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EVALUATION OF PHYTOCHEMICAL COMPOSITION AND ANTHELMINTIC EFFICACY OF AQUEOUS EXTRACTS FROM THREE BANGLADESHI MEDICINAL PLANTS

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ABSTRACT: The evolution of helminthes drug resistance and the increased expense of traditional medications have spurred investigation into medicinal plants as potential alternatives to anthelmintics. Utilizing *P. posthuma*, the present study focuses on the anthelmintic activity of three traditionally used plants in Bangladesh. *Mimosa pudica* (family: fabaceae), *Terminalia bellirica* (family: Combretaceae), *Calotropis gigantea* (family: Apocynaceae). At all tested concentrations (25, 50 and 100 mg/ml), of the plant extract showed a significant anthelmintic effect. The highest activity or effectiveness was observed at 100 mg/ml. At these maximum concentrations, *Calotropis gigantea* showed the most potent anthelmintic effect among the plants, achieving paralysis in 19 ± 5 minutes and death time in 86 ± minutes. This was succeeded by *Terminalia bellirica* (paralysis at 43 ± 5min and death at 185 ± 5 minutes) and *Mimosa pudica* (paralysis at 58 ± 5 minutes and death at 229 ± 5 minutes). This result supports the traditional use of these plant-based anthelmintic agents.

INTRODUCTION: Helminths are parasite worms that infect humans and mammals, leading to disease commonly referred to as helminthiasis. Intestinal helminthic diseases are extensively common worldwide. Especially in hot climates and warm regions of underdeveloped countries with low hygiene and sanitation systems. Usually produce indications such as abdominal pain, diarrhea, anemia deficiency, or low hemoglobin, nutrient deficiency, weight loss and weakness, resulting in considerable illness among the affected population ¹.

In addition, the growing incidence of antimicrobial resistance has become a critical issue in the management of parasite-related disease in both humans and animals ². As a result, helminthiasis is recognized as a significant or a crucial public health as well as animal health problem, leading to growing attention in exploration for different plant-based anthelmintic agents ³.

Anthelmintic drugs exist as chemical-based compounds used to destroy parasite helminths in humans as well as animals. Synthetic anthelmintics like albendazole work by damaging metabolic as well as neuromuscular functions of the parasite, which leads to paralysis and death ^{1, 2}. Chemotherapy is a primary technique for managing parasite worm infection because efficient vaccines continue to be unbelievable because of complicated life cycle and host immune avoidance mechanism



of helminthes¹. On the other hand, prolonged and uncontrolled application of synthetic drugs can lead to adverse effects such as nausea, stomach pain, discomfort and hepatotoxicity². Repeated contact of parasites with the same drug has also contributed to anthelmintic resistance, in which parasites become less sensitive to treatment³. Increasing drug resistance, the evaluation of treatment, cost, toxicity and insufficient access to synthetic drugs in developing regions or countries have encouraged the investigation of safer natural alternatives^{1,3}.

Medicinal plants have been commonly applied in conventional medicine for the management of infectious and parasite disease from early times. Plant-based and fermented products are receiving significant interest because they are safe and affordable, as well as easily reachable compared to synthetic drugs¹. Various medicinal plants contain biologically active phytochemicals such as tannins, alkaloids, flavonoids and saponins, which show considerable anthelmintic activities or effects. The following compounds can inhibit the biochemical metabolism and neuromuscular regulation of helminths, leading to paralysis and death of helminths. Natural medicines are also recognized as eco-friendly and can cause minimal adverse effects, as well as lower drug resistance issues than conventional synthetic drugs¹. Hence, medicinal plants are considered as a potential alternative source for the preparation of new anthelmintics or drugs.

Mimosa pudica L. (Mimosaceae) is a prostrate annual or perennial blossom-bearing plant found in the Asian region. *Mimosa pudica* has been recognized as Lajjalu in Ayurveda and has been found to have hepatoprotective, hypolipidemic, antifertility, antiasthmatic, aphrodisiac, analgesic, sedative, emetic, tonic properties and antidepressant properties⁴. This plant contains phytochemicals like alkaloids, flavonoids, tannins and glycosides and has shown antimicrobial, antioxidant and anthelmintic effects in past research⁴. Previous investigations have described the anthelmintic effects of various *Mimosa pudica* extracts towards parasite research models. Similarly, *Calotropis gigantea* is commonly used in conventional medicines for fever, pain, skin disease and parasite disease. This plant also contains flavonoids, saponins, terpenoids and

glycosides that show documented pharmacological and anthelmintic properties⁵. *Terminalia bellierica* is a significant medicinal plant conventionally applied in treating cough, diarrhea, liver disease and parasite infections. The plant includes tannin, flavonoids, glycosides and phenolic compounds and it shows antimicrobial, antioxidant and anthelmintic properties⁶. *Mimosa pudica*, *Calotropis gigantea* and *Terminalia bellirica* Act as potential candidates for the development of natural anthelmintic compounds due to their conventional uses and documented effectiveness. The study aims to investigate the *in-vitro* anthelmintic.

MATERIALS AND METHODS: The experiment was performed with normal saline and albendazole. Within all groups, only various doses of water extract of *Mimosa pudica*, *Terminalia bellirica* and *Calotropis gigantea* leaves were considered. Saline water was used as the control in the normal group. *In-vitro* anthelmintic activity comparison was made with the standard drug albendazole, which was procured from Eskayef Pharmaceuticals Ltd. Anthelmintic action of the aqueous extract was established in the studies.

Earthworms: To evaluate the anthelmintic activity, adult earthworms (*P. posthuman*) were taken *in-vitro*. The earthworms were collected from the ASA University of Bangladesh after they were picked up from the National Botanical Garden of Bangladesh. The earthworms were 2-4cm in length and 0.2-0.3 cm in diameter.

Collection and Identification of Plants: The plants *Mimosa pudica*, *Calotropis gigintia* and *Terminalia bellerica* were collected from the National Botanical Garden, Mirpur, Dhaka. Their identities were confirmed at the Bangladesh National Herbarium, Mirpur, Dhaka, with the following numbers: *Mimosa pudica* DACB-142203, *Calotropis gigintia* DACB-143246 and *Terminalia bellerica* DACB-142200. The collected plant parts were obtained and cut into small pieces. They were completely cleaned with distilled water and air -dried under shade at room temperature for a few days to preserve their phytochemicals.

Anthelmintic Activity: In the primary evaluation of anthelmintic activity, the experiment was

conducted *in-vitro*, applying adult local earthworms, *P. posthuma*. These specific earthworms were selected because of their own strong structural and functional similarity to human-related intestinal nematodes. Extract preparations were freshly prepared in normal saline with concentrations of 25, 50 and 100 mg/ml. For every concentration, six earthworms of approximately similar size were kept in a container containing 50 ml of the assigned liquid solution. The standard positive control was albendazole, a well-known benzimidazole compound that is effective in paralyzing and destroying helminths by blocking microtubule formation⁷.

Normal saline was applied as the inactive control group to make certain that any action was due to the extract or standard medication and not an osmosis-related effect. The earthworms were examined carefully throughout the experiment. Paralysis was observed during the time when the earthworms stopped movement except for occasional reaction after forceful movement⁸. Death was verified after the arm bones could not react even after being treated to gentle shaking or immersion in heated water 50°C.

Phytochemical Screening: Alkaloids, tannins, flavonoids, glycosides, saponins and terpenoids were included among the bioactive metabolites observed from the plant extracts of *Mimosa pudica*, *Calotropis gigantean* and *Terminalia bellirica* according to qualitative phytochemical screening^{4,9}.

RESULTS:

Phytochemical Screening: These three medicinal plants *Mimosa pudica*, *Calotropis gigantean* and *Terminalia bellirica*, phytochemical screening analysis of the Aqueous extracts showed the existence of phytochemical compounds like alkaloids, tannins, flavonoids, terpenoids, saponins and glycosides **Table 1, 2** and **3**.

TABLE 1: RESULT OF PHYTOCHEMICAL SCREENING OF MIMOSA PUDICA

Constituents	Aqueous
Alkaloids	+
Glycosides	-
Flavonoid	-
Saponin	+
Steroids	-
Tannin	+

TABLE 2: RESULT OF PHYTOCHEMICAL SCREENING OF CALOTROPIS GIGANTEA

Constituents	Aqueous
Carbohydrate	+
Saponins	+
Phenolic Compounds and Tannins	+
Phytosterols	-
Alkaloids	-
Flavonoids	+
Glycosides	-

TABLE 3: RESULT OF PHYTOCHEMICAL SCREENING OF TERMINALIA BELLIRICA

Constituents	Aqueous
Carbohydrates	+
Saponins	+
Alkaloids	-
Glycosides	+
Terpenoids	-
Steroids	-
Flavonoids	+
Phenolics and Tannins	+

Anthelmintic Activity: At the concentration of 25, 50 and 100 mg/mL, of *Mimosa pudica*, *Terminalia bellirica* and *Calotropis gigantea* were evaluated against adult earthworms, *P. posthuma*. Greatest results were generally observed at the highest concentration. Every three plants revealed concentration-dependent activity. The earthworms were paralyzed due to *Calotropis gigantea* at 100 mg/ml within nearly 19 minutes and they expired within approximately 86 minutes **Table 4**. Similarly, *Terminalia bellirica* extract induced paralysis at approximately 43 minutes and also death at 185 minutes **Table 5**. Afterward, *Mimosa pudica* required almost the same duration of time and all and needed 58 minutes to cause paralysis, but the worms survived much longer and died at 229 minutes **Table 6**. During the experiments, all three plants aqueous extracts revealed improved anthelmintic potency compared to the conventional standard drug albendazole, that lead to paralysis at around 7 minutes and also death at around 242 minutes. Overall, *Calotropis gigantea* and *Terminalia bellirica* showed quicker, also more powerful anthelmintic activity compared to mimosa pudica, which caused paralysis in a comparatively delayed duration and showed a slower killing effect. These findings show that although all three of them contain considerable anthelmintic effects, *Calotropis gigantea* and *Terminalia bellirica* could contain a stronger combination of active phytochemicals, supporting their efficacy.

TABLE 4: ANTHELMINTIC ACTIVITY OF CALOTROPIS GIGANTEA

Animal group	No. of worms	Concentration	Time taken for Paralysis (min)	Time taken for death (min)
Control Group	6	-----	-----	-----
Standard (Albendazole)	6	10 mg/ml,	7 ± 2	242 ±5
<i>Calotropis gigantea</i>	6	25 mg/ml	61 ±5	177 ±5
	6	50 mg/ml	40 ±5	109 ±5
	6	100 mg/ml	19 ±5	86 ±5

TABLE 5: ANTHELMINTIC ACTIVITY OF TERMINALIA BELLIRICA

Animal group	No. of worms	Concentration	Time taken for Paralysis (min)	Time taken for death (min)
Control Group	6	-----	-----	-----
Standard (Albendazole)	6	10 mg/ml,	7 ± 2	242 ±5
<i>Terminalia bellirica</i>	6	25 mg/ml	55 ±5	214 ±5
	6	50 mg/ml	49 ±5	197 ±5
	6	100 mg/ml	43 ±5	185 ±5

TABLE 6: ANTHELMINTIC ACTIVITY OF MIMOSA PUDICA

Animal group	No. of worms	Concentration	Time taken for Paralysis (min)	Time taken for death (min)
Control Group	6	-----	-----	-----
Standard (Albendazole)	6	10 mg/ml,	7 ± 2	242 ±5
<i>Mimosa pudica</i>	6	25 mg/ml	95±5	274 ±5
	6	50 mg/ml	61 ±5	255 ±5
	6	100 mg/ml	58 ±5	229 ±5

DISCUSSION: This aqueous extracts of *Mimosa pudica*, *Terminalia bellirica* and *Calotropis gigantea* had been evaluated under comparison to adult earthworms, *P. posthuma*, with concentrations of 25, 50 and 100 mg/ml. The findings indicated a clear concentration-dependent anthelmintic effect, showing maximum efficacy. *Calotropis gigantea* showed the most immediate, strong, powerful anthelmintic activity at this concentration, leading to paralysis at 19 min and death at 86 min. *Terminalia bellirica* induced paralysis is 43 minutes and death at 185 minutes, meanwhile, *Mimosa pudica* induced paralysis at 58 minutes and death at 229 minutes. Albendazole (10 mg/ml) applied as the control drug produces paralysis at 7 minutes and death at 242 minutes. Therefore, although albendazole results in fast paralysis, the water-based extracts, especially *Calotropis gigantea*, showed significantly decreased death time, indicating stronger killing activity. The present findings are consistent with previous studies demonstrating significant dose-dependent anthelmintic activity of *Mimosa pudica* extract against *P. posthuma*¹¹. Similarly, *Terminalia bellirica* has been reported to possess notable antiparasitic activity due to its rich tannin and phenolic content¹². *Calotropis gigantea*

exhibited potent pharmacological and anthelmintic properties attributed to its bioactive phytochemicals¹³. Overall, these observations deliver strong evidence that *Mimosa pudica*, *Calotropis gigantea* and *Terminalia bellirica* possess significant anthelmintic activity, with *Calotropis gigantea* exhibiting the most pronounced activity among the three plants. The proportional paralysis and their death time observed with *Calotropis gigantea* suggest that its phytochemical constituents function rapidly on the neuromuscular and metabolic system of the helminths. *Terminalia bellirica* also exhibited significant activity, while *Mimosa pudica* demonstrated modest but notable anthelmintic effect. This result emphasizes the therapeutic potential of these medicinal plants, particularly *Calotropis gigantea*, as a quick and efficient plant-based substitute to traditional anthelmintic agents, such as Albendazole. The observed activity may be associated with the presence of tannins, flavonoids, alkaloids, saponins and other bioactive phytochemicals that disrupt with biological function in parasite worms. Future studies should focus on the isolation purification and structure characterization of this active constituent, followed by the *in-vivo* investigation to confirm their potency, safety and mode of action.

CONCLUSION: The findings of the present study demonstrate that the aqueous extracts of *Mimosa pudica*, *Calotropis gigantea* and *Terminalia bellirica* possess significant anthelmintic activity against earth worms. The observed pharmacological effects may be attributed to the presence of biologically active phytoconstituents including flavonoids, tannins, saponins and terpenoids which are widely recognized for their antiparasitic potential. Among the tested plant extracts, *Mimosa pudica* exhibited comparatively rapid and potent anthelmintic activity. These findings scientifically support the traditional use of these medicinal plants in the treatment of anthelmintic infections and highlight their potential as promising natural alternatives to conventional synthetic anthelmintic agents. Nevertheless, further comprehensive investigations, including *in-vivo* studies, phytochemical characterization, toxicity assessment and mechanistic evaluations are required to validate their safety, efficacy and therapeutic applicability for future pharmaceutical development.

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