International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page:www.iscientific.org/Journal.html

Dose-Dependent Piscicidal Potential of Fresh Bark Aqueous Extracts of *Acacia pennata* (L.) Willd. on *Carassius auratus, Danio rerio* and *Cyprinus carpio* Fishes in-Aquaria

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Abstract

This study was conducted to investigate the dose-dependent acute-toxicity study of the fresh bark aqueous extract of *Acacia pennata* (L.) Willd. plant on three types of fish species, namely; *Carassius auratus* (gold fish), *Danio rerio* (zebra fish) and *Cyprinus carpio* (common carp) over a period of 24 hours in-aquaria. The mortality rate and stressful behaviour of fishes were monitored throughout the experimental period and recorded. The experimental fishes *C. auratus, D. rerio* and *C. carpio* started to show stressful behaviour towards the fresh bark aqueous extract of *A. pennata* at concentration 75 mg/L, 50 mg/L and 100 mg/L; whereas, the complete mortality was observed at concentration 225 mg/L, 400 mg/L and 500 mg/L over the period of 24 hours. The LD_{50} values of the fresh bark aqueous extract of *A. pennata* for the *C. auratus, D. rerio* and *C. carpio* were found to be 144.34 mg/L, 98.67 mg/L and 194.00 mg/L respectively. These results shows that the fresh bark aqueous extract of *A. pennata* plant is more toxic to the *D. rerio* fishes at low dose (LD_{50}: 98.67 mg/L) as compared with other two types of fish species (*C. auratus* and *C. carpio*). The phytochemical screening study of the methanol bark extract of *A. pennata* was also done and its screening results shows that saponins, tannins, terpenoids, flavonoids and glycosides were present. This piscicidal dose-dependent study reveals that the fresh bark aqueous extract of *A. pennata* is more toxic to fishes at lower concentrations. Therefore, this plant bark can be extremely helpful to fish farmers for catching fish rather than using man-made hazardous chemicals.

Keywords: Aqua-culture; fisheries; *Acacia pennata* plant bark extracts; Dose-dependent Acute-toxicity

Full length article **Corresponding Author*, e-mail: maddelap.org@gmail.com Doi # https://doi.org/10.62877/52-IJCBS-24-25-19-52

1. Introduction

Natural products have been used since time immemorial as a source of therapeutic substances, which are regarded as traditional medicines as well as modern medications for humankind [1-6]. Many plants are said to be valued for their individual significance as medicines, molluscicides, insecticides, preservatives and piscicides [7-8]. Toxins from plants are used to capture fish in small water bodies [9] known as botanical toxins or natural biocides and when they are toxic to fish, they are called piscicides [10-11]. Fishing is a practice that people do all over the world by utilizing various piscicidal plants [9,13-14,17]. Generally, barks, leaves, fruits, flowers, roots, and even the entire plant are the most often used plant parts for fishing [15]. *Acacia* plants are widely distributed in warm, arid and sub-arid areas of the world. Fresh bark of *Acacia pennata* (L.) Willd. plant has been used as piscicide for fishing in the river of Nagaland, India as a traditional practice. *A. pennata* plant is a very common climbing shrub having numerous prickles, also known as 'climbing Acacia' which belongs to Mimosaceae family [16]. This plant is popularly known as "sakro" in chokri dialect of Chakhesang tribe, Nagaland, India. According to reports, the indigenous people of Nagaland have historically employed 17 different piscicidal plants to catch fish in small rivers and other water bodies [18]. The leaves of *A. pennata* is known to possess anti-nociceptive, anti-inflammatory [19] and antioxidant activities [20], whereas the bark also showed antioxidant effect [16]. The butanolic fraction of dried *A. pennata* leaves were tested for anti-inflammatory and analgesic properties in animal models, and the results were demonstrated significant advantages against chemical stressors [19]. The methanol and acetone extracts of the bark of different *Acacia* species like *A. leucophloea, A. ferruginea, A. dealbata* and *A. pennata* were investigated for their phytochemical screening and antioxidants properties [16]. Various plants have been used by fishermen or fish farmers to catch fish but determining an appropriate extracts concentration is necessary in order to understand its toxicity level, because an excessive dosage can

harm the non-targeted organisms [21-23]. Indigenous people of Nagaland, traditionally use fresh bark of *A. pennata* for catching fish in rivers without any measured quantity, which may have negative impact on the fishes and other aquatic biota as well as on human health when fishes were consumed due to excess piscicide dose. Therefore, the current investigation focuses on the dose-dependent piscicidal potential of the fresh bark aqueous extracts of *A. pennata* on two ornamental fish species *viz*., *Carassius auratus* (gold fish) and *Danio rerio* (zebra fish) and one indigenous fish i.e., *Cyprinus carpio* (common carp) in aquaria to examine its toxicity level and mortality rate of fishes at minimum lethal dose.

2. Materials and Methods

2.1. Collection of test plant (Acacia pennata)

Fresh Acacia pennata (L.) Willd. bark was collected from Lumami campus, Nagaland University, Nagaland, India. 200 g of fresh bark were cut into smaller pieces and then mash it with a pestle and mortar to create macerated samples. The macerated bark material was transferred into 2 L of water, shaken well and strained to obtain A. pennata bark aqueous extracts. To preserve its freshness, the extracts were stored in an air tight container and used for the present piscicidal study.

2.2. Test fish species

Healthy and live fishes of Carassius auratus (gold fish), Danio rerio (zebra fish) having average length and weight of 10±1 cm, 5±0.5 cm and 20±1 g, 0.578±0.020 g were purchased from aquarium shop, Mokokchung town, Nagaland; Cyprinus carpio (common carp) were sourced separately at different times as per our study requirement from fishery pond Ungma village, Nagaland, India, measuring 5±1 cm in length and 5±0.5 g in weight, and the fishes were put in 60 L large rectangular aquarium. Continuous aeration of the water was maintained and the fishes were given commercial floating meal 'Tokyu' twice daily during their 14 days acclimatization period. Despite the fact that the water was changed often, waste feed and faeces were siphoned away to prevent water contamination. To empty the fish gastro intestinal tract, feeding was halted 24 hours before the experiments. Ten fishes of each species were selected randomly from the large aquarium (60 L) for the experiment.

2.3. Experimental set-up for dose-dependent fish-poisoning study

For the present piscicidal study, two translucent rectangular aquariums (20 L capacity) were set-up, like, one for experimental and the other one for control. Ten fishes of Carassius auratus (gold fish) were transferred into the 20 L experimental aquarium during the experiment and another ten C. auratus (gold fish) were also transferred into the control aquarium with no bark aqueous extract solution added to it and all other conditions held constant. The different concentrations of aqueous bark extract of A. pennata (Table 1) were added in the experimental aquarium, and the piscicidal effects were monitored and recorded during the 24 hours of experimental time. Overall, the aqueous fresh bark extracts concentrations of A. pennata were used at different concentrations starting from 5 mg/L to 225 mg/L (maximum concentration for 100 % mortality) for C. auratus (Table 1). The same experimental procedure was followed for the other two types of fish species. For D. rerio fishes, the aqueous fresh bark extracts of A. pennata were used starting from concentration 5 mg/L to 400 mg/L (maximum concentration for 100 % mortality) whereas, for C. auratus fishes, the concentrations used was from 5 mg/L to 500 mg/L (maximum concentration for 100 % mortality) (Table 1). The fishes in the control aquarium did not exhibit any behavioural changes during the experimental period, however the piscicidal effects on fishes were observed which includes irrational swimming, leaping, instability behaviour patterns and death time were recorded.

2.4. Phytochemical study

The methanol extract of A. pennata fresh bark was subjected to phytochemical screening to test the presence of phytoconstituents. Phytochemical test was carried out following standard procedures [36-40].

2.4.1. Saponins

Few ml of the plant extract was added in 10 ml of water by shaking vigorously. Formation of bubbles indicates the presence of saponins [36,38].

2.4.2. Steroids

About 1 ml of the plant extract dissolves in 10 ml of chloroform ($CHCl_3$) and equal volume of conc. H_2SO_4 was added by the sides of the test tubes. Formation of red colour on the upper layer and yellow with green fluorescence on H_2SO_4 layer indicates the presence of steroid [40].

2.4.3. Terpenoids

Take 1 ml of extract and add 0.5 ml of chloroform followed by few drops of conc. H_2SO_4. Formation of reddish-brown precipitate indicates the presence of terpenoids [36,38].

2.4.4. Alkaloids

To 1 ml of the plant extract add 2 ml of hexane and 2 % of HCl. Formation of yellow ppt indicates the presence of alkaloids [36,38].

2.4.5. Tannins

Few ml of the extract was treated with 3-4 drops of 10 % alcoholic ferric chloride ($FeCl_3$). Formation of brownish blue or black colour indicates the presence of tannins [36,38].

2.4.6. Phenols

To 2 ml of extract, 2 ml of aqueous ferric chloride was added. Formation of blue colour indicates the presence of phenols [36,38].

2.4.7. Flavonoids

Few ml of the extract was treated with 1-2 drops of sulphuric acid (H_2SO_4). Formation of intense yellow colour indicates the presence of flavonoids [36,38].

2.4.8. Glycosides

To 1 ml of the extract 0.5 mg of glacial acetic acid (CH_3COOH) and 3 drops of 1 % aqueous ferric chloride solution was added. Formation of brown ring at the interface indicates the presence of glycosides [37,39]. Phytochemical analysis of methanol bark extract of A. pennata revealed the presence of phytoconstituents like saponins, tannins, terpenoids, flavonoids, glycosides and absence of steroids, phenols and alkaloids as shown in Table 8.

2.5. Water parameters

The physicochemical parameters of the lab water used for acclimatization as well as for experiments were determined by APHA (1998). Dissolve oxygen meter (Lutron Do-5509), total dissolved solids meter (Konvio Neer) and pH meter (Ionix) were purchased for water testing. The parameters were monitored in accordance to the standard procedures recommended by [24] and the values recorded is given in Table 9.

2.6. Lethal concentration

The fish mortality within 24 hours of the experiment was plotted against the logarithm concentration to estimate the lethal concentration (LD_{50}) of A. pennata fresh bark aqueous extract. The median lethal concentration (LD_{50}) is the concentration at which 50 % of the test fish survived and 50 % died, while the concentration at which 100 % of the fish died is called LD_{100}.

2.7. Statistical analysis

A probit statistical test was performed on the collected data and logarithm of A. pennata concentration was being employed. Additionally, the response of each treatment mortality percentage was calculated against their probit values. Regression was used to construct a linear relationship between the probit values and logarithm concentration. This linear relationship was determined as follows:

$$y = bx + a \quad (1)$$

$$b = \sum \frac{(x-\bar{x})(y-\bar{y})}{(x-\bar{x})^2} = slope \quad (2)$$

The equation for the intercept of the regression line, a is;

$$a = \bar{y} - b\bar{x} \quad (3)$$

Where, y = intercept (constant by regression)

The plotted graph was used to determine the relationship equation. The 24 hours LD_{50} of A. pennata bark extract was then calculated based on the relationship. This was achieved by setting y to LD_{50} and using the following values for a and b:

$$y = bx + a \quad (4)$$

3. Results and discussion

3.1. LC_{50} of A. pennata bark extract for C. auratus, D. rerio and C. carpio at 24 hours

The mortality of C. auratus, *D. rerio* and *C. carpio* fishes were calculated for the various concentrations of *A. pennata* bark extract over the course of experimental time (24 hours) and the matching logarithmic value for each concentration used and the probit value of mortality percentage is described in the following sections and displayed in Table 2, 4 and 6.

3.2. Relationship for C. auratus between probit and log concentration of A. pennata bark extract

For each treatment, the log concentration was plotted against the probit value, which represents mortality (Table 3). The mortality and applied concentration are correlated, as shown by a regression formula. As a result, the following regression equation was obtained to represent the relationship between the probit and log concentration over a 24 hours period (Figure 2A).

$$y = mx + c$$
$$y = 2.6439x - 0.7095$$
$$5 = 2.6439x - 0.7095$$
$$5 + 0.7095 = 2.6439x$$
$$5.7095 = 2.6439x$$
$$x = 5.7095/2.6439$$
$$x = 2.1594$$

The antilogarithm of the value of x is found to be 144.34 mg/L. This value is taken as the LD_{50} of fresh bark aqueous extract of A. pennata for C. auratus at 24 hours.

3.3. Relationship for D. rerio between probit and log concentration of A. pennata bark extract

For each treatment, the log concentration was plotted against the probit value, which represents mortality (Table 5). The mortality and applied concentration are correlated, as shown by a regression formula. As a result, the following regression equation was provided to represent the relationship between the probit and log concentration over a 24 hours period (Figure 2B).

$$y = mx + c$$
$$y = 2.0873x + 0.8375$$
$$5 = 2.0873x + 0.8375$$
$$5 - 0.8375 = 2.0873x$$
$$4.1625 = 2.0873x$$
$$x = 4.1625/2.0873$$
$$x = 1.9942$$

The antilogarithm of the value of x is found to be 98.673 mg/L. This value is taken as the LD_{50} of aqueous bark extract of A. pennata for D. rerio at 24 hours.

3.4. Relationship for C. carpio between probit and log concentration of A. pennata bark extract

For each treatment, the log concentration was plotted against the probit value, which represents mortality (Table 7). The mortality and applied concentration are correlated, as shown by a regression formula. As a result, the following regression equation was provided to represent the relationship between the probit and log concentration over a 24 hours period (Figure 2C).

$$y = mx + c$$
$$y = 2.2433x - 0.1324$$
$$5 = 2.2433x - 0.1324$$
$$5 + 0.1324 = 2.2433x$$
$$5.1324 = 2.2433x$$
$$x = 5.1324/2.2433$$
$$x = 2.2878$$

The antilogarithm of the value of x is found to be 194.00 mg/L. This value is taken as the LD_{50} of aqueous bark extract of A. pennata for C. carpio at 24 hours. In this study, the three fish species, viz., C. auratus, D. rerio and C. carpio were exposed to varying concentrations of the fresh bark aqueous extract of A. pennata and they exhibited various behavioural changes. This clearly indicates that A. pennata fresh bark aqueous extract is extremely potent against the three fishes and the impacts were examined in a controlled aquarium setting. The fishes displayed unusual reactions during the experiment as they were gradually exposed to higher stress levels until they passed away from the effects of the plant extract. Their behaviours were observed simultaneously over a 24 hours period, and the results showed a variety of reactions and abnormal responses. During the acclimatization period and throughout the whole experiment, the fishes that were kept in the control aquarium remained the same and no mortality was recorded. All three different types of fish species viz., C. auratus, D. rerio and C. carpio exhibit normal swimming behaviour in the control aquarium, but erratic swimming of the treated fishes was observed in the experimental aquarium. When the fishes are treated with less concentration, they seem to be relatively unaffected by the plant bark extract since they show no sign of stressful behaviour. However, at increasing dosages, they began to exhibit various forms of discomfort. The treated fishes showed slow movement with the tendency of settle at the bottom of the aquarium, lying motionless for almost an hour, while in some cases the fishes exhibit inconsistent jumping, gulping of air on the surface and loss of balance. These abnormal behaviours of the fishes observed were due to the effect of the fresh bark aqueous extract of A. pennata. Indications of the sluggish impact of plant extracts when exposed to fishes include mucus secretion, loss of scales, stiff fin rays, gasping for air, irregular swimming and haemorrhages. Several studies revealed that the effect of the plant extract on the toxicity of test animal is because of the presence of the bioactive compounds [25-29]. It is reported that A. indica fruit mesocarp is known to have piscicidal potential [30]. Likewise, T. peruviana leaf and bark extracts also have high piscicidal activity and these poisonous effects may be due to the presence of the flavonoidapigenin-5-methyl ether and the triterpenoid glycosides in this plant [31]. In our current investigations, the fishes were swimming normally up to some extent even with the addition of the fresh aqueous bark extract of *A. pennata*,but after reaching a certain dose, they started showing response to the plant extract. For C. auratus, it started to show response to the fresh bark aqueous extract of A. pennata at the dose of 75 mg/L and complete mortality was observed at 225 mg/L (Table 1), whereas D. rerio responded at the dose of 50 mg/L and complete mortality was seen at 400 mg/L (Table 1) and for C. carpio, the fishes started to affect at the dose of 100 mg/Land complete mortality was observed at 500 mg/L(Table 1). The mortality rates of C. auratus, D. rerio and C. carpio against the concentrations of the bark extract are shown in graphs (Figure 1A, Figure 1B and Figure 1C). Abalaka et al., Obdoni and Ochuko [32-33], were reported that the toxicity might have also been due to an impairment of oxygen consumption, as saponins are known to lower the surface tension of reconstituted extracts by forming colloidal substances within them. It has also been reported that saponins are known to cause structural damage in the intestinal epithelia and respiratory of the exposed fishes, which favours our work, where saponins were present in the bark extract of A. pennata plant from phytochemical screening. We also used the methanol extract of A. pennata bark in phytochemical screening to identify the secondary metabolites that were present in this plant. The phytochemical screening of the methanol bark extract of A. pennata revealed the presence of saponins, terpenoids, tannins, flavonoids, and glycosides, while steroids, alkaloids and phenols were found to be absent, as shown in (Table 8). The observed restlessness and mortalities of the fishes might be due to the effects of tannins, terpenoids, flavonoids, glycosides and saponins that were present in the A. pennata fresh bark extract. Of all the three different types of fish species, D. rerio and C. auratus are seen to be more sensitive to the bark extract at lower concentrations like 50 mg/L and 75 mg/L as compared to C. carpio which is recorded at 100 mg/L. This clearly indicates that the higher the concentration of the bark extract of A. pennata, the higher the mortality of the fishes, which was similar to the reported study of T. tetraptera leaf powder [34-35]. Similar observations in fishes were reported when the experimental fishes showed stressful behaviours like rapid movement from top to bottom, imbalanced movement, surface floating, mucus secretion, damage to the skin, swelling of the body and subsequently leading to death when exposed to a higher concentration of the extract [27-28,34]. The correlation between probit and log concentration was demonstrated by plotting the probit value for mortality against the concentration of each treatment. C. auratus, D. rerio and C. carpio have a positive correlation between probit and log concentration, according to regression equation, which is given as y=2.6439x-0.7095 (Figure 2A), y=2.0873x+0.8375 (Figure 2B), y=2.2433x-0.1324 (Figure 2C).

Table 1. Piscicidal screening of fresh bark aqueous extract of *A. pennata* with various concentrations on *C. auratus, D. rerio* and *C. carpio* fishes in 20 L aquarium for 24 hours

Sl. No	Type of fish	Concentration (mg/L)	No. of fish	Mortality	Control
1	*Carassius auratus* (Gold fish)	0	10	0	10
2		25	10	0	10
3		50	10	0	10
4		75	10	1	10
5		100	10	2	10
6		125	10	3	10
7		150	10	5	10
8		175	10	7	10
9		200	10	8	10
10		225	10	10	10
11	*Danio rerio* (Zebra fish)	0	10	0	10
12		25	10	0	10
13		50	10	2	10
14		100	10	4	10
15		150	10	4	10
16		200	10	5	10
17		250	10	6	10
18		300	10	7	10
19		350	10	8	10
20		400	10	10	10
21	*Cyprinus carpio* (Common carp)	0	10	0	10
22		50	10	0	10
23		100	10	2	10
24		150	10	3	10
25		200	10	5	10
26		250	10	5	10
27		300	10	5	10
28		350	10	6	10
29		400	10	7	10
30		450	10	8	10
31		500	10	10	10

Table 2. Mortality and probit values for *C. auratus* at various concentrations of *A. pennata* bark extract.

Concentration (mg/L)	Mortality	% Mortality	Probit	Log concentration
0	0	0	1.03	0
25	0	0	1.03	1.39
50	0	0	1.03	1.69
75	1	10	3.72	1.87
100	2	20	4.16	2
125	3	30	4.48	2.09
150	5	50	5	2.17
175	7	70	5.52	2.24
200	8	80	5.84	2.30
225	10	100	8.95	2.35

Table 3. Log concentration and probit relationship for *C. auratus*.

Probit (Y)	Log concentration (X)
1.03	0
1.03	1.39
1.03	1.69
3.72	1.87
4.16	2
4.48	2.09
5	2.17
5.52	2.24
5.84	2.30
8.95	2.35

Table 4. Mortality and Probit values for *D. rerio* at various concentrations of *A. pennata* bark extract

Concentration (mg/L)	Mortality	% Mortality	Probit	Log concentration
0	0	0	1.03	0
25	0	0	1.03	0
50	2	20	4.16	1.69
100	4	40	4.75	2
150	4	40	4.75	2.17
200	5	50	5	2.30
250	6	60	5.25	2.39
300	7	70	5.52	2.47
350	8	80	5.84	2.54
400	10	100	8.95	2.60

Table 5. Log concentration and probit relationship for *D. rerio*.

Probit (Y)	Log concentration (X)
1.03	0
4.16	1.69
4.75	2
4.75	2.17
5	2.30
5.25	2.39
5.52	2.47
5.84	2.54
8.95	2.60

Table 6. Mortality and probit values for *C. carpio* at various concentrations of *A. pennata* bark extract.

Concentration (mg/L)	Mortality	% Mortality	Probit	Log concentration
0	0	0	1.03	0
50	0	0	1.03	1.69
100	2	20	4.16	2
150	3	30	4.48	2.17
200	5	50	5	2.30
250	5	50	5	2.39
300	5	50	5	2.47
350	6	60	5.25	2.54
400	7	70	5.52	2.60
450	8	80	5.84	2.65
500	10	100	8.95	2.69

Table 7. Log concentration and probit relationship for *C. carpio*.

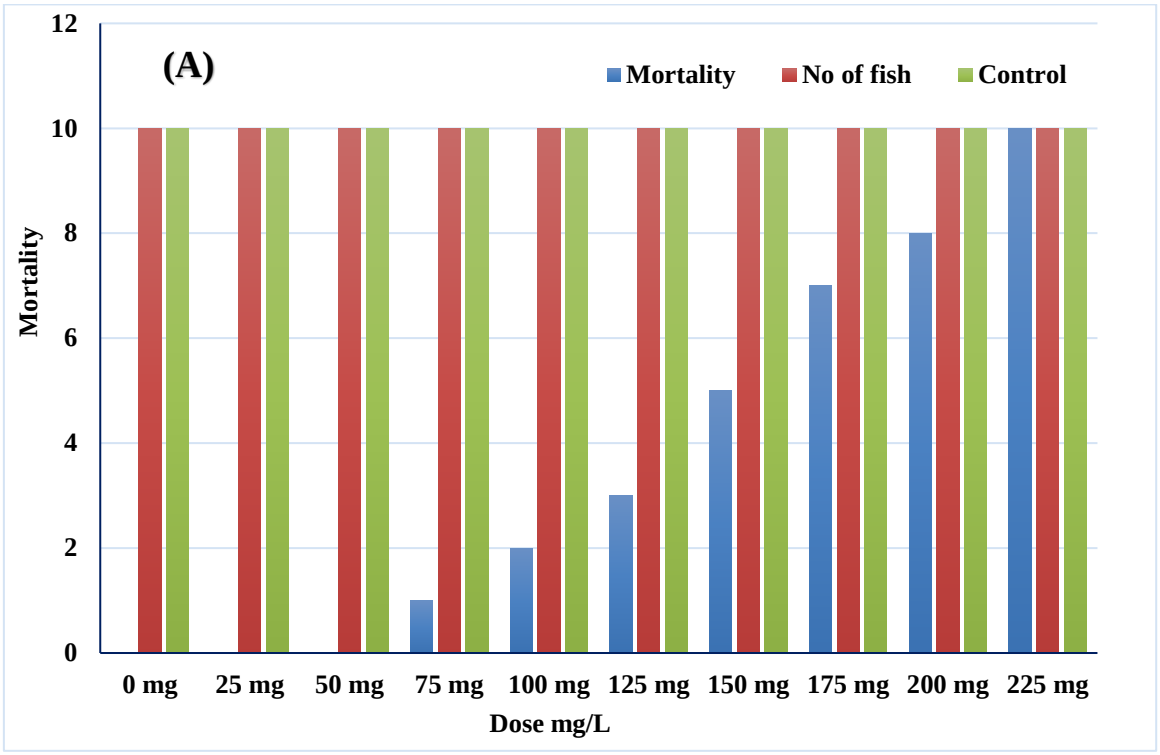
Probit (Y)	Log concentration (X)
1.03	0
1.03	1.69
4.16	2
4.48	2.17
5	2.30
5	2.39
5	2.47
5.25	2.54
5.52	2.60
5.84	2.65
8.95	2.69

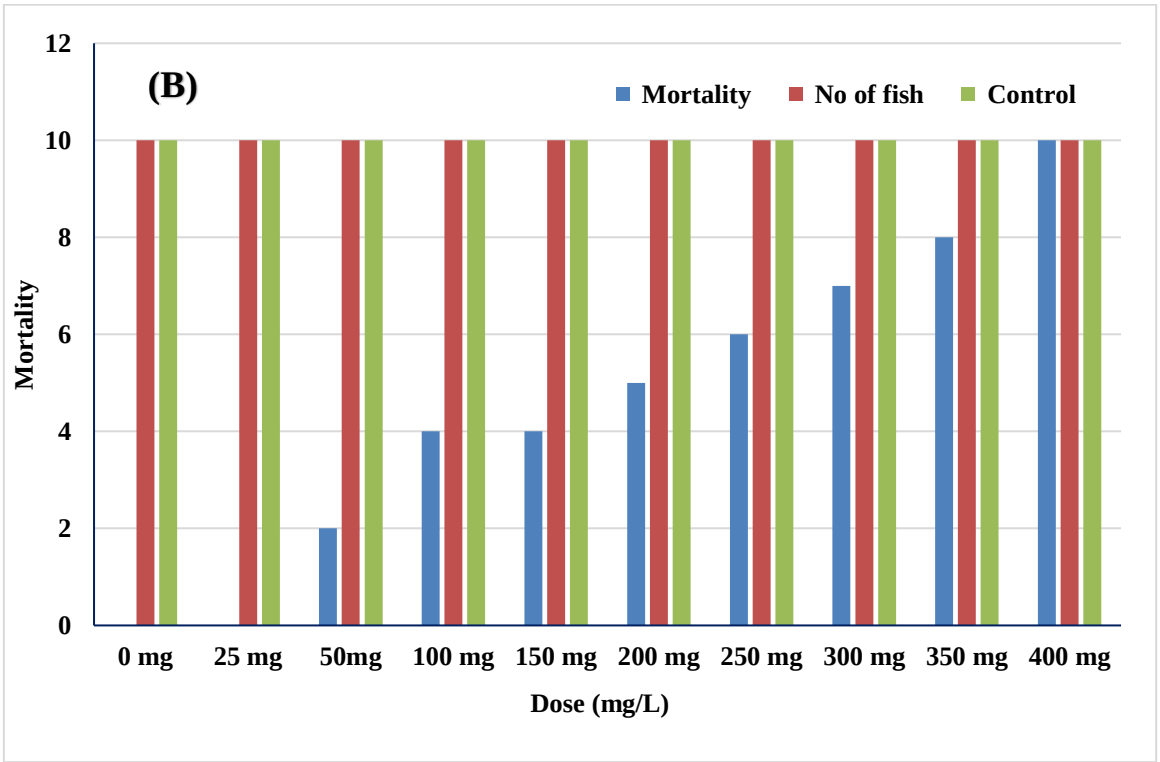
Table 8. Phytochemical constituents of methanolic bark extract of *A. pennata*.

Sl. No	Phytoconstituent	Observation
1	Saponins	+
2	Steroids	-
3	Terpenoids	+
4	Alkaloids	-
5	Tannins	+
6	Phenols	-
7	Flavonoids	+
8	Glycosides	+

Table 9. Physicochemical parameters of the lab water used for experiment.

Sl. No	Parameter	Value
1	Water temperature	30 ± 20 ℃
2	pH	7.5 ± 7
3	Dissolved oxygen	7.9 ± 7.5 ppm
4	Total dissolved salts	75 ± 70 ppm


(A)
Mortality
No of fish
Control
Mortality
12
10
8
6
4
2
0
0 mg
25 mg
50 mg
75 mg
100 mg
125 mg
150 mg
175 mg
200 mg
225 mg
Dose mg/L


(B)
Mortality
No of fish
Control
Mortality
12
10
8
6
4
2
0
0 mg
25 mg
50mg
100 mg
150 mg
200 mg
250 mg
300 mg
350 mg
400 mg
Dose (mg/L)

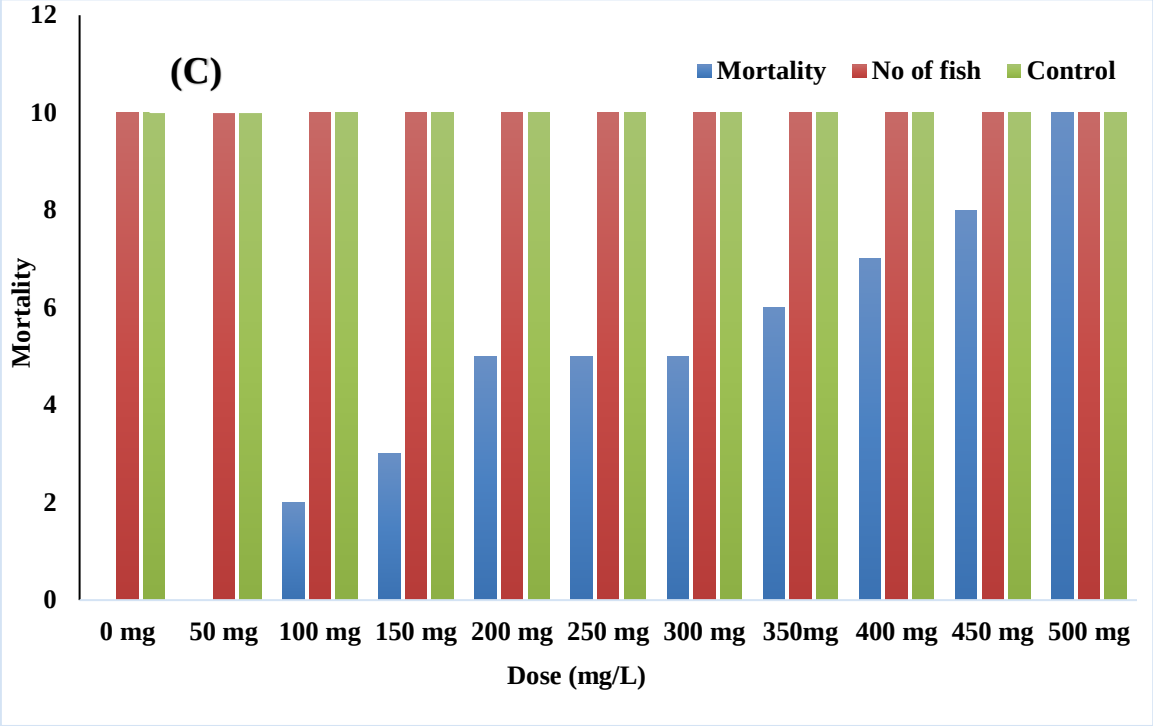
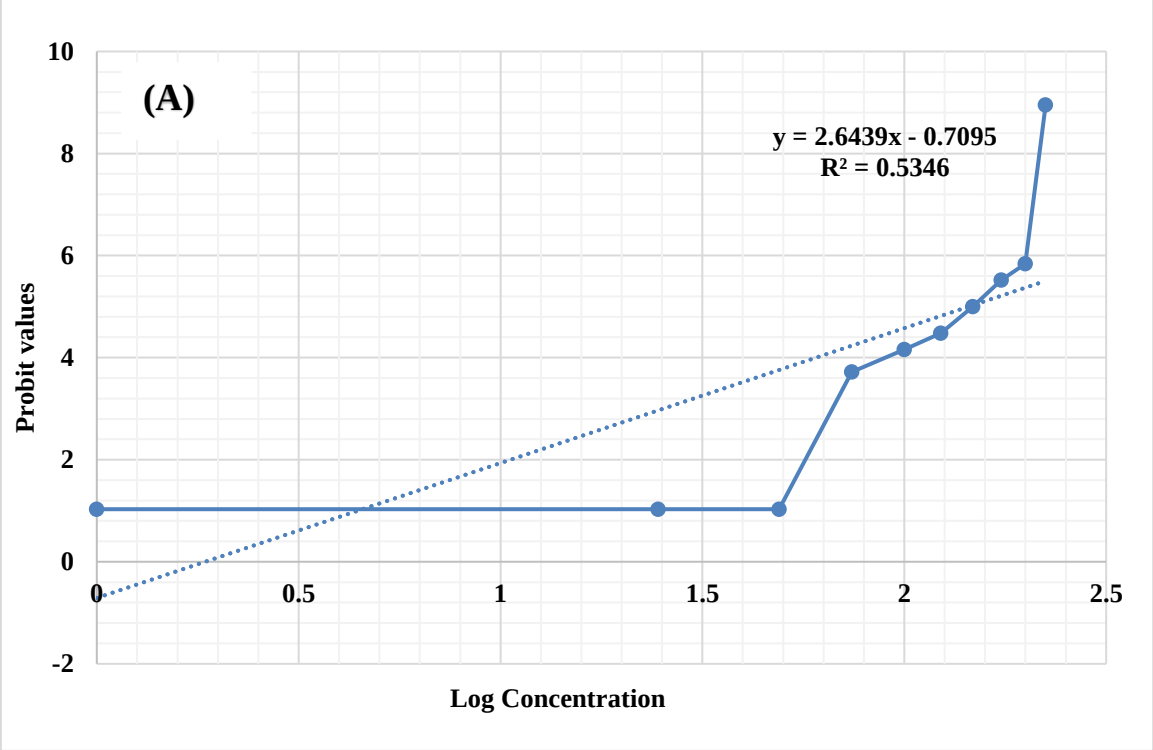
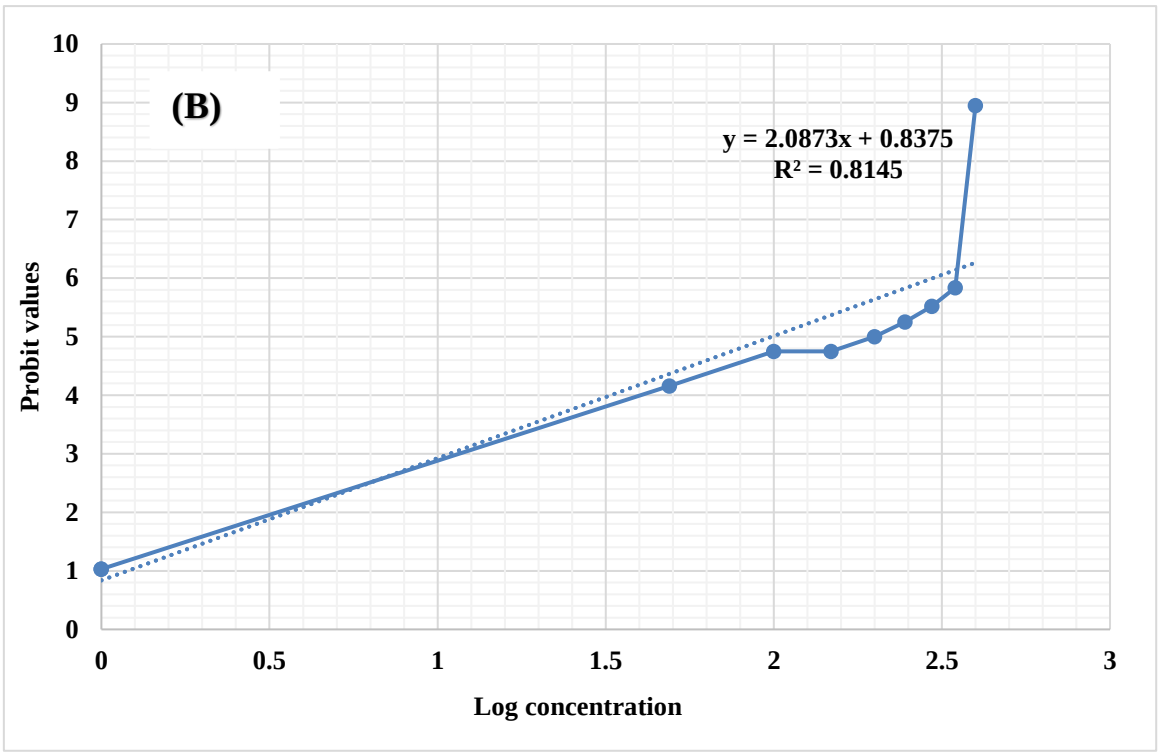


Figure 1. The mortality rate of (**A**) *C. auratus*, (**B**) *D. rerio* and (**C**) *C. carpio* at different concentrations of *A. pennata* fresh bark aqueous extract.





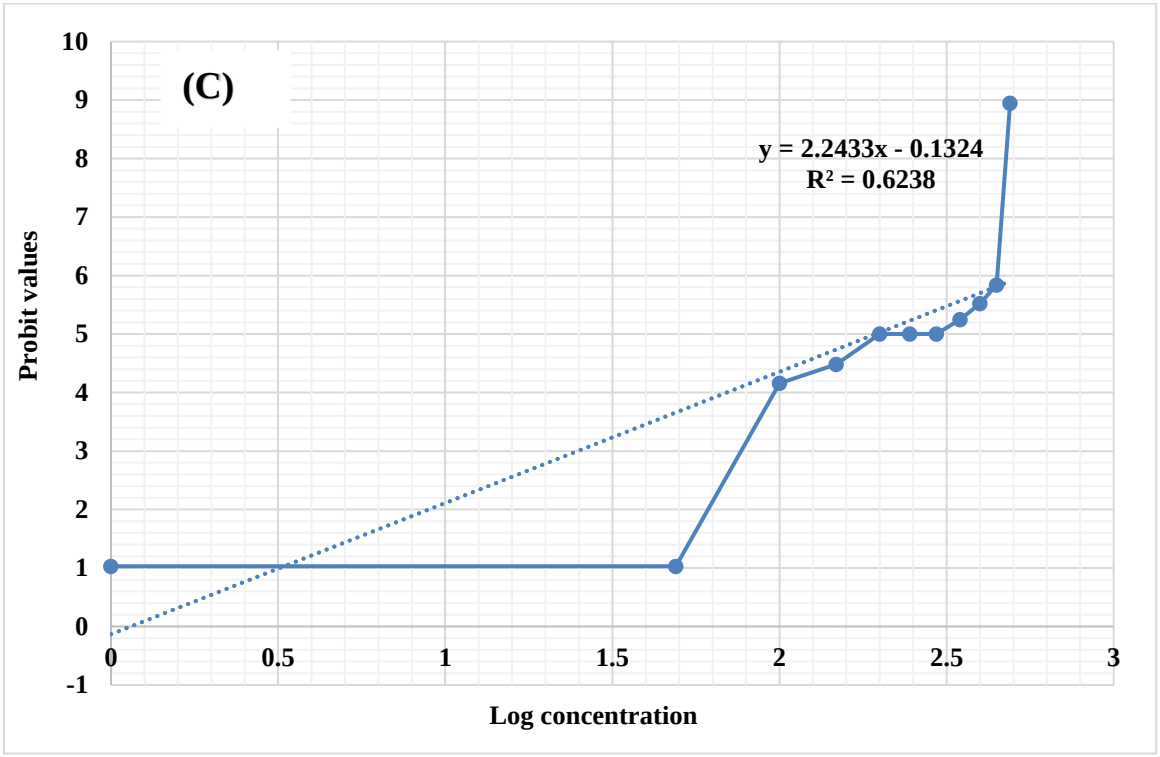


Figure 2. 24 hours LD_{50} of *A. pennata* fresh bark aqueous extract on (**A**) *C. auratus*, (**B**) *D. rerio* and (**C**) *C. carpio*

The LD_{50} of A. pennata bark against C. auratus, D. rerio and C. carpio fishes were found to be 144.34 mg/L, 98.67 mg/L and 194.00 mg/L, respectively, during the 24 hours of the experimental period. It is very clear from our study that the survival time for the fishes declined with an increase in the concentration of the fresh aqueous bark extract of A. pennata. This study revealed that A. pennata exerts piscicidal properties and is highly toxic to the fishes even at a very low concentration. Additional work was also done to evaluate the potential toxicity of methanol bark extract of A. pennata on the same fishes. The result shows that the methanol bark extract of A. pennata was found to be toxic and started to affect the fishes only at the dose of 250 mg/L and complete mortality was observed at 400 mg/L, which is comparatively higher than the dose of the aqueous bark extract of A. pennata. Among the two extracts, aqueous and methanol bark extract that was performed on the fishes, we have come to conclusion that the aqueous bark extract of A. pennata seems to be more effective at a much lower dose as compared to the methanol bark extract of A. pennata. Further, we extended our work to check the effectiveness of dry bark and fresh leaf extracts of A. pennata against the same fishes, C. auratus, D. rerio and C. carpio, but surprisingly, we observed that the fishes did not exhibit any piscicidal effect even at higher concentrations over the course of 24 hours of exposure.

4. Conclusions

The piscicidal study of the aqueous bark extract of A. pennata against the C. auratus, D. rerio and C. carpio fishes determines the potential efficacy of A. pennata fresh bark extracteven at lower concentrations. The resultant findings of the experimental fishes revealed distinct behavioural changes and abnormalities, emphasizing the toxic potential of the fresh bark aqueous extract of A. pennata, which starts at concentrations as low as 75 mg/Lfor C. auratus, 50 mg/L for D. rerio and 100 mg/L for C. carpio. Whereas, complete mortality was observed for C. auratus, D. rerio and C. carpio at concentrations of 225 mg/L, 400 mg/L and 500 mg/Lover a period of 24 hours. The LD_{50} values of the fresh bark aqueous extract of A. pennata for C. auratus, D. rerio and C. carpio were found to be 144.34 mg/L, 98.67 mg/L and 194.00 mg/L respectively, which showed significant piscicidal potential of the fresh bark extract of A. pennata. The methanol bark extract of A. pennata was also done to evaluate the potential toxicity on the fishes and we have found out that it started to affect the fishes at 250 mg/L and complete mortality was observed at 400 mg/L, which shows that the doses are comparatively higher than the aqueous bark extract. Therefore, the fresh bark aqueous extract of A. pennata emerges as a promising option for fishermen in aquaculture practices as a natural fishing agent. It's easy accessibility, eco-friendliness and potential to replace the use of expensive or harmful chemicals in aquaculture make it a favourable choice for aqua-farmers or aqua-industry for fishing practices. From our study, we can conclude that A. pennata could be used in aquaculture since the extracts are very effective even at a very low concentration, thereby, it can help reduce pollution in aquatic ecosystems, safeguard aquatic biodiversity, as well as promote human health.

Conflict of interest

The authors have no conflicts of interest regarding this investigation.

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