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INVESTIGATIONS OF ANTHELMINTIC ACTIVITY OF AQUEOUS EXTRACT OF THREE MEDICINAL PLANTS OF BANGLADESH

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ABSTRACT: The anthelmintic activity of *Nigella sativa* (family: Ranunculaceae), *Lawsonia inermis* (family: Lythraceae) and *Syzygium cumini* (Family: Myrtaceae) 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. *Nigella sativa*, *Lawsonia inermis* and *Syzygium cumini* caused paralysis and death of the earthworms at this concentration in about 7 and 37, 25 and 55 and 61 and 101 min, respectively. However, the extract of *Nigella sativa* at 100 mg/ml induced paralysis and death at about 7 and 37 min, respectively.

INTRODUCTION: Helminths are a group of worms which cause a major health problem on animals worldwide. Even though controlling pastures for domestic animals could reduce the impact of parasites, these techniques are not adequate to get rid of these parasites. People have mostly relied on pharmaceutical anthelmintics to control helminthiasis, and honestly, in a lot of countries, these drugs eat up most of the animal health budget [1]. Anthelmintics are medicines that treat and control infections from parasitic nematodes, trematodes, and cestodes both in animals and people.

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Because we don't have effective vaccines yet, and sanitation isn't always up to par in areas where these parasites are common, it's tough to break the cycle and really stop these infections. Instead, treatment and prophylaxis have had to rely on a limited number of chemical classes of anthelmintics. The frequent use of these anthelmintics has led to concerns about the development of anthelmintic resistance inhelminths of companion and production animals, with reports of resistance to multiple classes a clear threat to our existing control strategies. The evidence from veterinary medicine and reports of reduced anthelmintic efficacy in human helminths also raise concerns about the risk of resistance in mass drug administration efforts for humans [2].

Earthworms and helminths such as the trematode *Schistosoma mansoni* are effectively inhibited by *Nigella sativa* seeds and its aqueous and ethanolic

extracts, which are known to signficantly reduce worm load, motality and egg count [3]. Besides anthelmintic effects, *N. sativa* is also reputed to exhibit antidiabetic, antihistaminic, antihypertensive, anti-inflammatory, antimicrobial, antitumour, galactagogue and insect repellent effects [4]. When exposed to *S. cumini* leaf extracts, tapeworms and red worms lost all their mobility. The crude extract caused a paralyzing effect which resulted in eventual death and since the concentration of the extract increased, the same caused its enhanced power as well [5]. The fruits and seeds are used to treat diabetes, pharyngitis, splenopathy, urethritis, and ringworm infection. Human beings have employed leaves to heal numerous unimaginable conditions, which include diabetes, constipation, leucorrhea, stomach-aches, fever, gastropathy, strangury, dermopathy [6] and prevent hematochezia [7]. The aqueous solution of the crude extracts of the leaves of *Lawsonia inermis* were researched on their anthelmintic activity. The extracts produced a paralyzing effect in adult earthworms and the onset of paralysis was much faster and death occurred sooner than in the control [8]. *Lawsonia inermis* is a typical herb used in the treatment of many human illness problems, such as ulcers, strangled, cough, bronchitis, lumbago, hemicrania, leukoderma, scabies, boils, eye disorders, hair loss, and jaundice [9].

N. sativa, *S. cumini*, and *L. inermis* are promising candidates for the creation of natural anthelmintic agents due to their historical use and documented effectiveness. The purpose of this study is to examine the *in-vitro* anthelmintic.

MATERIALS AND METHODS: The experiment was performed with normal saline and albendazole. Within all groups, only various in doses of water extract of *Lawsonia inermis*, *Syzygium cumini* and *Nigella sativa* 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 earthworms (*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 Comilla district. The earthworms measured 2-4 cm in length and 0.2-0.3 cm in width.

Collection and Identification of Plants: Collections of *Lawsonia inermis*, *Syzygium cumini* and *Nigella sativa* plants were from Comilla district of Bangladesh. Authenticity of the specimens was confirmed at the Bangladesh National Herbarium, Mirpur, Dhaka, under accession *Lawsonia inermis* DACB 135490, *Syzygium cumini* DACB 135494 and *Nigella sativa* DACB 135495. 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.

Preparation of Fresh 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: We followed the method from Ajaiyeoba *et al* [10], with a few tweaks, to test the anthelmintic activity of *Lawsonia inermis*, *Syzygium cumini* and *Nigella sativa* plants extracts. Fresh test solutions were prepared in normal saline with the plant extract of concentration 25, 50 and 100 mg/mL. In order to retain reproducible experimental conditions, six worms of the same size were placed into different beakers with 50 mL of each test solution. Since the well-established benzimidazole derivative albendazole could paralyse and kill the helminth by blocking microtubule polymerisation, it was used as the positive control [11]. Normal saline served as the (negative) control in order to confirm that any observed activity was through the extract or routine drug and not osmotic effects of the extract. After the standardly described protocol, paralysis was deemed to occur when the worms were unable to move in response to light shaking. The lack of movement after mechanical stimulation or after dipping in 50 °C hot water (a final verification step) was the final method used to confirm death. Comparable approaches have been described for

validation of mortality end points in previous anthelmintic screening studies [12].

Phytochemical Screening: Alkaloids, flavonoids, tannins, steroids, terpenoids, glycosides, and saponins were among the secondary metabolites found in the aqueous extracts of *Nigella sativa* [13], *Lawsonia inermis* [14], and *Syzygium cumini* [15].

TABLE 1: RESULT OF PHYTOCHEMICAL SCREENING *N. SATIVA, S. CUMINI* AND *L. INERMIS*

Extract	Alkaloids	Flavonoids	Glycosides	Steroids	Saponins	Tannins	Terpenoids
N. sativa extract	+	+++	-	-	+	++	-
S. cumini extract	-	+	-	+	+	+	+
L. inermis extract	-	-	-	+	+	-	-

Present (+), Strongly present (++), Highly present (+++), Absent (−)

TABLE 2: ANTHELMINTIC ACTIVITY OF *N. SATIVA*

Animal group	No of worms	Concentration	Time taken for paralysis (min)	Time taken for death (min)
Control Group	6	100mg/ml	-	-
Standard Group (Albendazole)	6	10mg/ml	180± 2	203±5
N. sativa	6	100mg/ml	7±2	37±4
		50mg/ml	16±3	41±2
		25mg/ml	24±2	45±3

TABLE 3: ANTHELMINTIC ACTIVITY OF *L. INERMI*

Animal group	No of worms	Concentration	Time taken for paralysis (min)	Time taken for death (min)
Control Group	6	100mg/ml	-	-
Standard Group (Albendazole)	6	10mg/ml	180± 2	203±5
L. inermi	6	100mg/ml	25±2	55±5
		50mg/ml	42±3	223±2
		25mg/ml	54±4	257±3

TABLE 4: ANTHELMINTIC ACTIVITY OF *S. CUMINI*

Animal group	No of worms	Concentration	Time taken for paralysis (min)	Time taken for death (min)
Control Group	6	100mg/ml	-	-
Standard Group (Albendazole)	6	10mg/ml	180± 2	203±5
S. cumin	6	100mg/ml	61±3	101±2
		50mg/ml	87±2	231±2
		25mg/ml	100±5	250±5

RESULT: Phytochemical screening revealed the presence of alkaloids, tannins, reducing sugar, flavonoids, steroids, terpenoids, and saponins in the aqueous extracts of *Nigella sativa*, *Lawsonia inermis*, and *Syzygium cumini*.

Anthelmintic Activity: Anthelmintic activity *in-vitro* anthelmintic activity was performed by using adult Bangladesh earthworms (*Pheretima posthuma*) at concentrations of 25, 50, and 100 mg/mL of aqueous extracts of *Nigella sativa*, *Lawsonia inermis*, and *Syzygium cumini*. All extracts exhibited dose-dependent effects, with 100 mg/mL exhibiting the greatest activity. At this dose, *Nigella sativa* seed extract had the most potent effect with paralysis in approximately 7 min and death in 37 min. At about 25 minutes and 55 minute, *L. inermis* leaf extract induced paralysis and death, showing that the action was slower but still significant. In contrast, *Syzygium cumini* extract was slower action effective, with paralysis and death times of roughly 61 and 101 minutes, respectively. The plant extracts exhibited more anthelmintic efficacy than reference anthelmintic drug, albendazole (10 mg/ml), which caused paralysis at 180 minutes and death at 203 minutes, during the experiments.

DISCUSSION: The extracts of *Nigella sativa*, *Lawsonia inermis* and *Syzygium cumini* have been claimed as effective anthelmintic agents in a dose dependent manner in higher concentrations (up to 100 mg/mL). Of these *Nigella sativa* 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 [16, 17]. Similarly, the extract of *Lawsonia inermis* 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 death [18, 19]. On the other hand, *Syzygium cumini* showed relatively lower anthelmintic potential which could be owing to its disparity in phytochemical constitutes or lesser concentration of bioactive compounds like polyphenols and tannins [20, 21].

These results are consistent with earlier reports, suggesting that tannins and polyphenolic compounds affect helminth's structural integrity and metabolism negatively [22]. They have exhibited anthelmintic effects either similar or in some cases greater than the standard chemical synthetic drug albendazole [23], indicating that these phytochemicals may serve as natural replacements of synthetic anthelmintics and alleviate the issue of drug resistance [24]. The more rapid time to paralysis and death in the plant-treated groups may also represent a therapeutic benefit. 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 dosages [25].

CONCLUSION: Results of this study indicate that the extracts of *Lawsonia inermis*, *Nigella sativa* and *Syzygium cumini* have marked anthelmintic activity against *Pheretima posthuma in-vitro*. Most potent amongst all the tested extracts was *Nigella sativa*, followed by *Lawsonia inermis* and *Syzygium cumini*. All the extracts exhibited concentration-dependent response, 100 mg/mL was most active. They were more effective than the reference drug albendazole in the same experimental condition. This work indicates that these plant-based candidates may offer drug leads especially in the light of safer, more economical anthelmintics. Further studies, which include phytochemical isolation, toxicity profiling, and animal experiments are also warranted to confirm their pharmacological potential and facilitate them to be formulated as herbal preparations.

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