In a beaker, 300ml of hot distilled water was used to extract 3g of each dry plant powder after it had been weighed. The aqueous extracts were filtered, chilled, and then mixed before being kept for 24 hours at 4°C in an automatic refrigerator. Five milliliters of the plant extract were put into a test tube and mixed with five milliliters of distilled water. For two minutes, the mixture was forcefully shook. The presence of saponins was established by the formation of an emulsion upon the addition of olive oil or by the persistence of foam for at least fifteen minutes[4].

Wet foam test: After diluting the test solution with water and aggressively shaking it for one to two minutes, a stable, foamy lather formed on the top of the sample test tube[5].
Dry foam test: In a test tube, approximately 0.5 grams of the plant's crude powder were shaken with 5 milliliters of distilled water and heated in a water bath. The stable, persistent froth was then combined with three drops of olive oil and agitated rapidly. Emulsion development is a sign that saponins are present[6].
Foam test for fresh samples: An electrical mixer was used to mix about 2 grams of fresh plant material (leaves) with 20 milliliters of distilled water (w/w = 1:10). The mixture was then filtered, and the filtrate was concentrated by evaporating it in a water bath to half its initial volume before being put into a test tube. Three drops of olive oil were added to the stable, persistent froth, and it was agitated violently before the emulsion formed, indicating the presence of saponin[7]

Experiment
Experimental animal model
Because of their morphological and physiological similarities to human intestinal roundworm parasites, healthy adult Indian earthworms (Pheretima Posthuma) were employed in this investigation[8].

Worm Collections
Adult Indian earthworms (Pheretima posthuma) were gathered from the Anantapuram region's damp soil and thoroughly cleaned with water to get rid of any debris. Every earthworm that was used measured roughly 4–8 cm in length and 0.1–0.2 cm in breadth[9].

Experimental Design
In the study, seven groups of earthworms of almost comparable size were used, with five earthworms in each group.

Preparation of doses of drug
To prepare the dose of drug at concentrations of 5, 10, 20 mg/mL; 50, 100, 200 mg of sample powder were taken and dissolved in little amount of water. Final volume was made up to 10mL using water to respective concentrations[10].

Preparation of doses of Albendazole
50, 100, and 200 mg of powdered albendazole were taken and triturated with 0.2% v/v of Tween 80 as a suspending agent to create a standard dose of the drug at concentrations of 5, 10, and 20 s mg/mL. The final volume was made up to 10 mL using water for the corresponding concentrations[11].

The study used seven groups of roughly equal size earthworms, with 4-6 earthworms in each group.

1	Group 1	Served as (Receives normal saline)
2	Group 2	Received 5mg/mL of QS
3	Group 3	Received 10mg/mL of QS
4	Group 4	Received 20mh/mL of QS
5	Group 5	Received standard drug Albendazole 5mg/mL,
6	Group 6	Received standard drug Albendazole 10mg/mL
7	Group 7	Received standard drug Albendazole 20mg/mL

The amount of time it took for the worms to become paralyzed and to die (the time of mortality) was noted. When there was no movement at all until the worms were shaken briskly, a period of paralysis was recorded. When the worms lost their ability to move despite shaking violently and their body color faded, the time of death (or time of mortality) was determined. A Mean ± SEM of three worms per group was used to express all results.

Statistical Analysis:
Each group's mean± SEM is displayed, followed by a one-way ANNOVA. Results were deemed statistically significant if $p < 0.005$

RESULT:

Table 1: Identification test for Saponins

S.No	Tests	
1.	Typical foam test	+
2.	Test of wet foam	+
3.	New foam test sample	+
4.	Test of foam	+

Table 2: Effect of QS **and Albendazole on Pheretima posthuma.**

Group	Paralysis time(min)	Mortality time (min)
Control	-	-
Test-I (5mg/ml)	6.30± 0.00577***	12.4± 0.0252
Test-II (10mg/ml)	5.98±0.0418^{ns}	12.2± 0.0176 ^{ns}
Test-III (20mg/ml)	5.02±0.00882***	8.15± 0.0208***
Std-I (5mg/ml)	6.37±0.0882***	16.9± 0.406***
Std - II (10mg/ml)	6.37±0.186 ^{ns}	13.6± 0.208***
Std -III (20mg/ml)	5.53±0.0333***	10.2±0.0549***

Data generated represented as mean ±SEM (n=5) and significant changes were considered as When * (p value < 0.05), ** (p value < 0.01), *** (p value < 0.001)

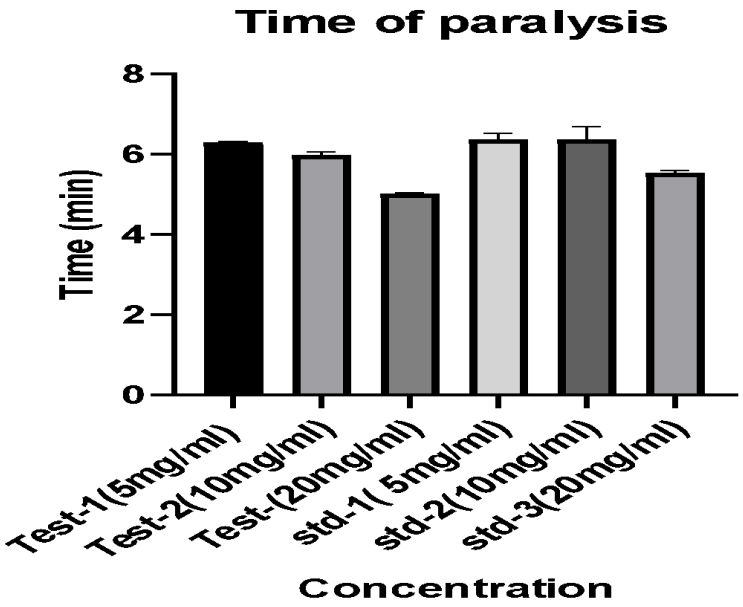

Figure 1: Time of Paralysis

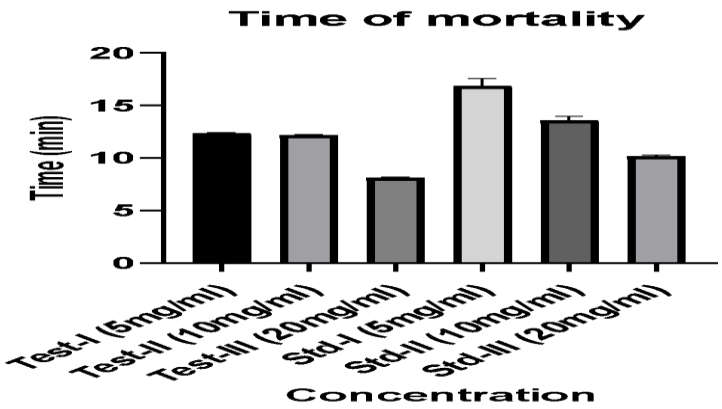

Figure 2: Time of Death

DISCUSSION

In the present work, the *Quillaja Saponaria* is evaluated to have Anthelmenthic activity against the adult Earthworms (Pheretima Posthuma) *Quillaja Saponaria* showed increase in the time of paralysis and time of mortality with dose dependent in comparison to standard drug (Albendazole).

CONCLUSION

Helminthiasis is one of the most frequent infections which is caused by the worms in GIT, Liver, and other organs. As currently available marketed drugs having many drawbacks with hepatotoxicity, loss of appetite, dizziness, headache, mental confusion, abdominal pain etc., So 80% of people on the planet rely on traditional medicinal plants and their extracts as they contain active constituents,secondary metabolites which meets the people health care needs with less adverse effects. The present study is on Quillaja saponaria bark of saponins are found to have low anthelminitic activity when compared to Albendazole.

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