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WATER-BASED EXTRACTS FROM A VARIETY OF BANGLADESHI MEDICINAL PLANTS AND THEIR ANTIHELMINTIC EFFECTS

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ABSTRACT: The antihelmintic activity of *Causonis trifolia* (Family: Vitaceae), *Alstonia scholaris* (Family: Apocynaceae), *Trewia nudiflora* (Family: Euphorbiaceae), *Echinochloa crus-galli* (Family: Poaceae), *Cynodon dactylon* (Family: Poaceae), *Clerodendrum viscosum* (Family: Lamiaceae) sometimes Verbenaceae, *Aegle marmelos* (Family: Rutaceae), *Mentha australis* (Australian mint) (Family: Lamiaceae) was studied against adult earthworms (*Pheretima posthuma*). The extract showed anthelmintic activity at the doses of 25 mg/ml, 50 mg/ml, and 100 mg/ml, although the reference standard used was albendazole (10 mg/ml). But the 100 mg/ml had the higher strong anthelmintic effect. *Causonis trifolia*, *Alstonia scholaris*, *Trewia nudiflora*, *Echinochloa crus-galli*, *Cynodon dactylon*, *Clerodendrum viscosum*, *Aegle marmelos*, and *Australian mint* caused paralysis and death of the earthworms at this concentration in about 88 min and 229min, 423min and 461min, 247min and 319min, 468min and 540 min, 440 min and 520 min, 61 min and 215 min, 270 min and 346 min, 328 min and 366 min, respectively. However, the extract of *Clerodendrum viscosum* at 100 mg/ml induced paralysis and death at about 61 min and 215 min, respectively.

INTRODUCTION: Often referred to as anthelmintics, antihelminthics are medications that either kill or incapacitate parasitic worms (helminths) to enable their removal from the body. The WHO estimates that two billion people are affected with parasitic worms ¹. Parasitic worms also affect crops and cattle, and their alteration of food production has an economic impact. Research on anthelmintic medications is scarce, despite the high prevalence of parasite infections.

Only a few number of drugs are regularly used to treat certain parasite illnesses, according to the WHO. According to traditional medicine, a number of natural plants can effectively eradicate worms ².

Causonis trifolia (L.) Mabb., commonly known as “fox grape” (synonym: *Cayratia trifolia* (L.) Domin), is an ethnomedicinally important twining herb belonging to the Vitaceae family. The whole plant is used as a diuretic and in tumours, neuralgia, and splenopathy. Leaf decoctions, or the juice of the fresh leaves, are used to cure scurvy. The roots are used as an astringent and as an antidote to snake bites ³. To control blood sugar levels, diabetic patients are administered with an aqueous extract of the tuber along with the infusion of seeds, whereas the powdered root tuber is administered orally with milk for the early healing

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of fractured bones⁴. Very few scientific explorations have been conducted previously to investigate the phytochemical and pharmacological potential of the studied plant parts. *Alstonia scholaris* Linn. (Family: Apocynaceae), commonly known as Saptaparna or devil tree. The bark extract is traditionally used as an antiperiodic, febrifuge, and astringent. The bark is bitter, astringent, acrid, thermogenic, digestive, and laxative. In Ayurveda, bark soaked overnight in water is reported to reduce blood glucose levels⁵. *Trewia nudiflora* is a fast-growing, dioecious plant found in Bangladesh and throughout South Asia. Extracts and decoctions of different parts are used for removal of bile and phlegm, cerebroprotection, wound healing, hyper locomotion, flatulence, gout, rheumatism, tumor, and fungal growth inhibition. *Cynodon dactylon* (Family: Poaceae) is a widely distributed perennial medicinal herb⁶. It is traditionally used for intestinal worm infections, diarrhea, vomiting, burning sensation, fever, skin diseases, headache, malaria, kidney stones, prostate ailments, rheumatism, and gonorrhea. It possesses antioxidant, antidiabetic, antimicrobial, hepatoprotective, wound healing, and antiarthritic properties. *Clerodendrum viscosum* leaves and roots are used traditionally as bitter tonic, antiperiodic, vermifuge, laxative, and cholagogue. They are externally applied for tumors and skin diseases⁷. Fresh leaf juice is reported for treating ascariis. Preliminary phytochemical screening of ethanolic and aqueous extracts has been conducted, and anthelmintic activity was evaluated against *Ascaridia galli*. *Aegle marmelos* (Family: Rutaceae), commonly known as Bael, Bengal quince, Bilva, Indian quince, Golden apple, and Holy fruit. Various parts (leaves, roots, bark, seeds, fruit) contain coumarins, alkaloids, sterols, essential oils, terpenoids, phenols, glycosides, and tannins. Reported activities include antimicrobial, antifungal, analgesic, anti-inflammatory, antipyretic, hypoglycaemic, antidyslipidemic, immunomodulatory, anti-fertility, and insecticidal properties. It is used traditionally for diabetes, cardiovascular disorders, and inflammatory conditions⁸.

MATERIALS AND METHODS: The experiment was performed with normal saline and albendazole. Within all groups, only various in doses of water extract of *Causonis trifolia*, *Alstonia scholaris*,

Trewia nudiflora, *Echinochloa crus-galli*, *Cynodon dactylon*, *Clerodendrum viscosum*, *Aegle marmelos*, and *Mentha australis* (Australian mint) leaves were considered. Saline water was used as the control in the normal group. In vitro anthelmintic activity comparison was made with standard drug albendazole which was procured from Albion Laboratories Ltd. Anthelmintic action of the aqueous extract was established in the studies.

Earthworms: To evaluate the anthelmintic activity, adult earth worm (*P. posthuman*) were taken *in-vitro*. The earthworms were discovered at Bangladesh's Primeasia University after they had been gathered from a village in the Kurigram district. The earthworms measured 2-4 cm in length and 0.2-0.3 cm in width.

Collection and Identification of Plants: Collections of *Causonis trifolia*, *Alstonia scholaris*, *Trewia nudiflora*, *Echinochloa crus-galli*, *Cynodon dactylon*, *Clerodendrum viscosum*, *Aegle marmelos*, and *Mentha australis* (Australian mint) plants were from Pabna district of Bangladesh. Authenticity of the specimens was confirmed at the Bangladesh National Herbarium, Mirpur, Dhaka. Plant parts were collected and were chopped into small pieces, they were thoroughly washed with distilled water and dried under shade at room temperature for a few days to prevent their phytoconstituents. The identification numbers of those plants were *Causonis trifolia* DACB 10370, *Alstonia scholaris* DACB 10371, *Trewia nudiflora* DACB 10372, *Echinochloa crus-galli* DACB 10373, *Cynodon dactylon* DACB 10374, *Clerodendrum viscosum* DACB 10375, *Aegle marmelos* DACB 10376 and *Australian mint* DACB 10377.

Preparation of Fresh Leaf Extract: 100 mg/ml, 50 mg/ml, and 25 mg/ml of the collected leaves were weighed and mixed with a maximum of 50 ml of normal saline to obtain a solution. The supernatant was sieved into a conical flask through a sterile filter paper.

Anthelmintic Activity: The anthelmintic activity was evaluated using adult Bangladeshi earthworm namely *Pheretima posthuma* having close anatomical and physiological resemblance with the

intestinal roundworm parasites of human beings⁹. Test samples of extract were prepared at 25, 50 and 100mg/ml concentration in normal saline water and approximately equal size of six earthworms (*Pheretima posthuma*) were placed in each beaker containing 50 ml of above test solutions of extract. Albendazole (10 mg/ml) was used as a reference standard and normal saline water as control.

All the test and reference solutions were freshly prepared before initiating the experiment¹⁰. The time taken for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50°C)¹¹.

RESULTS:

TABLE 1: ANTHELMINTIC ACTIVITY OF *C. TRIFOLIA*

Animal group	No of worms	Concentration	Time taken for paralysis (min)	Time taken for death (min)
Control Group	6	----	-	-
Standard Group (Albendazole)	6	10mg/ml	27± 0.05	68.95±0.41
<i>C. trifolia</i>	6	100mg/ml	74±4	229±5
		50mg/ml	82±1	265±3
		25mg/ml	88±2	300±2

TABLE 2: ANTHELMINTIC ACTIVITY OF *A. SCHOLARIS*

Animal group	No of worms	Concentration	Time taken for paralysis (min)	Time taken for death (min)
Control Group	6	----	-	-
Standard Group (Albendazole)	6	10mg/ml	27± 0.05	68.95±0.41
<i>A. scholaris</i>	6	100mg/ml	423±1	461±5
		50mg/ml	489±3	582±3
		25mg/ml	557±2	627±2

TABLE 3: ANTHELMINTIC ACTIVITY OF *T. NUDIFLORA*

Animal group	No of worms	Concentration	Time taken for paralysis (min)	Time taken for death (min)
Control Group	6	----	-	-
Standard Group (Albendazole)	6	10mg/ml	27± 0.05	68.95±0.41
<i>T. nudiflora</i>	6	100mg/ml	247±2	319±3
		50mg/ml	269±3	344±2
		25mg/ml	300±1	369±3

TABLE 4: ANTHELMINTIC ACTIVITY OF *E. CRUS-GALLI*

Animal group	No of worms	Concentration	Time taken for paralysis (min)	Time taken for death (min)
Control Group	6	----	-	-
Standard Group (Albendazole)	6	10mg/ml	27± 0.05	68.95±0.41
<i>E. crus-galli</i>	6	100mg/ml	468±2	540±1
		50mg/ml	481±1	550±3
		25mg/ml	494±2	569±2

TABLE 5: ANTHELMINTIC ACTIVITY OF *C. DACTYLON*

Animal group	No of worms	Concentration	Time taken for paralysis (min)	Time taken for death (min)
Control Group	6	----	-	-
Standard Group (Albendazole)	6	10mg/ml	27± 0.05	68.95±0.41
<i>C. dactylon</i>	6	100mg/ml	440±2	520±1
		50mg/ml	479±3	524±1
		25mg/ml	487±3	539±4

TABLE 6: ANTHELMINTIC ACTIVITY OF *C. VISCOSUM*

Animal group	No of worms	Concentration	Time taken for paralysis (min)	Time taken for death (min)
Control Group	6	----	-	-

Standard Group (Albendazole)	6	10mg/ml	27± 0.05	68.95±0.41
<i>C. viscosum</i>	6	100mg/ml	61±2	215±1
		50mg/ml	74±3	265±1
		25mg/ml	82±1	300±2

TABLE 7: ANTHELMINTIC ACTIVITY OF *A. MARMELOS*

Animal group	No of worms	Concentration	Time taken for paralysis (min)	Time taken for death (min)
Control Group	6	----	-	-
Standard Group (Albendazole)	6	10mg/ml	27± 0.05	68.95±0.41
	6	100mg/ml	278±1	346±2
		50mg/ml	320±3	427±1
		25mg/ml	336±2	480±1

TABLE 8: ANTHELMINTIC ACTIVITY OF *A. MINT*

Animal group	No of worms	Concentration	Time taken for paralysis (min)	Time taken for death (min)
Control Group	6	----	-	-
Standard Group (Albendazole)	6	10mg/ml	27± 0.05	68.95±0.41
	6	100mg/ml	328±2	366±2
		50mg/ml	351±1	396±3
		25mg/ml	356±2	405±2

Anthelmintic Activity: *In-vitro* anthelmintic activity was performed by using adult Indian earthworms (*Pheretima posthuma*) at concentrations of 25, 50, and 100 mg/mL of aqueous extracts of *Causonis trifolia*, *Alstonia scholaris*, *Trewia nudiflora*, *Echinochloa crus-galli*, *Cynodon dactylon*, *Clerodendrum viscosum*, *Aegle marmelos*, and *Mentha australis*. All extracts exhibited dose-dependent effects, with 100 mg/mL exhibiting the greatest activity. At this dose, *Clerodendrum viscosum* extract had the most potent effect with paralysis in approximately 61 min and death in 215 min. The plant extracts exhibited more anthelmintic efficacy than reference anthelmintic drug, albendazole (10 mg/ml), which caused paralysis at 27 minutes and death at 68.95 minutes, during the experiments.

DISCUSSION: The aqueous extracts of *Causonis trifolia*, *Alstonia scholaris*, *Trewia nudiflora*, *Echinochloa crus-galli*, *Cynodon dactylon*, *Clerodendrum viscosum*, *Aegle marmelos*, and *Mentha australis* (Australian mint) have been claimed as effective anthelmintic agents in a dose dependent manner in higher concentrations (up to 100 mg/mL)¹². *Clerodendrum viscosum* was most effective, its action being a little quicker than that of the other extracts in causing paralysis and death of helminths. These potent activities might be due to the presence of bio-active chemicals like thymoquinone, alkaloids, saponin and essential oil

which disrupt the neurotransmission system in parasites leading to paralysis and death. Similarly, the extract of *Causonistrifol* showed significant anthelmintic potential to its flavonoids, tannins and phenolic compounds that interfere with parasite metabolism and affect cuticular surface resulting on structural breakdown and death¹³. On the other hand, *Alstonia scholaris*, *Trewia nudiflora*, *Echinochloa crus-galli*, *Cynodon dactylon*, *Aegle marmelos*, and *Mentha australis* (Australian mint) showed relatively lower anthelmintic potential which could be owing to its disparity in phytochemical constitutes or lesser concentration of bioactive compounds like polyphenols and tannins¹⁴. These results are consistent with earlier reports, suggesting that tannins and polyphenolic compounds affect helminth's structural integrity and metabolism negatively. Nevertheless, the clinical applications of the most active compounds need to be validated by bioassay-guided separation, *in-vivo* studies and toxicological tests. With the growing interest worldwide in herbal drugs used to treat parasitic diseases, there is an urgent need for more knowledge about their mechanisms of action and standardized dosages¹⁵.

CONCLUSION: Results of this study indicate that the some of *Causonis trifolia*, *Alstonia scholaris*, *Trewia nudiflora*, *Echinochloa crus-galli*, *Cynodon dactylon*, *Clerodendrum viscosum*, *Aegle marmelos*, and *Mentha australis* (Australian mint)

have marked anthelmintic activity against *Pheretima posthuma in-vitro*. Most potent amongst all the tested extracts was *Clerodendrum viscosum*, followed by *Causonis trifolia*. All the extracts exhibited concentration dependent response, 100 mg/mL was most active. They were less effective than the reference drug albendazole in the same experimental condition.

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