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Acute toxicity of Albizia procera (Roxb.) Benth. bark extract on Carassius auratus and Danio rerio Fishes

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Acute toxicity of *Albizia procera*(Roxb.) Benth. bark extract on *Carassius auratus* and *Danio rerio* Fishes

Manuscript Info

Manuscript History

Received: xxxxxxxxxxxxxxxxx

Final Accepted: xxxxxxxxxxxxx

Published: xxxxxxxxxxxxxxxxx

Key words:-

Natural product, *Albizia procera* (Roxb.) Benth., bark extract, fisheries, fishes, toxicity, LC₅₀, mortality.

This study aims to determine the lethal concentration (LC₅₀) of *Albizia procera* (Roxb.) Benth. bark extract on two different fish species; *Carassius auratus* (Gold fish) and *Danio rerio* (Zebra fish) through the acute toxicity test, focusing on the concentration-dependent changes for 24-hours exposure. The acute toxicity study conducted on *C. auratus* and *D. rerio* have revealed that the *A. procera* bark extract was effective to the fish under test, beginning with a dosage 150

Abstract

mg/L and 100 mg/L for *C. auratus* and *D. rerio*. The fishes that were exposed to the fresh bark extract of *A. procera* displayed signs of abnormal behaviours like erratic swimming patterns, positional imbalance, excessive mucus production, surface gulping of air, and settles at the bottom motionless before death. While, the fishes in the control aquarium, without *A. procera* bark extract survived the entire 24-hours exposure period. Among the two different fish species used, the bark extract of *A. procera* showed remarkable piscicidal activities on *D. rerio* as compared to *C. auratus*. The LC₅₀ values of the aqueous bark extract of *A. procera* for *C. auratus* and *D. rerio* fishes were 243.05 mg/L and 169.78 mg/L. Also, the LC₅₀ of the methanol bark extract of *A. procera* for *C. auratus* fishes was found to be 193.95 mg/L. A high concentration range of *A. procera* extract has the potential to become toxic to fishes, hence this study was done to identify the safety margin of *A. procera* bark extract to guarantee that its use is limited in order to reduce the likelihood of hazardous intake and contamination of the aquatic environment. Since no toxicity studies were investigated on this plant, the present study was done to evaluate the toxicity of the bark of *A. procera* on two fishes *D. rerio* and *C. auratus*. Additionally, *A. procera* methanol bark extract was screened for phytochemicals, revealing the presence of compounds such as saponins, phenols, tannins, alkaloids, flavonoids, steroids, terpenoids, and glycosides. health.

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Introduction:- Natural products derived from bacteria, plants, minerals, and animals have long been the foundation for treating a wide range of human illnesses¹. For primary healthcare, the majority of underdeveloped

nations rely on traditional medicines made from botanical and herbal sources. Approximately 80% of people worldwide receive their primary medical treatment from non-traditional therapies, with herbal products being the most commonly used². Due to the absence of side effects, herbal medications are currently in high demand and is growing commercially³. Plant-derived medications can effectively prevent or treat a wide range of infectious and non-infectious disorders, regardless of whether they are made from the decoctions of plants or their parts or after bioactive ingredients have been extensively enriched⁴. Around 10 % of the world's vascular flora is used medicinally, and there are secondary metabolites in many of these plants that are toxic to both people and animals⁵ of which some are toxic to fishes are classified as amino acids, cyanogen, alkaloids, phenolics, terpenoids, tannins, and saponins⁶. Plants are rich in secondary metabolites, that have pharmacological action against a number of illnesses. Due to their accessibility, cost, perceived efficacy, and safety, the use of herbal medications continues to grow popularity in many societies^{7,8}. Although the people believes that herbal treatments are safe and free, however there are potential toxicities associated with the use of herbal treatment⁹. Some of the common toxicities include acute eosinophilic pneumonia, seizures, adult respiratory distress syndrome, neurotoxicity, lung toxicity, cardiac toxicity, liver toxicity, and renal toxicity^{10,11}. The procedure used to make the herbal product, variations in the active or poisonous ingredients, or the existence of naturally occurring harmful secondary metabolites can all result in toxicity or toxic components as a result of soil chemistry and growth conditions^{12,13}. The World Health Organisation (WHO) advises that in order to safeguard the population from exposure to harmful phytochemicals, herbal treatments should undergo thorough scientific testing for both efficacy and safety. Numerous natural chemical compounds with a variety of pharmacological and therapeutic qualities can be found in medicinal plants. In order to identify potential hazards and guarantee their safe use, it is crucial to evaluate the toxicity of therapeutic herbs¹⁴. Utilising these natural resources can be very advantageous for maintaining aquaculture's sustainable growth in terms of social, economic, and environmental effectiveness^{15,16}. Piscicide may be able to help aquaculture firms overcome their problems. Synthetic piscicide is typically used to prevent bacteria, fungus, or non-target fish spawning. Synthetic pesticide, on the other hand, is chemically manufactured, non-biodegradable, harmful to aquatic life, and indirectly pollutes the marine environment^{17,18}. As a result, plant-based pesticide has emerged as a substitute and is employed in aquaculture. Plant-based pesticides are organic, natural, and have no effect on the fish they are intended for. Additionally, it is environmentally safe for both consumers and the environment. Fish farmers are mostly interested in natural piscicide because of its eco-toxic qualities^{22,23}. Herbs and medicinal plants may provide alternative antimicrobial agents, particularly piscicide, for use in fish farming and aquaculture because plant-based natural solutions are inexpensive and have a lesser potential for toxicity²³. Overall, natural products provide an alluring blend of efficacy, safety, and ecological friendliness. They align very well with the worldwide movement towards sustainable aquaculture and agriculture, which aims to preserve output while reducing negative impacts on the environment and the health of the people²⁴.

Albizia procera is a tree that is a member of the Fabaceae family. The vernacular names of *A. procera* include white siris, acacia, albizia, brown albizia. Tropical and subtropical regions are where it is most frequently found. It has many economic importance like timber, fuelwood, fodder and also has various medicinal properties in treating many ailments. All the plant parts are known to exhibit anti-cancer activity²⁵. Decoction of *A. procera* bark is used in

treating rheumatism, sinus stomach-ache, diabetes mellitus. The powdered seeds are used in treating amoebiasis and for curing urinary tract infections²⁶. The leaves are used to cure ulcers²⁷. *A. procera* leaves are said to have piscicidal and insecticidal properties, and fish poison is made from the crushed bark. *A. procera* is known to have a potential source of antioxidant property,²⁸ bark of *A. procera* has a good anti-HIV-1 integrase activity,^{29, 30} antidiabetic activity,³⁰ and *A. procera* leaf extract has the ability as target linked to Alzheimer's disease³¹. From the previous works, phytochemical study of *A. procera* leaves methanolic and aqueous extracts shows the presence of saponins, tannins, steroids, flavonoids, glycosides^{32, 33}.

Investigating concentration-dependent variations in the acute toxicity of *A. procera* bark extracts was the goal of our study in *C. auratus* and *D. rerio*. Plant extracts can also be fatal and become poisonous to different organs in a concentration-dependent manner, according to numerous studies³⁴. In the current investigation, toxicity tests were conducted on fishes that showed mortality at different dosages. More bark extract concentrations are thought to harm fish and may affect their behaviour, which might be investigated in accordance with that prediction. Due to the lack of research on the toxicity assessments of fish exposed to different doses of the *A. procera* bark extracts, this study was carried out to understand *A. procera* toxic level. This study aims to assess the toxicity of the fish-poisoning plant of *A. procera* (Roxb.) Benth. aqueous bark extract on *C. auratus* (Gold fish) and *D. rerio* (Zebra fish) in an aquarium setting to find the median lethal concentrations (LC₅₀) following a 24-hours exposure period.

MATERIALS AND METHODS:-

Collection and preparation of *A. procera* bark aqueous extract

Fresh *A. procera* bark were collected from Nagaland University, Lumami campus under Zunheboto district, Nagaland, India. 200 g of *A. procera* bark were measured, cut into smaller pieces and pounded using a clean mortar and pestle. The pounded bark materials were mixed with 2L of water, filtered and the extract was stored in an airtight container and used for the dose-dependent piscicidal experiments.

Methanol extraction of *A. procera* bark

50 g fresh bark of *A. procera* was extracted in Soxhlet apparatus for 72 hours, using 150 ml of methanol solvent. The extracts obtained were dried and measured. The yield was found to be 3.531 g and the colour of the crude extract was dark-brown shiny solid.

Test fish collection and acclimatization

The test fishes *C. auratus* (Gold fish) and *D. rerio* (Zebra fish) were purchased from Dimapur, Nagaland, India, as per our study requirements. The fishes were transferred into 60 L rectangular aquarium and acclimatized to the laboratory conditions for 14 days before piscicidal experiments. The aquarium was maintained with a continuous well aerated condition and other water parameters as per the standard procedures.⁵⁹ The fishes were fed 'Tokyu' meal twice a day during the 14 days acclimatization period. Despite the fact that the water was regularly changed, waste feeds and faeces were siphoned away to prevent water contamination. Fish feeding was stopped for 24 hours before the piscicidal experiment in order to clear the fish digestive system. 10 fishes of each species were randomly selected from the 60 L aquarium, and their length and weight were measured. The *C. auratus* fish species were similar in size with a weight of 20±1 g and length of 10±1 cm. Similarly, *D. rerio* fish species have a weight of 0.578±0.01 g, and a length of 5±1 cm respectively.

78

79 Experimental set-up for dose-dependent toxicity study

21 80 The experimental set-up consisted of two rectangular-shaped glass aquariums, measuring 38 cm x 26 cm x 26 cm
1 81 were used in this study. Each aquarium was filled with 20 L of tap water; one used for experimental aquarium and
1 82 the other used as control aquarium. Ten fishes of *C. auratus* were transferred into the experimental aquarium, and
83 another ten fishes of *C. auratus* were also transferred into the control aquarium with no *A. procera* bark extract
13 84 solution and the other standard conditions like pH, temperature, dissolved oxygen and total hardness of the aquarium
85 water were analysed as per the standard methods.⁵⁹ The different concentrations of *A. procera* aqueous bark extract
86 were added to the experimental aquarium, and the toxic effects were monitored and recorded for 24-hours
1 87 experimental time (Table 1.1). The *A. procera* aqueous bark extract was utilised for *C. auratus* fishes at
88 concentrations ranging from 5 mg/L to 350 mg/L. *D. rerio* was treated in the same way and the concentration of *A.*
89 *procera* bark aqueous extract started from 5 mg/L to 300 mg/L (Table 1.1). Same methodology was followed for the
90 *A. procera* bark methanol extract against *C. auratus* (Table 1.2).

91

92 Phytochemical screening

93 The *A. procera* bark methanol extract was carried out for phytochemical screening. The test was conducted using
94 standard procedures⁵⁴⁻⁵⁸.

95 Saponins

96 5 mg of the *A. procera* methanol bark extract added to 10 ml of water and shaken well. Formation of bubbles confirms
97 that saponins are present.

98

99 Steroids

100 10 ml of chloroform (CHCl_3) and concentrated sulphuric acid (H_2SO_4) have been added to 3 mg of the *A.*
101 *procera* methanol bark extract. Steroids are present when the upper layer becomes red, yellow and fluoresces green on
102 the H_2SO_4 layer.

103 Terpenoids

104 When 0.5 ml CHCl_3 , few drops of concentrated H_2SO_4 are added to 3 mg of the *A. procera* methanol bark extract,
105 reddish-brown precipitate formation indicates that terpenoids are present.

106 Alkaloids

8 107 After mixing 3 mg of *A. procera* methanol bark extract with 2 ml of hexane and 2% hydrochloric, a yellow precipitate
108 was formed indicating the presence of alkaloids.

109 Tannins

110 3-4 drops of 10 % alcoholic ferric chloride (FeCl_3) were mixed 3 mg of *A. procera* methanol bark extract. Brownish
111 blue or black colour formation indicates that tannins are present.

112 Phenols

29 113 3 mg of *A. procera* methanol bark extract was added to 2 ml of aqueous ferric chloride (FeCl_3). Formation of blue
114 colour confirms that phenols are present.

115 Flavonoids

116 A few drops of concentrated H_2SO_4 are added to 3 mg of *A. proceramethanol* bark extract, intense yellow colour was
117 formed which indicates the presence of flavonoids.

118

119 Glycosides

120 0.5 ml of the glacial acetic acid (CH_3COOH) with 2-3 drops of 1 % aqueous (FeCl_3) was mixed to 3mg of *A.*
121 *proceramethanol* bark extract. Brown ring appearance at the interface determines that glycosides are present.

122 Lethal concentration

123 The LC_{50} of the aqueous bark extract of *A. procera* bark was determined by plotting fish mortality during a 24-hours
124 period against the logarithm concentration. The dose at which 100 % of the test fish died is known as the LC_{100} , while
125 the median lethal concentration, or LC_{50} , is the concentration at which 50 % of the fish survived and 50 % died.

126 Statistical analysis

127 The logarithm of *A. procera* concentration was used in a probit statistical analysis of the data that was gathered. The
128 percentage of each treatment mortality response was computed in relation to the values of probit. Regression analysis
129 revealed a linear relationship between the logarithm concentration and the probit values. This is how linear
130 relationship was established.

$$131 \quad y = bx + a \quad (1)$$

$$132 \quad b = \frac{\sum (x - \bar{x})(y - \bar{y})}{\sum (x - \bar{x})^2} = \text{slope} \quad (2)$$

133 The following is the regression line intercept equation, a, is,

$$134 \quad a = \bar{y} - b\bar{x} \quad (3)$$

135 Where, y = intercept (constant)

136 Using this relationship equation, the 24-hours LC_{50} of the aqueous bark extract of *A. procera* was determined. This
137 was accomplished by using a and b values and setting y to LC_{50} :

$$138 \quad y = bx + a$$

139

140 RESULTS:-

141 Phytochemical screening of *A. proceramethanol* bark extract

142 Phytochemicals screening of *A. procera* bark methanol extract reveals the presence of bioactive compounds such as
143 saponins, phenols, tannins, alkaloids, flavonoids, steroids, terpenoids, and glycosides.

144 LC_{50} of *A. procerabark* extract for *C. auratus* and *D. rerio* fishes at 24 hours

145 The mortality rate of *C. auratus* and *D. rerio* fishes was determined for a 24-hours period using varying
146 concentrations of *A. procera* bark extract. The corresponding logarithmic value for each treatment utilisation,
147 together with the mortality % probit value, are explained and displayed in the sections that follow as given in Table
148 1.3 and Table 1.4.

1 Relationship for *C. auratus* between probit and log concentration of *A. procera* aqueous bark extract

Mortality of *C. auratus* was calculated using the concentration of log of *A. procera* aqueous bark extract which will be plotted against the probit value for each treatment (Table 1.3). A regression formula shows a correlation between applied concentration and mortality. The correlation between log concentration and the probit value over a 24-hours period was represented by the regression equation that follows (Figure 1.4).

$$y = mx + c$$

$$y = 6.5274x - 10.573$$

$$5 = 6.5274x - 10.573$$

$$5 + 10.573 = 6.5274$$

$$10.573 = 6.5274$$

$$x = 10.573/6.5274$$

$$x = 2.3857$$

The antilogarithm of the value of x is found to be 243.05 mg/L. This value is taken as the LC_{50} of aqueous bark extract of *A. procera* for *C. auratus* at 24-hours.

Relationship for *D. rerio* between probit and log concentration of *A. procera* aqueous bark extract

The log concentration of *A. procera* aqueous bark extract in each treatment was plotted against mortality-measurement probit values of *D. rerio* (Table 1.4). A regression formula shows that the mortality and applied concentration are associated. The correlation between the log concentration and the probit over a 24-hours period was represented by the regression equation that follows (Figure 1.5).

$$y = mx + c$$

$$y = 7.1772x - 11.005$$

$$5 = 7.1772x - 11.005$$

$$5 + 11.005 = 7.1772x$$

$$16.005 = 7.1772x$$

$$x = 16.005/7.1772$$

$$x = 2.2299$$

The antilogarithm of the value of x is found to be 169.78mg/L. This value is taken as the LC_{50} of aqueous bark extract of *A. procera* for *D. rerio* at 24-hours.

Relationship for *C. auratus* between probit and log concentration of *A. proceramethanol* bark extract

The probit value calculates mortality of *C. auratus* which was plotted against the log concentration of *A. proceramethanol* bark extract in each treatment (Table 1.5). The regression formula shows a correlation between applied concentration and mortality. The link between the log concentration probit over a 24-hours period was reflected in the regression equation that follows (Figure 1.6).

For *C. auratus* (methanol bark extract)

$$y = mx + c$$

$$y = 6.5095x - 9.8922$$

$$5 = 6.5095x - 9.8922$$

$$5 + 9.8922 = 6.5095x$$

187 14. 8922 = 6.5095x

188 x = 14. 8922/6.5095

189 x = 2.2877

190 The antilogarithm of the value of x is found to be 193.95 mg/L. This value is taken as the LC₅₀ of methanol bark
191 extract of *A. procera* for *C. auratus* at 24-hours.

192 Table 1.1. Piscicidal screening of *A. procera* bark aqueous extract with different concentrations on *C. auratus* and *D. rerio* fishes in 20 L
193 aquarium for 24-hours

Exp. No.	Type of fish	Dose (mg/L)	No of fish	Mortality	Control
1	<i>Carassius auratus</i> (Gold fish)	5 mg	10	0	10
2		25 mg	10	0	10
3		125 mg	10	0	10
4		150 mg	10	1	10
5		175 mg	10	1	10
6		200 mg	10	1	10
7		225 mg	10	2	10
8		250 mg	10	3	10
9		275 mg	10	5	10
10		300 mg	10	7	10
11		325 mg	10	8	10
12		350 mg	10	10	10
1	<i>Danio rerio</i> (Zebra fish)	5 mg	10	0	10
2		25 mg	10	0	10
3		50 mg	10	0	10
4		100 mg	10	1	10
5		125 mg	10	1	10
6		150 mg	10	2	10
7		175 mg	10	4	10
8		200 mg	10	5	10
9		225 mg	10	5	10
10		250 mg	10	7	10
11		275 mg	10	8	10
12		300 mg	10	10	10

194

195 Table 1.2. Piscicidal screening of *A. procera* bark methanol extract with different concentrations on *C. auratus* fishes in 20 L aquarium for
196 24 hours

Exp. No.	Type of fish	Dose (mg/L)	No of fish	Mortality	Control
1	<i>Carassius auratus</i> (Gold fish)	5 mg	10	0	10
2		25 mg	10	0	10
3		50 mg	10	0	10
4		100 mg	10	1	10
5		150 mg	10	2	10
6		200 mg	10	3	10
7		250 mg	10	5	10
8		300 mg	10	6	10
9		350 mg	10	8	10
10		400 mg	10	10	10

197

198 Table 1.3. Probit values obtained for different concentrations of *A. procera* aqueous bark extract on *C. auratus* fishes for 24-hours

Sl. No.	Concentration (mg/L)	Mortality	% Mortality	Probit	Log concentration
1	5	0	0	0	0.69
2	25	0	0	0	1.39
3	125	0	0	0	2.09
4	150	1	10	3.77	2.17

5	175	1	10	3.77	2.24
6	200	1	10	3.77	2.30
7	225	2	20	4.23	2.35
8	250	3	30	4.56	2.39
9	275	5	50	5.13	2.43
10	300	7	70	5.74	2.47
11	325	8	80	6.18	2.51
12	350	10	100	8.95	2.54

Table 1.4. Mortality and probit values for *D. rerio* fishes at different concentrations of *A. procera* aqueous bark extract for 24-hours

Sl. No.	Concentration (mg/L)	Mortality	% Mortality	Probit	Log concentration
1	5	0	0	0	0.69
2	25	0	0	0	1.39
3	50	0	0	0	1.69
4	100	1	10	3.77	2
5	125	1	10	3.77	2.09
6	150	2	20	4.23	2.17
7	175	4	40	4.85	2.24
8	200	5	50	5.13	2.30
9	225	5	50	5.13	2.35
10	250	7	70	5.74	2.39
11	275	8	80	6.18	2.43
12	300	10	100	8.95	2.47

Table 1.5. Probit values obtained for different concentration of *A. procera* methanol bark extract on *C. auratus* fishes for 24-hours

Sl. No.	Concentration (mg/L)	Mortality	% Mortality	Probit	Log concentration
1	5	0	0	0	0.69
2	25	0	0	0	1.39
3	50	0	0	0	1.69
4	100	1	10	3.77	2
5	150	2	20	4.23	2.17
6	200	3	30	4.56	2.30
7	250	5	50	5.13	2.39
8	300	6	60	5.41	2.47
9	350	8	80	6.18	2.54
10	400	10	100	8.95	2.60

Table 1.6. Phytochemical screening of *A. procera* bark methanol extract

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

Table 1.7. Solubility test of methanol bark extraction of *A. procera*

Sl. No.	Solvent	Observation
1	Water (H ₂ O)	Soluble
2	Methanol (CH ₃ OH)	Soluble
3	Ethanol (C ₂ H ₅ O)	Almost soluble
4	Chloroform (CHCl ₃)	Almost soluble

5	Hexane (C ₆ H ₁₄)	Partially soluble
6	Dimethyl sulfoxide (DMSO)	Soluble
7	Acetone (C ₃ H ₆ O)	Not soluble
8	Ethyl acetate (EtOAc)	Not soluble
9	Dichloromethane (CH ₂ Cl ₂)	Not soluble

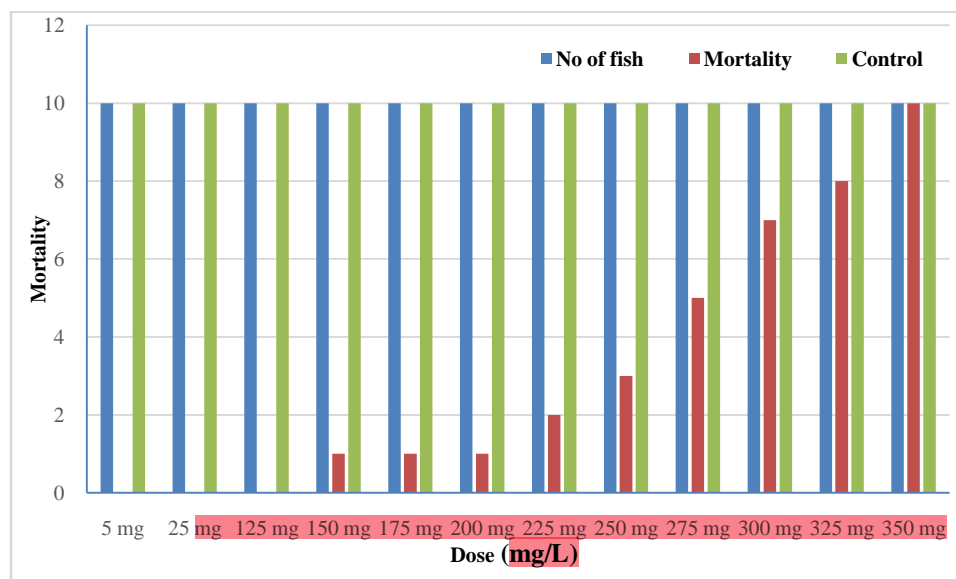


Figure 1.1. The mortality rate of *C. auratus* at different concentrations of *A. proceraaqueous* bark extract for 24-hours

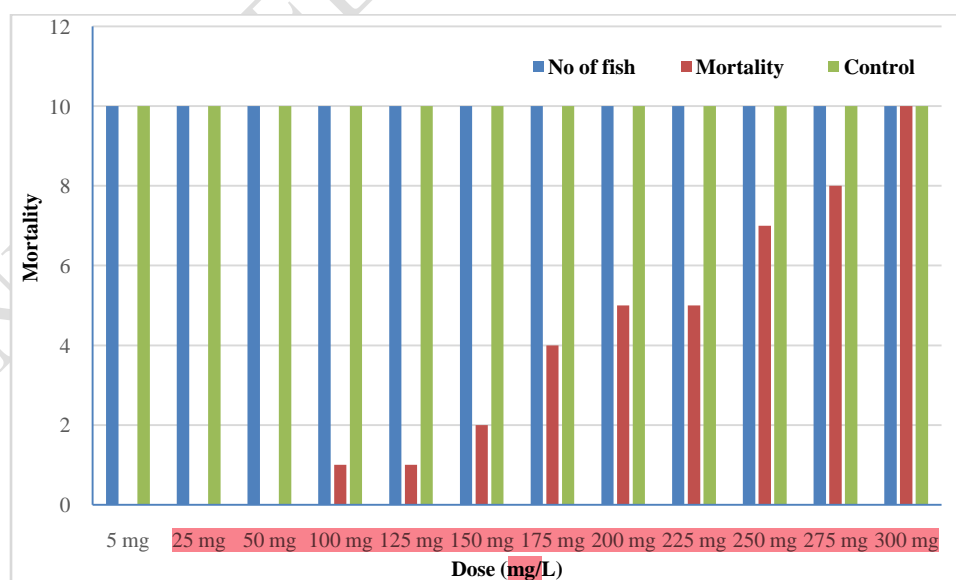
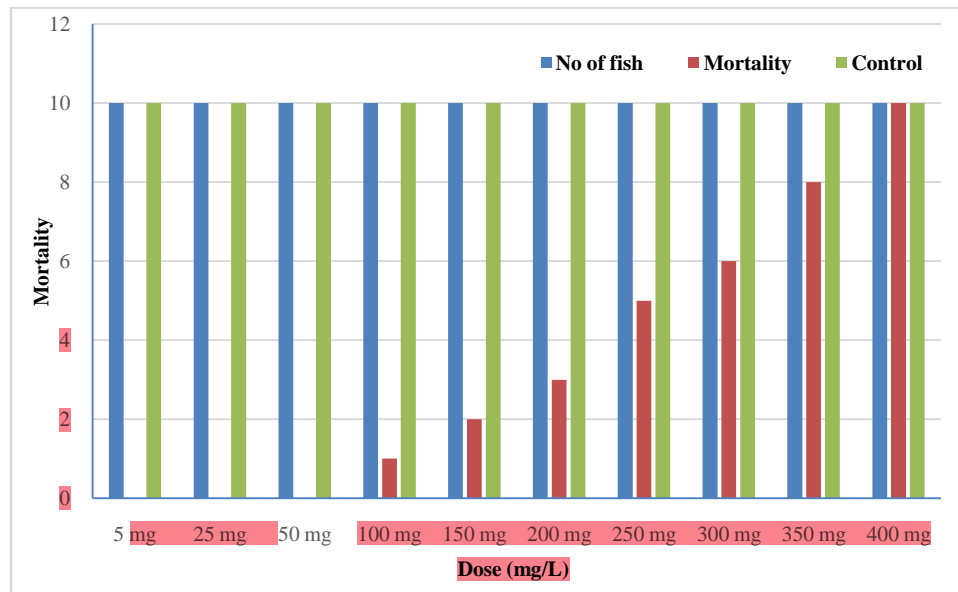


Figure 1.2. The mortality rate of *D. rerio* at different concentrations of *A. proceraaqueous* bark extract for 24-hours

220

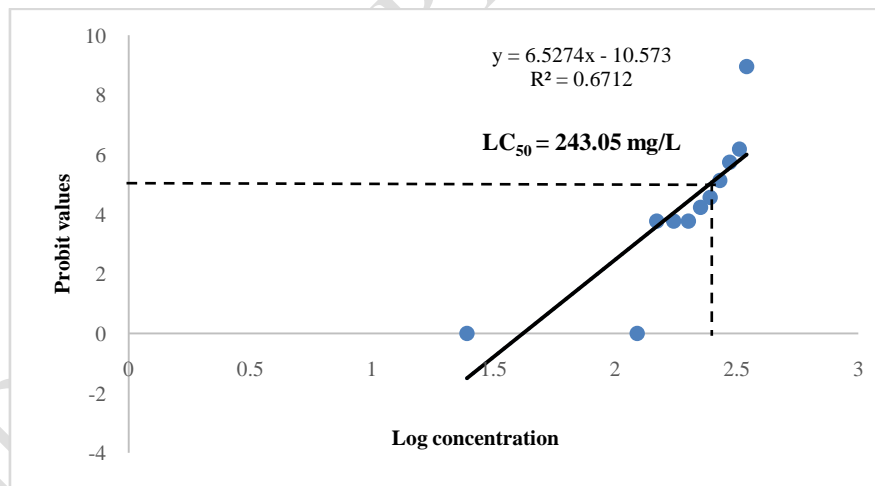


221

Figure 1.3. The mortality rate of *C. auratus* at different concentrations of *A. procera* methanol bark extract for 24-hours

223

224



225

Figure 1.4. 24-hours LC_{50} of *A. procera* fresh aqueous bark extract on *C. auratus* fishes

227

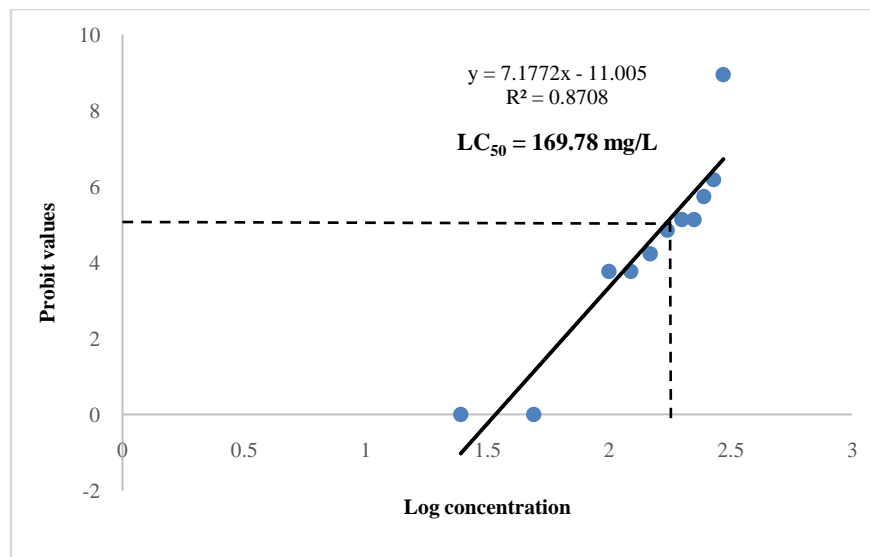


Figure 1.5. 24-hours LC₅₀ of *A. procera*freshaqueous bark extract on *D. rerio*fishes

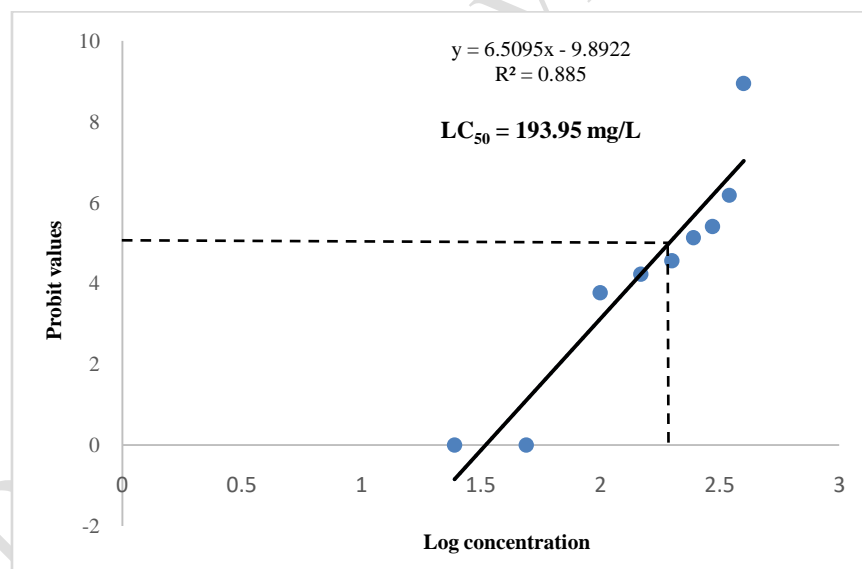


Figure 1.6. 24-hours LC₅₀ of *A. procera*freshmethanol bark extract on *C. auratus*fishes

DISCUSSION:-

In this experiment, the aqueous bark extract of *A. procera* was tested for acute toxicity to two different fish species *C. auratus* and *D. rerio*. Ten *C. auratus* were selected randomly from the 60 L rectangular aquarium and transferred into the experimental aquarium (20 L) and another ten more *C. auratus* were also transferred into the control aquarium (20 L) with no aqueous bark extract added to it and all other conditions kept constant.⁵⁹ Likewise, same

procedure was also followed for *D. rerio*. The water was continuously aerated in both the experimental and control aquarium from the starting till the end of the experiment. The fishes were starved for 24-hours prior to experiment and they were not fed throughout the whole experiment. Both the fishes, *C. auratus* and *D. rerio* were stressed progressively with time before death and the mortality rates were closely monitored and documented.

Different concentrations of *A. procera* aqueous bark extract (5, 25, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350 mg/L) for *C. auratus* (Table 1.1 and Figure 1.1); and (5, 25, 50, 100, 125, 150, 175, 200, 225, 250, 275, 300 mg/L) for *D. rerio* (Table 1.1 and Figure 1.2) were used for the piscicidal experiment, like each one of the concentration was added to the experimental aquarium to check its toxicity effect on fishes and monitored for 24-hours times. Fish mortalities were observed at the end point of toxicity experiment and recorded at every hour throughout the 24-hours exposure. The percentage mortalities recorded for the aqueous bark extract of *A. procera* exposed to *C. auratus* and *D. rerio* for 24-hours is shown in Table 1.1, and for the methanol bark extract of *A. procera* is represented in Table 1.2 and Figure 1.3. During the observation period, any dead fish found under any circumstances were removed from the experimental aquarium to prevent fouling. When *C. auratus* were exposed to the aqueous bark extract of *A. procera*, they did not show any distressed behaviour from the first few initial concentrations, but instead they were swimming normally from the starting concentration of 5 mg/L till 125 mg/L. Gradually, with the increase in concentration, *C. auratus* started to show response to the aqueous bark extract of *A. procera* at a concentration of 150 mg/L onwards and we observed 10 % mortality under 24-hours exposure. While there were no much fluctuations in the mortality of *C. auratus* until they reach up to a certain concentration of 200 mg/L. However, some fishes started to show stressful and abnormal behaviours and we recorded 20 % mortality from 225 mg/L and 30 % mortality was observed at a concentration of 250 mg/L. But, interestingly at this time, these abnormal and restlessness responses subsided to some fishes after 10 to 20 minutes. Later, at a higher concentration of 275 mg/L we recorded 50 % mortality for *C. auratus* and at this time, the fishes struggled to recover and showed more suffocation and distressful movements. The remaining fishes were also affected because of the aqueous bark extract of *A. procera*, but after a few hours they recover and regain back to their normal behaviour again. This shows that the fishes have the mechanism to detoxify the bioactive compounds present in the aqueous extracts of *A. procera* that allows them to recover from the initial stress. Lastly, the fishes showed signs of extreme stress by repeatedly swimming up and down or along the sides of the aquarium, becomes inactive, lying motionless at the aquarium floor or at the surface of the water, bodies become stiffened and that was how 100 % mortality was recorded at 350 mg/L respectively.

There were significant differences in the toxicity of the aqueous bark extract of *A. procera* when exposed to *D. rerio*. This signifies that the toxicity of *A. procera* differs with the concentration and the type of the test fish species used in the experiment. There was a slight fluctuation in the mortality of *D. rerio* as compared to *C. auratus* because *D. rerio* did not show any effect to the aqueous bark extract of *A. procera* from 5 mg/L till 75 mg/L. From then on, *D. rerio* encountered distressed symptoms, started to show some abnormal response and we recorded 10 % mortality at a concentration of 100 mg/L which is relatively lower than *C. auratus*. Likewise, we recorded 50 % mortality of *D. rerio* at a concentration of 200 mg/L and 100 % mortality at 300 mg/L. No mortalities were observed in the control aquarium throughout the all experiments for both *C. auratus* and *D. rerio* fishes as shown in Table 1.1. By exposing the aqueous bark extract of *A. procera* at various effective concentrations, both *C. auratus* and *D. rerio* fishes

exhibited several behavioural activities which were different from the fishes in the control aquarium. This clearly shows that these abnormal stressful behavioural responses which leads to the fish mortality. As fish mortality increases with the increase in concentrations of the *A. procera* aqueous bark extract (dose-dependant). We have carefully examined the effects of aqueous bark extracts of *A. procera* when exposed to *C. auratus* and *D. rerio*. Gradually, the fishes became inactive at a basic effective concentration and subsequently loses their balance as the concentration of aqueous bark extract of *A. procera* goes higher. The affected fishes after exposing to aqueous bark extract of *A. procera* shows hyperactivity, hyperventilation, swimming erratically, gulping for air and lying motionless at the bottom till death. The total death fishes throughout 24-hours in the respective extract concentrations were systematically recorded for determining the LC_{50} or lethal concentrations of the *A. procera* bark extracts. Also, the mortality of *C. auratus* when exposed to methanol bark extract of *A. procera* is represented in Table 1.2. For methanol bark extract of *A. procera*, no mortalities were observed from 5 till 50 mg/L for *C. auratus*; but the mortalities notably increased from 100 to 400 mg/L. *C. auratus* displayed a variety of behaviours in response to varying concentrations of the methanol bark extract of *A. procera* and we recorded 10 % mortality at 100 mg/L. Due to this piscicidal action of methanol bark extract of *A. procera*, various abnormal behaviours of *C. auratus* were observed again in most of the fishes such as breathing problems, irregular swimming, restlessness, loss of equilibrium, gulping for air at the surface, mouth wide open or laterally extended fins, but at this point, it was hard for them to recover and that was how we recorded 50 % mortality at a concentration of 250 mg/L and 100 % mortality at 400 mg/L. In the treated experimental aquarium, all the fishes displayed aggressive tendencies after exposure to the aqueous and methanol bark extracts of *A. procera* at the effective concentrations. Our findings are in agreement with,^{35,36} which stated that any herbal or natural plant source could either be detrimental or beneficial to any fish since the toxicity level mainly depends on the applied extract concentrations and targeted fish species. Here, both *C. auratus* and *D. rerio* became lethargic to the aqueous and methanol bark extracts of *A. procera*, lost their balance and their fins get stiffened, bulged eyes occurred, their scales fell off, tails and fins were broken, and ultimately those conditions led them to death. While some dead fishes float vertically or parallel to the bottom of the aquarium, others stay flat on the surface of the water. Prior to death, the fishes showed changes in body colour and slowed movements or responses as compared with the normal fishes in the control aquarium. Since the fishes in the control aquarium exhibit no such behavioural abnormalities, it is evident that the aqueous and methanol bark extracts of *A. procera* was the sole cause of mortality to all the fishes used in the piscicidal experiment. These abnormal behavioural responses we observed in our study are comparable to the findings of *Latifa et al.*, (1993), (1997); *Nasiruddin et al.*, (1998), (2002); *Ashraf et al.*, (2010); *Chowdhury et al.*, (2014); *Nasiruddin et al.*, (2014)³⁷⁻⁴². Excessive mucus secretion in exposed fish was consistent with the findings by *Jothivel and Paul* (2008), *Abalaka and Auta* (2010), and *Orji et al.* (2014)⁴³⁻⁴⁵. The gills began to release mucus after five to ten hours on average, and a film of mucus covered the entire body. Excessive mucus secretion is known to be the form of defence mechanisms to coat their body surface when the fishes are exposed to the toxicants so as to stop or lessen the plant toxicants uptake⁴⁶. Fish respiratory functions are known to be lowered by such extensive mucus productions⁴⁷. Piscicidal abilities and phytotoxic properties of *A. procera* aqueous and bark extracts was observed and similar studies has reported by a number of researchers, including *Fafioye* (2012), *Akinwande et al.*, (2007), and

Adewole *et al.*, (2002)⁴⁸⁻⁵⁰. With the passing of time, the normal colours of the eyes also changed drastically. *C. auratus* and *D. rerio* remained in the state of exhaustion and their body were fully discoloured, which afterwards failed to react to the outside stimuli. Fish were considered dead if they showed no signs of movement and did not react to physical contact. After recording mortalities, dead fish were removed. Negative physiological impacts of *C. auratus* and *D. rerio* after exposing to the aqueous and methanol bark extract of *A. procera* were evident in this study that results in behavioural deviation which was followed by death. Failure of the neurological system could be the cause of the aqueous and methanol bark extracts of *A. procera* as reported by Ufodike and Omeregbe, 1994⁵¹. We examined the sensitivity of the fresh aqueous and methanol bark extracts of *A. procera* in this work, and the results indicated that throughout the 24-hours exposure period, *C. auratus* fish had a greater fatal effect than *D. rerio* fish. The mortality probit value is plotted versus each treatment concentration of *C. auratus* and *D. rerio* has shown that the regression equation shows a positive relationship between probit and log concentration, which is provided as, $y = 6.5274x - 10.573$, $y = 7.1772x - 11.005$, whereas, for the methanol bark extract of *A. procera* against *C. auratus* is given as $y = 6.5095x - 9.8922$. Probit analysis was applied to determine the relative LC₅₀ or lethal concentration of aqueous and methanol bark extracts of *A. procera* that caused death at 50 % from the tested fishes in the exposure period for 24-hours. Through this analysis, LC₅₀ of aqueous bark extract of *A. procera* for *C. auratus* and *D. rerio* were found to be 243.05 mg/L and 169.78 mg/L as shown in Figure 1.4 and Figure 1.5. Additionally, the LC₅₀ of the methanol bark extract of *A. procera* for *C. auratus* was found to be 193.95 mg/L as shown in Figure 1.6. In this study, the LC₅₀ value was relatively lesser for *D. rerio* as compared to *C. auratus*. This implies that *A. procera* aqueous bark extract is more toxic to *D. rerio* fishes as compared to *C. auratus* fishes. However, it is crucial to remember that the variations between this study and the previously stated studies may result from variations in species, size, age, and experimental settings. Likewise, the effectiveness and the differences may also depend on various factors like the duration of the treatment, specific plant parts used, its concentrations and the kind of fish species involved. We found that the mortality of *C. auratus* and *D. rerio* was clear and positively correlated with the concentrations of the aqueous and methanol bark extract of *A. procera*. These outcomes are comparable to those of Tiwari and Singh (2003) and Dan and Sogbesan (2007)^{52,53} who found that exposure to dried *Euphorbia heterophylla* (L) stem water extract and ethanol extract of *Nerium indicum* increased the concentration and mortality of *C. punctatus* on *Barbus occidentalis*. Phytochemical analysis of methanol bark extract of *A. procera* was also done and this analysis reveals the presence of phytochemicals like glycosides, flavonoids, alkaloids, steroids, tannins, phenols, terpenoids and saponins (Table 1.6). Solubility was also done by using the methanol bark extract of *A. procera* and from this, we found out that the crude extract is soluble in solvents like methanol (MeOH), water (H₂O) and dimethyl sulfoxide (DMSO); almost soluble in solvents like chloroform (CHCl₃) and ethanol (C₂H₆O); partially soluble in hexane (C₆H₁₄); and not soluble in dichloromethane (CH₂Cl₂), acetone (C₃H₆O) and ethyl acetate (EtOAc) as shown in Table 1.7.

CONCLUSION:-

Traditionally, the people of Nagaland use different piscicidal plants in fishing practices. The excessive use of synthetic pesticides in water bodies can result in serious environmental hazards so the plant extracts can be a good

alternative because for a healthy aquaculture environment, the removal of unwanted or weed fish also becomes a necessary step for the success of sustainable aquaculture. Although, herbal piscicides are more eco-friendly compared to those chemicals used in fishing, knowing the effective dose is necessary to allow efficient and optimal utilization of the plant without harming the fish species and even humans after consumption. So, to understand the minimum required dose for fishing, this scientific experimental study was conducted to evaluate the toxicity of *A. procera* bark extract against the *C. auratus* and *D. rerio* fish species. The aqueous bark extract of *A. procera* was able to show toxicity to fishes at concentrations as low as 100 mg/L and 150 mg/L for *D. rerio* and *C. auratus* respectively. The LC₅₀ values of aqueous bark extract of *A. procera* were found at 243.05 mg/L and 169.78 mg/L for *C. auratus* and *D. rerio* fishes. Whereas, the LC₅₀ value of methanol bark extract of *A. procera* was recorded at 193.95 mg/L for *C. auratus*. Complete mortality of the fishes was observed at conc. 300 mg/L for *D. rerio* and conc. 350 mg/L for *C. auratus* fish. Additionally, the phytochemical screening results of *A. procera* bark methanol extract reveals the presence of saponins, phenols, tannins, alkaloids, flavonoids, steroids, terpenoids, and glycosides compounds. The results of this scientific study shows that the aqueous fresh bark extract of *A. procera* has piscicidal effect on *C. auratus* and *D. rerio* fishes at a very low concentration. Therefore, these research findings may be useful for aquaculture-industry to use the minimum concentrations of the *A. procera* bark extract for fishing as it is a natural resource, ease of availability, high efficiency, safe and biodegradability and not hazardous to the aquatic eco-system.

ACKNOWLEDGEMENTS:-

The authors acknowledge the DBT-Govt. India for their financial assistance. MT thank DBT for fellowship. We thank the Department of Chemistry, Nagaland University for providing necessary facilities.

CONFLICT OF INTEREST:-

The authors declares that there is no conflict of interest.

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