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Investigation of Anthelmintic Activity on Seed Coat of *Caesalpinia bonduc* L. Roxb.

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ABSTRACT

Helminths are parasitic worms. Intestinal helminths and protozoan parasites are two of the most prevalent diseases affecting people in impoverished nations. Protozoan parasites cause gastrointestinal disorders more frequently than helminths do in developed countries. In countries where they are endemic, intestinal parasites considerably raise morbidity and mortality rates. Antihelmintic medications work systemically to eliminate adult helminths or developing forms that infect organs and tissues, but they can also operate locally to remove worms from the GIT. The current work aims to examine the anthelmintic activity of an ethanolic extract of Caesalpinia bonduc seed coat. **Objectives:** to perform a qualitative analysis to check for the presence of phytoconstituents in the crude extract. to look at Caesalpinia bonduc L. Roxb.'s seed coat test extract's anthelmintic properties. **Methodology:** On Indian adult earthworms (Eisenia foeitida), the seed coat of Caesalpinia bonduc extract was compared to the control group (Mebendazole). **Result:** The timing of the worms' paralysis and death was determined as part of the investigation. When compared to the conventional medicine Mebendazole 100mg/ml, the ethanolic extract at concentrations of 10, 20, and 50 mg/ml demonstrated extremely considerable efficacy. The observed times for paralysis and death were 4.3330.1453 and 6.2000.1764, respectively. **Conclusion:** According to the data, EESCB exhibited anthelmintic action at different concentrations (10 mg/ml, 20 mg/ml, and 50 mg/ml, respectively). When compared to the standard solution, the greatest anthelmintic activity was attained at (50 mg/ml) concentration.

INTRODUCTION:

Many of the wonderful types of natural materials that nature has to offer are among the innumerable gifts that have made human history possible, as they satisfy many of our most fundamental needs.1

India offers an extensive geographical area with strong potential for Ayurvedic, Siddha, Unani, and other traditional medicine, as well as a rich culture of medicinal herbs that contain more than 2000 species.2-3Since the dawn of time, plants have been used to treat a wide range of diseases and enhance human health and welfare. The foundation of traditional or indigenous health systems continues to be medicinal plants, notwithstanding the age of the practice.

The word "helminth" comes from the Greek word "helminths," which means "worm" A broad "categorical term" used to describe numerous kinds of parasitic worms that live inside the body is helminth. According to the World Health Organization (WHO), about two billion individuals have parasitic worm infections7. By 2025, it is predicted that 57% of people living in developing nations will be affected. Within an infected population, the prevalence of parasitic helminths often exhibits a negative binomial distribution, meaning that only a small number of people have significant parasite burdens. Those people are more prone to falling ill and spreading infection across their group if they are not treated. Drugs called anthelmintics can either act locally to remove adult worms from the gastrointestinal tract or systemically to do so.

The majority of currently available anthelmintics have adverse effects including abdominal pain, lack of appetite, nausea, vomiting, headaches, and diarrhoea11. Helminths or developing forms that penetrate organs and tissues. Natural anthelmintics may be crucial in the management of certain parasite infestations. 12. Growing issues with helminth resistance to anthelmintics have prompted the suggestion of testing medicinal herbs for their anthelmintic action. 13. Numerous biologically active chemical groups found in Caesalpinia bonduc. L. Roxb. include desired phytochemical elements including flavonoids, alkaloids, saponins, and others. These chemical groups have a variety of pharmacological actions, including anti-inflammatory, anti-diabetic, analgesic, and antipyretic activity.

The present study was conducted to examine the anthelmintic effect of an ethanolic extract of the seed coat of Caesalpinia bonduc L. Roxb. using adult earthworms (Eisenia foetida).

However, the anthelmintic activity of Ceasalpinia bonduc L. Roxb. seed coat has not yet been scientifically proven. The majority of currently available anthelmintics have adverse effects including abdominal pain, lack of appetite, nausea, vomiting, headaches, and diarrhoea11. Helminths or developing forms that penetrate organs and tissues. Natural anthelmintics may be crucial in the management of certain parasite infestations. 12.

The suggestion of testing medicinal herbs for their anthelmintic activity has resulted from growing issues with the development of resistance in helminths against anthelmintics 13. Bonduc Caesalpinia. Many biologically active chemical groups are found in L. roxb. include desired phytochemical elements such flavonoids, alkaloids, saponins, and others. These chemical groups have a variety of pharmacological actions, including anti-inflammatory, anti-diabetic, analgesic, and antipyretic activity.

REVIEW OF LITERATURE

- **Kingdom** : Plantae
- **Phylum** : Magnoliophyte
- **Division** : Magnoliopsida
- **Class** : Angiospermae
- **Order** : Fabales
- **Family** : Caesalpiaceae
- **Genus** : Caesalpinia
- **Species** : bondac
- **Part used** : Seed coat

Vernacular names:

- **English name** : Fever nut
- **Hindi Name** : Kantkarej,

- **Sanskrit Name** : Kakachika,
- **Kannada Name** : Gajjiga,
- **Telugu Name** : Mulluthige,
- **Tamil Name** : Kalarchi Kaai,
- **Malayalam Name** : Kalamchikuru

Fig no 01: Leaf of *Caesalpinia*. *bonducL. Roxb*

Fig no 02 : Bark of *Caesalpinia bonduc* L. Roxb.

Fig no 03: *bonduc* L. Roxb

Fig no 04 :Fruit of *Caesalpinia*

Fig no 05: Fruit with seed of *Caesalpin*

Fig no 06: Seed coat of *Caesalpinia*

PLANT DESCRIPTION:

Caesalpinia bonduc L.Roxb. is an extensive climber with a very thorny shrub, branches finely grey-downy, armed with hooked and straight hard yellow prickles. The stem usually grows as a vine but also flowers and fruits as a shrub. Occasional spines or numerous spines present on the stems. The blaze odor resembles that of fresh green beans (Phaseolus vulgar) is Pith white, quite large in diameter. Stipules are a pair of bipinnately reduced pinnae at the base of the leaf, each with a long mucronate point, and leaves have broad, leafy, branching, basal appendages. Leaflets are 2-3.8 by 1.3-2.2 and come in 6–9 pairs. cm tall, approximately 18-75 x 12-40 mm leaflet blades. leaflet stalks that are 1-2 mm in length. Pedicels are extremely short in buds, extending to 5 mm in flower and 8 mm in fruits, and there are brown downy bracts in the racemes of flowers that are dense (typically) long peduncled, terminal, and supra axillary, dense at the top and looser downward, 15-25 cm long. Rose is square, linear, acute,

and up to 1 cm long. The calyx is 6–8 mm long, fulvous, and hairy, and the lobes are obviated–oblong and acute. About 10 to 12 mm long, oblanceolate, yellow petals with declinate filaments that are flattened at the base are covered in long, white silky hairs. Fruit expanded into rigid-spined pods. Oblong, 5.0–7.5 by roughly 4.5 cm, heavily armed with wiry prickles, pods were short-stalked. 1–2 seeds per 14 pods. Seeds are 1-2, oblong, lead-colored, and 1.3 cm long. The seed coat is firm, glossy, and greenish to ash grey. Circular and vertical faint lines of the cracks produce uniform rectangular to squarish rectulations all over the surface. The kernel of a dried seed separates from the testa. Testa is roughly 1–11.25. mm in thickness and is composed of three distinct layers, the outermost – thin and brittle, the middle one – broad, fibrous and dark – brown and the innermost – white and papery and the seed is exalbuminous.

Chemical constituents

Several chemical constituents including Steroidal Saponin, Fatty Acids, Hydrocarbons, Phytosterols, Isoflavones, Aminoacids and Phenolic were present in the plant.

Medicinal uses:

Caesalpinia bonduc L. Roxb. A big, scandent, thorny shrub belonging to the family (Caesalpiniaceae) can be found in the interior of India, Sri Lanka, and the West Indies. It is widespread in the southern regions of India and frequently cultivated as a hedge plant16. This plant has profound medicinal use and is a proven anti-inflammatory. anti-helminthic and anti-malarial drugs. It has also been an effective stomachic, and digestive and is used as liver tonic in the treatment of jaundice and various liver disorders. used as a liver tonic to treat jaundice and other liver conditions. It is said to be an aphrodisiac and all-around tonic that aids in body renewal. As an anti-leprotic, roasted seed powder is utilized. The seeds have anti-diabetic, anti-pyretic, and other uses.

METHODOLOGY

Test for phytoconstituents12:

Following the common practices outlined in the literature, preliminary phytochemical screening was performed on the acquired Caesalpinia bonduc L. Roxb.

Preliminary phytochemical investigation:

The acquired extracts were put through a preliminary qualitative phytochemical examination and subsequent qualitative testing to see whether any chemical components were present.

Test for carbohydrates

a) Each extract was individually diluted in 5 cc of distilled water and then filtered. The presence of carbohydrates was examined in the filtrates.

b) Molisch's Test

A test tube containing filtrates was treated with 2 drops of an alcoholic -naphthol solution and 2 ml of concentrated sulphuric acid was gently applied along the test tube's walls. At the intersection, a violet ring forms, indicating the presence of carbohydrates.

Benedict's Test

c) Benedict's reagent was used to treat the filtrates, and they were heated in a water bath. As a result, a crimson precipitate forms, which denotes the presence of reducing sugars.

d) Fehling's Test

e) Filtrates were heated with Fehling's A and B solutions, neutralized with alkali, and hydrolyzed with diluted hydrochloric acids. As a result, a crimson precipitate forms, which denotes the presence of reducing sugars.

f) Barfoed's Test

Add 3 ml of the carbohydrate solution to around 2 ml of Barfoed's reagent, boil for 3 minutes, and then let stand. A red precipitate is produced, indicating that the extract contains monosaccharides.

Test of proteins

a) Biuret Test

b) After heating, 1ml of a 10% sodium hydroxide solution was added to the extract. To the aforementioned mixes, a drop of a 0.7% copper sulfate solution was added. When proteins are

present, a precipitate of purplish violet color forms.

c) Millon's Test

d) 2 ml of Millon's reagent were added to the extracts for treatment. Proteins were detected by the development of a white precipitate that turns red when heated.

e) Xanthoprotein Test

Add 1 ml of boiling nitric acid that has been made alkaline with sodium hydroxide or ammonium hydroxide to 1 ml of solutions. When the color of yellow changes to deep yellow or orange due to the presence of benzene or phenyl groups, protein is present.

Test for Amino acids

a) Ninhydrin Test

0.25% ninhydrin reagent was added to the extracts and heated for a short period. The extract takes on a blue hue, which denotes the presence of amino acids.

Worm collection13:

To investigate the anthelmintic properties of plant extracts, the adult Indian earthworm (Eisenia foeitida) was employed.

• The Horticulture Department, Mahadwvapura Farm 577536 is where the adult earthworm was found. Worms measuring 5 to 6 cm in length and 0.2 to 0.3 cm in width were used for the entire experiment.

• Due to the earthworms' automatic and physiological resemblance to human intestinal roundworm parasites, they were thought to have anthelmintic action.

Anthelmintic activity13:

• Using Hussain et al.'s approach, an anthelmintic investigation of the extract was conducted at doses of 10, 20, and 50 mg/ml against Indian earthworms (Eisenia foetida).

For the investigation, five groups of Indian earthworms were used, each including five worms that were almost the same size.

• Three groups of earthworms were examined using extracts at various concentrations (10 mg/ml, 20 mg/ml, and 50 mg/ml). One group received treatment with 10 mg/ml of mebendazole as the reference standard, and the other group served as the control and was given saline as usual.

• Earthworm anthelmintic activity was watched, and the length of time until paralysis and death were noted.

RESULTS

Table No (01): Preliminary Phytochemical screening of seed coat of

***Caesalpinia bonduc* L. Roxb.**

Sl no.	Phyto-constituents	Alcohol extract
1	Carbohydrates	+
2	Proteins	+
3	Tannins	+
4	Saponins	++
5	Triterpenoids	+
6	Flavonoids	+
7	Resins	+
8	Glycosides	-
9	Alkaloids	+
10	Steroids	+

+:Present;-: Absent;++ Higherconcentration

Table No.(02): -Effect of EESCB on earthworms : -

Sl.No.	Group	Treatment	Time of paralysis (min)	Time takento death(min)
1	Control	Saline	-	-
2	Standard	Mebendazole(10 mg/ml)	4.333 ± 0.1453	6.200 ± 0.1764
3	Test A	EESCB (10 mg/ml)	7.600 ± 0.1155	12.00 ± 0.1453
4	Test B	EESCB (20 mg/ml)	6.233 ± 0.1202	8.900 ± 0.2603
5	Test C	EESCB (50 mg/ml)	5.367 ± 0.088	7.200 ± 0.2404

EESCB: Ethanolic Extract of Seed Coat of *Caesalpinia bonduc* L. Roxb. values were expressed as Mean ± SEM (n=6); Significance values are: ***P < 0.001, **P< 0.01 and *P < 0.05. Stress group vs all groups. By one-way ANOVA followedby Tukey-Kramer Multiple comparison tests.

Time taken for paralysis:

The graph displays how long it took for ES to become paralyzed after being treated with the conventional treatment (10 mg/ml) and the test drug at concentrations of 10, 20, and 50 mg/ml, respectively. Test A (10 mg/ml) causes paralysis for a longer period than Test B (20 mg/ml), while Test C (50 mg/ml) causes paralysis for a shorter period when compared to the reference medication.

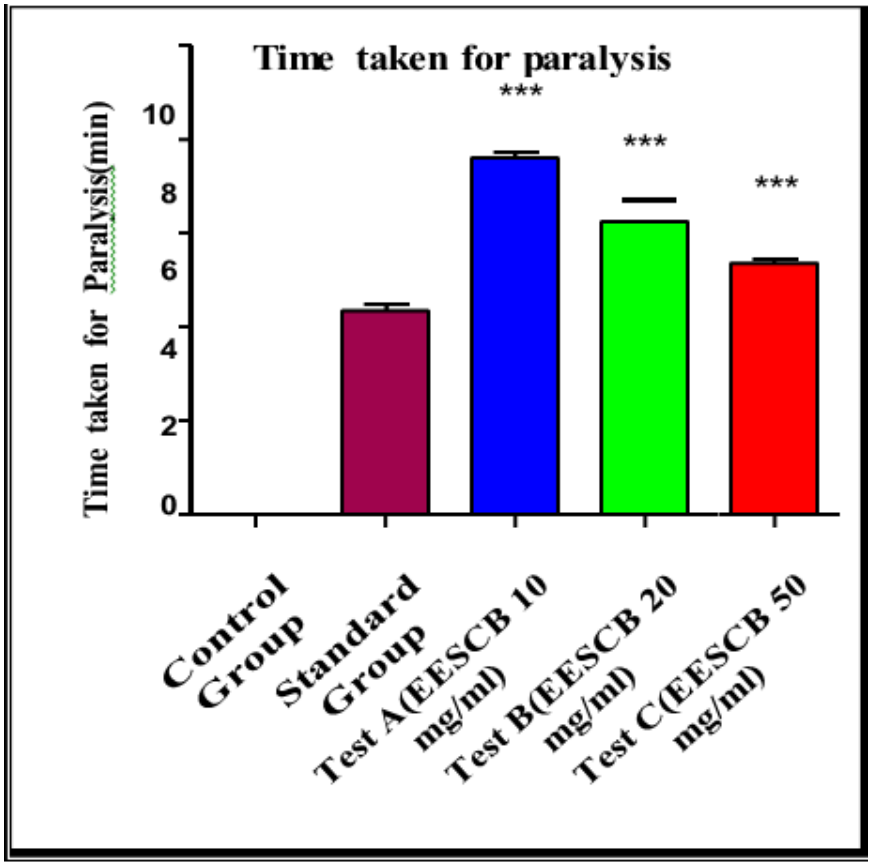

Fig No 10: Time taken for paralysis

Time taken for death:

The graph displays how long it took ES to die after receiving treatment with the reference drug (10 mg/ml) and the test drug at doses of 10, 20, and 50 mg/ml, respectively. Test A (10 mg/ml) shows a longer time for death than Test B (20 mg/ml), and Test C (50 mg/ml) displays the shortest time for death, respectively, when compared to Standard Drug. The Test Drug exhibits Significant Anthelmintic Activity in a Dose-Dependent Manner. The extract comprises sugars, alkaloids, phenols, glycosides, flavonoids, tannins, and steroids, according to preliminary phytochemical tests. The effects of ethanolic extracts of Caesalpinia bonduc and mebendazole at various concentrations on Eisenia foetida are shown in the table. Earthworms treated with the extract displayed dose-dependent paralysis and mortality when compared to the reference medication mebendazole. The paralysis time was 7.60001155, 6.2330.1202, and 5.3670.088 minutes, respectively, while the death time was 12.00.153, 8.9000.2603, and 7.2000.2404 minutes for the ethanolic extract at doses of 10, 20, and 50mg/ml. The highest concentration, which is comparable to mebendazole, quickly results in paralysis and death. At a dose of 10 mg/ml, the mebendazole-treated group experienced

paralysis for 14.23 min and death for 17.26 min. The amount of physical activity of the earthworms exposed to the regular saline solution did not alter.

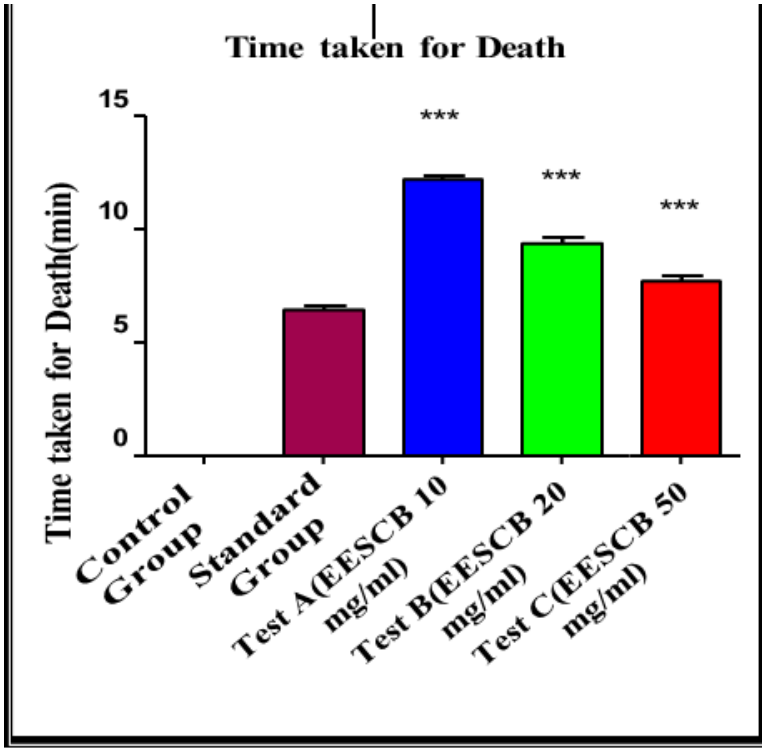

Fig no11:- Time taken for death

DISCUSSION

Eisenia foetida subjected to ethanolic extract of Caesalpinia bonduc seed coat at various concentrations L. Roxb found that changes in physical activity, paralysis, and mortality were dose-dependent. One-way ANOVA was used to analyze the data, followed by the Dunnett test and the calculation of mean and SEM for each extract concentration. The effectiveness of ethanolic extract as an anthelmintic against earthworms (Eisenia foetida) is summarised in table 2, where it was compared to the reference medicine mebendazole and paralyzed earthworms at various dosages. Mebendazole causes metabolic disruption at several distinct locations, the majority of which are involved in the parasite's energy synthesis and cause earthworm mortality.

The earthworms' movement changed at all concentrations of the ethanolic extract of Caesalpinia bonduc, and eventually, they died. However, the substantial effect was only seen at a concentration of 50 mg/ml. Caesalpinia bonduc's current anthelmintic components are unknown, but phytochemical screening literature claims that it contains carbohydrates,

alkaloids, phenols, glycosides, flavonoids, tannins, steroids, and several phytochemicals that have the potential to alter the earthworms' metabolic pathway and cause mortality. One of these probable chemical components, tannins, has anthelmintic properties and can interact with free proteins in the gastrointestinal tract of earthworms to kill them.

The formation of protein complexes by increasing the supply of digestible proteins, imposing larval starvation by utilizing free protein present in tubes, producing energy by uncoupling oxidative phosphorylation, and interfering with nematodes cuticle that results in paralysis are a few of the mechanisms that contribute to tannins' anthelmintic effect. Plants that include tannins as their primary chemical component have considerable anthelmintic action, making them a good choice to manage nematodes. Steroids are also known to impact the permeability of parasite membranes and the development of parasite pores, which results in parasite mortality. The central nervous system of parasites is affected by alkaloids, which limit nitrate production, which in turn reduces ribosomal and mitochondrial protein synthesis, interferes with DNA and RNA synthesis and activity, inhibits glucose delivery, and causes worms to become paralyzed.

The inclusion of phytochemicals like tannins may be the reason why Caesalpinia bonduc's ethanolic extract exhibits such strong anthelmintic action. However, more research is required to determine the exact anthelmintic mechanism.

Caesalpinia bonduc L. Roxb. has to undergo additional in vivo research to assess its efficacy and pharmacological basis as an anthelmintic agent. Even if further research is required, the plant nevertheless shows great anthelmintic potential that can be used to treat liver disease brought on by worms.

CONCLUSION

The test sample of seed coat extract in the current study significantly reduced the adult Indian earthworm (Eisenia foeitida) population. The active components responded to the protective against anthelmintic activity that may be present in the seed coat extract, it has been determined.

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