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Anti-leech activity of asparagus racemosus and anogeissus latifolia roxb extracts against Piscicola Geometra

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ABSTRACT

Piscicola geometra leeches are naturally parasitizing Cobia juveniles. The leeches adhere to cobia by suctioning and biting its surface, thereby facilitating the entry of a secondary infection. The anti-leech action An in vitro technique investigated the anti-leech action against Piscicola geometra using water extracts of Asparagus racemosus seeds and Anogeissus latifolia roxb leaves, preparing a grand total of 800 afflicted cobia leeches. We compared the anti-leech activity of water extracts from Asparagus racemosus seeds and Anogeissus latifolia leaves. We diluted the plant extracts to various concentrations and observed the activity over a duration of 96 hours. The seeds and leaves of Asparagus racemosus exhibited significant anti-leech activity, resulting in 80% of the leeches dying. Anogeissus latifolia leaves exhibited a greater degree of anti-leech action, resulting in the complete killing of leeches. The average duration required for Anogeissus latifolia leaves to immobilise and eliminate the leeches ranged from 8 hours to 96 hours, depending on the different concentrations of Anogeissus latifolia leaves used. This research has shown that the aqueous extract of Anogeissus latifolia leaves has strong potential as a novel anti-leech agent.

1. Introduction

Cultures centered around cobia (Rachycentron canadum) have recently gained popularity in over twenty-three countries in the Asia-Pacific region.[1] The fast growth and intensification of cobia farming, however, have led to a rise in disease outbreaks.[2] Parasite illnesses often infect the gastrointestinal system, gills, and skin of cobia, resulting in economic losses.[3,4] In addition, various diseases can enter the body through an infestation of leeches, which can cause secondary infections and ultimately death.[5] Research has shown that the severity of a leech infestation is directly proportional to the number of leeches present. The Piscicolidae family includes P. geometra and other marine leeches that feed on blood. Its body is cylindrical, and its front part resembles a bell. In addition, it is oligotrophic, has a pH range of 7.0 to 9.0, and consumes oxygen at a far greater rate than other bacteria. It also favors water that is moving. The leech's adaptability to a wide range of environments is likely based on its widespread distribution. Pleurone Researchers have previously found P. geometra in Pleuronectes platessa, Myoxocephalus, Blica bjoerkna, Tinca tinca, Esox lucius, Barbus rajanorum, Salmo salar, Salmo girdneri, Rutilus rutilus, Gasterosteus aculeatus, Cottus scorpius, and Platichtys flesus. e just a few of the previously reported species of P. geometra.

As a low-cost, environmentally friendly, and broadly active strategy for managing fish illnesses, medicinal plants contain bioactive components and have shown influence in this area.[6,7] Both the seeds of Asparagus racemosus and the leaves of Anogeissus latifolia roxb have a long history of therapeutic usage.[8,9] Naphthalene,

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a unique molecule present in high concentrations in asparagus racemosus seeds, possesses antibacterial, anti-inflammatory, and decanoic acid properties.[10] Among the many purported health benefits of asparagus racemosus seeds are their antibacterial, anti-toxoplasmic, anti-inflammatory, hemorrhage-preventing, and liver-supportive properties.[11] Researchers have investigated the antimicrobial, antioxidant, larvicidal, and anticancer properties of Anogeissus latifolia leaf.[9] In addition to its other uses, the plant extract possesses anti-helminthic and anti-parasitic properties. This study aimed to identify Piscicola geometra, a species of leech, as well as aqueous extracts from Asparagus racemosus seeds and Anogeissus latifolia leaves that exhibited anti-leech action.

2. Methodology

2.1. Sample collection

The Department of Biotechnology at Assam Down Town University's Faculty of Science gathered 800 leeches that were 1.27±0.44 cm long. The most common ways to identify a Piscicola geometra species were by looking at its distinctive bell-shaped anterior sucker, cylindrical body, and transparent segments adorned with white dots throughout the length of its body (Figure 1).

Figure 1: *Piscicola geometra*.

2.2. Plant extractions

We purchased this dried Asparagus racemosus seed from the Vatika Agro shop in Jaipur, Rajasthan, India. We gathered the fresh Anogeissus latifolia leaves from the Maharana Pratap College of Pharmacy's botanical garden in Kanpur, UP. We rinsed the leaves with distilled water, cut them into small pieces, and then placed them in the oven for 12 hours. We independently ground the dehydrated plant using a mechanical grinder and sealed it in a container to keep air out. We heated 150 mL of distilled water and 10 g of plant powder to 95 °C to make the water extract. We used a centrifuge to spin the supernatant for 10 minutes at 4C and 1000 g after extraction. After filtering, the transparent supernatant was frozen at -20 ℃.

2.3. Leech assay

We diluted Anogeissus latifolia leaves and Asparagus racemosus seeds with sea water to create dilutions of 50x, 100x, 200x, 400x, 500x, 700x, and 1000x. Each of the three sets of eight petri dishes had five leeches, and there was a total of seven concentrations plus sea water used as a control. We monitored their effects for 96 hours, with 8-hour intervals, to observe the fatality rates. We documented the time it took to paralyze and kill the leeches, as well as their behaviour. We changed the dilutions every twelve hours. In the future, the plant extract's anti-leech capabilities will be utilised to treat diseased cobia, as evidenced by its ability to kill the leeches between 1-96 hours. Using a one-way ANOVA, we compared the control and treatment groups.

3. Results

isplays the anti-leech activities of water extracts from Anogeissus latifolia leaves against P. geometra infestation, while Table 1 shows the anti-leech activities of Asparagus racemosus seeds. The introduction of a plant extract reduced P. geometra's swimming activity. The Asparagus racemosus seed water extracts resulted in the complete death of the leeches (Table 1). Throughout the experiment, the leeches' strength dwindled, and their skin wrinkled. They writhed feebly and succumbed to death. Conversely, an extract of Anogeissus latifolia leaves reduced leech mortality by around 60%. The statistical analysis revealed that the plant extracts were most effective at 50x and 100x dilutions, and least effective at 700x and 1000x dilutions.Table 2 shows that while the leeches submerged in Asparagus racemosus seeds perished at 500x, 700x, and 1000x dilutions, the Anogeissus latifolia leaf extract had a far lower survival rate. In both experiments, the control group did not exhibit any anti-leech effects. It was clear that the aqueous extract of Anogeissus latifolia leaves was more effective at killing leeches after seeing how quickly the Asparagus racemosus seed extract paralysed and killed them.

3.1. Leeches behaviors

When a leech (Piscicola geometra) attaches its posterior sucker, it will stretch its body to attach its oral sucker, allowing the leech to move around (Figure 2 A). Once the leech pulls forward, it reattaches the posterior sucker close to the anterior sucker before releasing it (Figure 2 B). This motion is quick and effective. Figures 2C–D illustrate the aggregate loop that results from applying the posterior sucker high on the ventral surface after separation, while

Table 1: Leeches die after 96 h in repeated dilutions of Anogeissus latifolia leaves (N = 5 per treatment in 3 replicates). a-e character exhibits no significant treatment changes.

Time observed (hour)	D ilution							
	Control^{e}	**1/50x**^{a}	**1/100x**^{a}	**1/200x**^{b}	**1/400x**^{bc}	**1/500x**^{c}	**1/700x**^{cd}	**1/1000x**^{d}
1	zero	6	6	zero	zero	zero	zero	zero
8	zero	6	6	zero	zero	zero	zero	zero
16	zero	-	-	3	zero	zero	zero	zero
24	zero	-	-	1	zero	2	2	zero
32	zero	-	-	zero	zero	zero	zero	zero
40	zero	-	-	3	zero	2	2	zero
48	zero	-	-	-	4	zero	zero	zero
56	zero	-	-	-	-	1	zero	2
64	zero	-	-	-	-	1	2	2
72	zero	-	-	-	-	1	1	zero
80	zero	-	-	-	-	-	-	2
88	zero	-	-	-	-	-	-	zero
96	zero	-	-	-	-	-	-	1
combined	zero	6	6	7	6	7	7	7

Table 2: Leeches die after 96 h in Asparagus racemosus seeds extract repeated dilutions (N=5 per treatment in 3 replicates). a–e character exhibits no significant treatment changes.

Time observed (hours)	D ilution							
	Control^{e}	**1/50x**^{a}	**1/100x**^{ab}	**1/200x**^{b}	**1/400x**^{c}	**1/500x**^{d}	**1/700x**^{d}	**1/1000x**^{de}
1	zero	zero	zero	zero	zero	zero	zero	zero
8	zero	2	zero	zero	zero	zero	zero	zero
16	zero	zero	2	zero	zero	zero	zero	zero
24	zero	2	zero	2	zero	1	zero	zero
32	zero	1	zero	zero	zero	zero	zero	zero
40	zero	-	zero	zero	1	2	1	zero
48	zero	-	2	1	1	zero	zero	zero
56	zero	-	-	1	zero	zero	zero	zero
64	zero	-	-	-	zero	zero	zero	zero
72	zero	-	-	-	zero	zero	1	zero
80	zero	-	-	-	zero	zero	1	1
88	zero	-	-	-	1	zero	zero	zero
96	zero	-	-	-	2	zero	zero	2
combined	zero	5	4	4	5	3	3	3

Figure 2 E depicts the body returning to its normal position. Submerged in various extract dilutions, the leeches moved erratically until the posterior sucker sank to the bottom of the petri dish. The leeches became weaker and more wrinkled the longer they submerged.

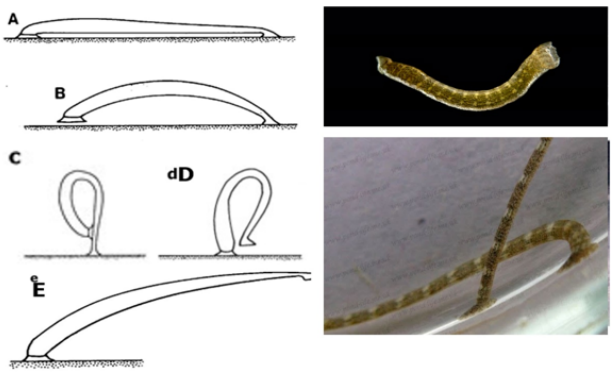

Figure 2: *Piscicola geometra* Behavior

4. Discussion

A significant P. geometra infestation can cause small circular lesions with blood on the skin, eyes, and gills of cobia. It is believed that fungal and bacterial infections can enter through the broken scale and lead to further infections.[12] Unless there is a significant infestation, leeches are usually harmless to their hosts. Currently, there is no approved medication for treating leech infestations. Conventional remedies for leech infections, such as antihistamines, bicarbonate, lidocaine, hypertonic saline, calcium hydroxide, and citrus extract, have proven to be useless. A number of natural antioxidants derived from

pharmaceutical plants have demonstrated safe therapeutic benefits in the treatment of fish diseases.

Traditional Chinese medicine (TCM) uses the seeds of the asparagus plant (Asparagus racemosus) and the leaves of the anogeissus plant (Anogeissus latifolia) to cure a variety of illnesses. Dried Asparagus racemosus seeds and Anogeissus latifolia leaves contain high concentrations of bioactive flavones, which have several beneficial effects on human health. There have been reports of immunostimulatory, anticancer, antibacterial, antiseptic, anti-fungal, anti-inflammatory, and anti-helminthic activities supported by Asparagus racemosus seeds and Anogeissus latifolia leaves, and these benefits have been found to be side effect free. [11,13]

This study has proposed the use of Asparagus racemosus seeds and Anogeissus latifolia leaves as a drug-free remedy for leech infestation.The study found that leeches immersed in a water extract from Anogeissus latifolia leaves died at a high rate within 96 hours, suggesting that the herb was successful in controlling leeches in cobia cultivation. The Anogeissus latifolia leaves killed the leeches after 8, 40, 48, 72, and 96 hours, respectively. With 50x, 100x, 200x, and 400x dilutions of Asparagus racemosus seeds, leech mortality rates were 32 hours, 48 hours, 56 hours, and 96 hours, respectively. Meanwhile, after 96 hours of immersion, only a small number of leeches were able to survive in Asparagus racemosus seed leaf dilutions of 500x, 700x, and 1000x. An initial study reported on the toxicity of a herbal extract on Piscicola geometra. Tetracer alnifolia concentrations of 5%, 10%, 15%, and 20% reduced leech swimming activity after 42 hours, 30 hours, 18 hours, and 12 hours, respectively. Previous research [14] found that Raphia vinifera had a toxic impact on Piscicola geometra. A water mixture of C. spathulifolia (62.6%), V. elaeagnifolia (63.0%), and S. rasak (82.6%) could reduce the number of terrestrial bloodsucking leeches (Haemadipsidae). [15] Anogeissus latifolia leaves demonstrated efficient anthelmintic activity in a study using earthworms (Pheritima posthuma). Despite this, no research has shown that Asparagus racemosus seeds or Anogeissus latifolia leaves had any effect on P. geometra in terms of parasites or leeches.

5. Conclusions

The research has shown that the water extract of dried leaves from Anogeissus latifolia has a more potent anti-leech effect compared to the water extract of dried seeds from Asparagus racemosus. Additionally, it has the potential to limit the swimming activity of P. geometra. The aqueous extract of dried leaves from Anogeissus latifolia exhibited a potent anti-leech action against P. geometra infection. In the future, more research will be necessary to investigate the efficacy of using bath therapy for infected cobia, as well as to analyse the active components present in the extract of dried leaves from Anogeissus latifolia.

6. Source of Funding

None.

7. Conflict of Interest

The authors declare no conflict of interest

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