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RESEARCH ARTICLE

ACUTE TOXICITY OF ALBIZIA PROCERA (ROXB.) BENTH.BARK EXTRACT ON CARASSIUS AURATUSAND DANIO RERIOFISHES

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Abstract

This study aims to determine the lethal concentration (LC_{50}) 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 in fishes for 24-hours exposure in-aquaria. The acute toxicity study conducted on C. auratus and D. reriofishes in-aquariarevealed that the A. procera bark extract was effective to the fish under test, beginning with a dosage 150 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 LC_{50} values of the aqueous bark extract of A.procera for C. auratus and D. reriofisheswere 243.05 mg/L and 169.78 mg/L. Also, the LC_{50} of the methanol bark extract of A. procera for C. auratusfishes was found to be 193.95 mg/L. A high concentration range of A. proceraextract has the potential to become toxic to fishes, hence this study was done to identify the safety margin of A. procerabark 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. proceraon two types of fishes D. rerio and C. auratus. Additionally, A. procera methanol bark extract was screened for phytoc hemicals, revealing the presence of compounds such as saponins, phenols, tannins, alkaloids, flavonoids, steroids, terpenoids and glycosi des.

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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 illnesses[1]. 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 used[2]. Due to the absence of side effects, herbal medications are currently in high demand and is growing commercially[3].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 enriched[4]. 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 animals[5] of which some are toxic to fishes are classified as amino acids, cyanogen, alkaloids, phenolics, terpenoids, tannins, and saponins[6]. 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 treatment[9]. 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 herbs[14].

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 toxicity[23]. 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 people[24].

Albizia procerais 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 activity[25]. Decoction of A. procerabark is used in treating rheumatism, sinus stomach-ache, diabetes mellitus. The powdered seeds are used in treating amoebiasis and for curing urinary tract infections[26]. The leaves are used to cure ulcers[27]. A. procera leaves are said to have piscicidal and insecticidal properties, and fish poison is made from the crushed bark. A. procerais known to have a potential source of antioxidant property,[28] bark of A. procerahas a good anti-HIV-1 integraseactivity,[29,30] antidiabetic activity,[30] and A. procera leaf extract have the ability as target linked to Alzheimer's disease[31].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 on C. auratusand D. rerio fishes. Plant extracts can also be fatal and become poisonous to different organs in a concentration-dependent manner, according to numerous studies[34]. 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 Albizia procera(Roxb.) Benth. aqueous bark extract on C. auratus (Gold fish) and D. rerio(Zebra fish) fishes in an aquarium settingto find the median lethal concentrations (LC_{50}) at the 24-hours exposure period.

Materials and Methods:-

Collection and preparation of A. procerabark aqueous extract:-

FreshAlbiziaprocerabark were collected from Nagaland University, Lumami campus under Zunheboto district, Nagaland, India. 200 g of A.procerabark 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 andthe extract was stored in an airtight container and used for the dose-dependantpiscicidal experiments.

Methanol extraction of A.procerabark:-

50 g fresh bark of Albiziaprocerawas 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 Carassiusauratus(Gold fish) and Danio rerio(Zebra fish) were purchased from Dimapur, Nagaland, India, as per our study requirements. The fishes were transferredinto 60 L rectangularaquarium and acclimatized to the laboratory conditions for 14 days before piscicidalexperiments. 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 fishesof each species were randomly selected from the 60 L aquarium, and their length and weight were measured. The C. auratusfish species were similar in size with a weight of 20±1 gand length of 10±1 cm. Similarly, D. reriofish species have a weight of 0.578±0.01 g, and a lengthof 5±1 cm respectively.

Experimental set-up for dose-dependent toxicity study:-

The experimental set-up consisted of two rectangular-shaped glass aquariums, measuring 38 cm x 26 cm x 26 cm were used in this study. Each aquarium was filled with 20 L of tap water; one used for experimental aquarium and the other used as control aquarium. Ten fishes of Carassiusauratuswere transferred into the experimental aquarium, and another ten fishes of Carassiusauratuswere also transferred into the control aquarium with noAlbiziaprocera bark extract solution and the other standard conditions like pH, temperature, dissolved oxygen and total hardness of the aquarium water were analysedas per the standard methods.[59] The different concentrations of Albizia proceraaqueous bark extract were added to the experimental aquarium, and the toxic effects were monitored and recorded for 24-hours experimental time (Table 1.1). The Albizia proceraaqueousbark extract was utilised for Carassiusauratusfishes at concentrations ranging from 5 mg/L to 350 mg/L. Danio reriofishes were treated in the same way and the concentration of Albizia procerabark aqueous extract started from5 mg/L to 300 mg/L(Table 1.1). Same methodology was followed for the Albizia procerabark methanol extract against Carassiusauratus(Table 1.2).

Phytochemical screening:-

The Albizia procerabark methanol extract was carried out for phytochemical screening. The test was conducted using standard procedures[54-58].

Saponins:-

5 mg of the A. proceramethanolbark extract added to 10 ml of water and shaked well. Formation of bubbles confirms that saponins are present.

Steroids:-

10 ml of chloroform ($CHCl_3$) and concentratedsulphuric acid (H_2SO_4) have been added to 3 mg of theA. proceramethanolbark extract. Steroids are present when the upper layer becomes red, yellow and fluoresces green on the H_2SO_4 layer.

Terpenoids:-
When 0.5 ml $CHCl_3$, few drops of concentrated H_2SO_4 are added to 3mg of theA. proceramethanolbark extract, reddish-brown precipitate formation indicates that terpenoids are present.

Alkaloids:-
After mixing 3 mgof A. proceramethanolbark extract with 2 ml of hexane and 2% hydrochloric, a yellow precipitate was form indicating the presence of alkaloids.

Tannins:-
3-4 drops of 10 % alcoholic ferric chloride ($FeCl_3$) were mixed 3 mg ofA. proceramethanolbark extract. Brownish blue or black colour formation indicates that tannins are present.

Phenols:-
3 mg ofA. proceramethanolbark extract was added to 2 ml of aqueous ferric chloride ($FeCl_3$). Formation of blue colour confirms that phenols are present.

Flavonoids:-
A few drops of concentrated $H_2SO_4$are added to 3 mgof A. proceramethanolbark extract, intense yellow colourwas formed which indicates the presence of flavonoids.

Glycosides:-
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. proceramethanolbark extract. Brown ring appearance at the interface determines that glycosides are present.

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

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

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

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

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

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

Where, y = intercept (constant)
Using this relationship equation, the 24-hours LC_{50} of the aqueous bark extract of A. procera was determined. This was accomplished by using a and b values and setting y to LC_{50}:
$y = bx + a$

Results:-

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

LC_{50} of Albiziaprocerabark extract for Carassiusauratus andDaniorerio fishes at 24-hours:-
The mortality rate of C. auratus and D. rerio fishes was determined for a 24-hours period using varying concentrations of A. procera bark extract. The corresponding logarithmic value for each treatment utilisation, together with the mortality % probit value, are explained and displayed in the sections that follow as given in Table 1.3 and Table 1.4.

Relationship forC. auratus between probit and log concentration ofA. proceraaqueous bark extract:-
Mortality of C. auratus was calculated using the concentration of log of A. proceraaqueous 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 forD. rerio between probit and log concentration of A. proceraaqueous bark extract:-
The log concentration of A. proceraaqueous 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 forC. auratus between probit and log concentration ofA. proceramethanol bark extract:-
The probit value calculates mortality of C. auratuswhich 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
14. 8922 = 6.5095x
x = 14. 8922/6.5095
x = 2.2877
The antilogarithm of the value of x is found to be 193.95 mg/L. This value is taken as the LC_{50} of methanol bark extract of A. procera for C. auratus at 24-hours.

Table 1.1. Piscicidal screening results of A. procerabark aqueous extract with different concentrations on C. auratusandD. reriofishes in20 L aquarium for 24-hours

Exp. No.	Type of fish	Dose (mg/L)	No of fish	Mortality	Control
1	Carassius auratus (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	Danio rerio (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

Table 1.2. Piscicidal screening results of A. procerabark methanol extract with different concentrations on C. auratusfishes in 20 L aquarium for 24-hours

Exp. No.	Type of fish	Dose (mg/L)	No of fish	Mortality	Control
1	Carassius auratus (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

Table 1.3. Probit values obtained for different concentrations of A. proceraaqueous 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. reriofishes at different concentrations of A. proceraaqueous 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. proceramethanol 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_2O)	Soluble
2	Methanol (CH_3OH)	Soluble
3	Ethanol (C_2H_6O)	Almost soluble
4	Chloroform ($CHCl_3$)	Almost soluble
5	Hexane (C_6H_{14})	Partially soluble
6	Dimethyl sulfoxide (DMSO)	Soluble
7	Acetone (C_3H_6O)	Not soluble
8	Ethyl acetate (EtOAc)	Not soluble
9	Dichloromethane (CH_2Cl_2)	Not soluble

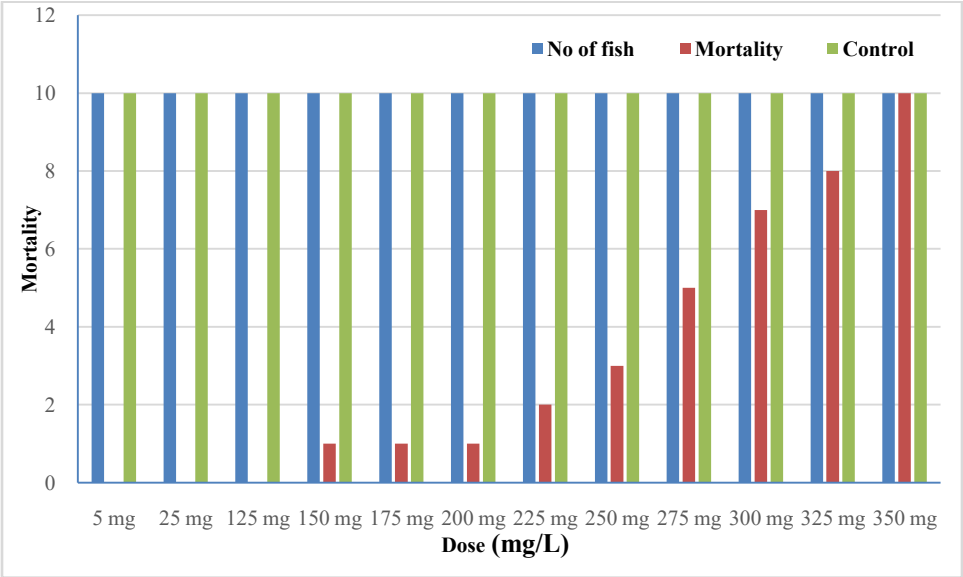

Figure 1.1. The mortality rate of C. auratus fishes at different concentrations of A. proceraaqueous bark extract for 24-hours

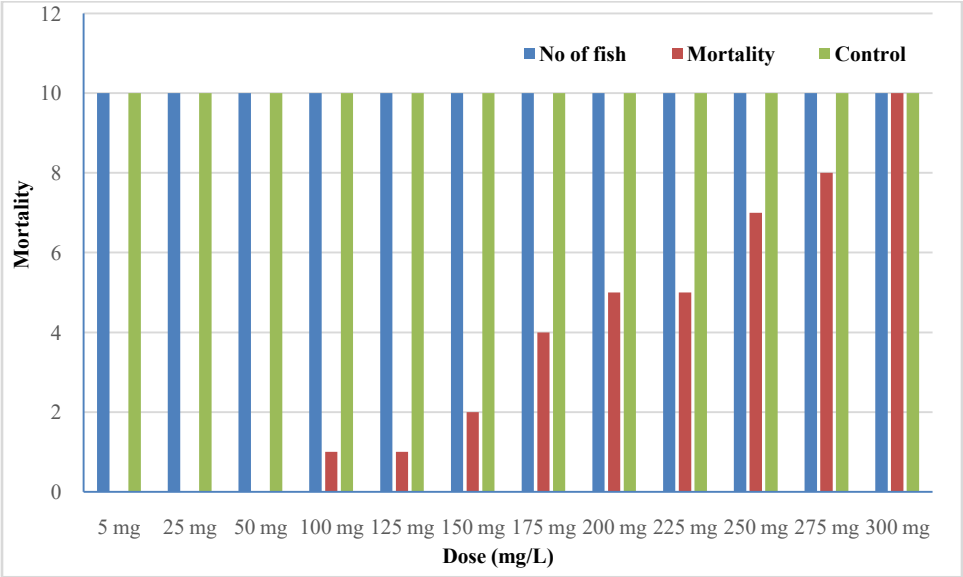

Figure 1.2. The mortality rate of D. reriofishesat different concentrations of A. proceraaqueous bark extractfor 24-hours

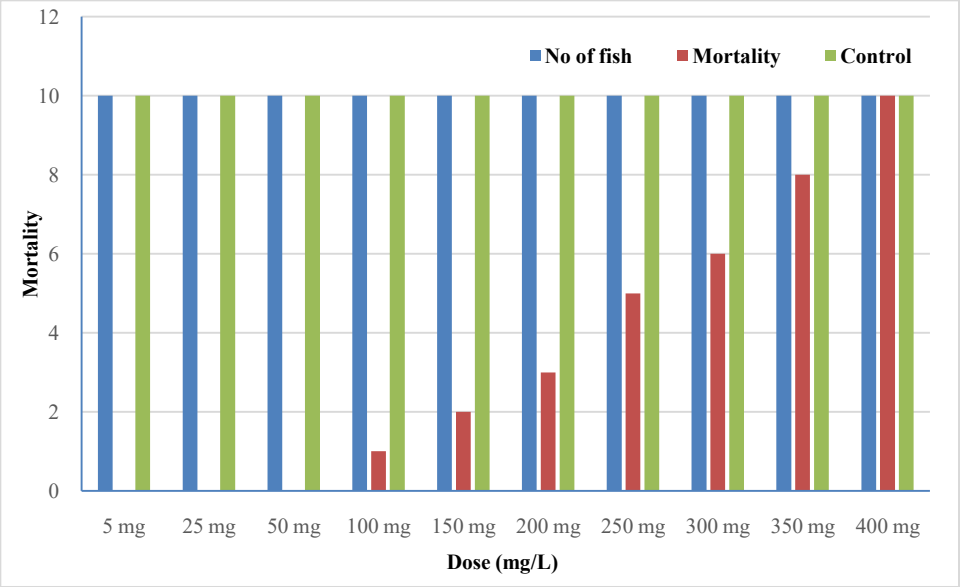

Figure 1.3. The mortality rate of C. auratusfishesat different concentrations of A. proceramethanol bark extractfor 24-hours

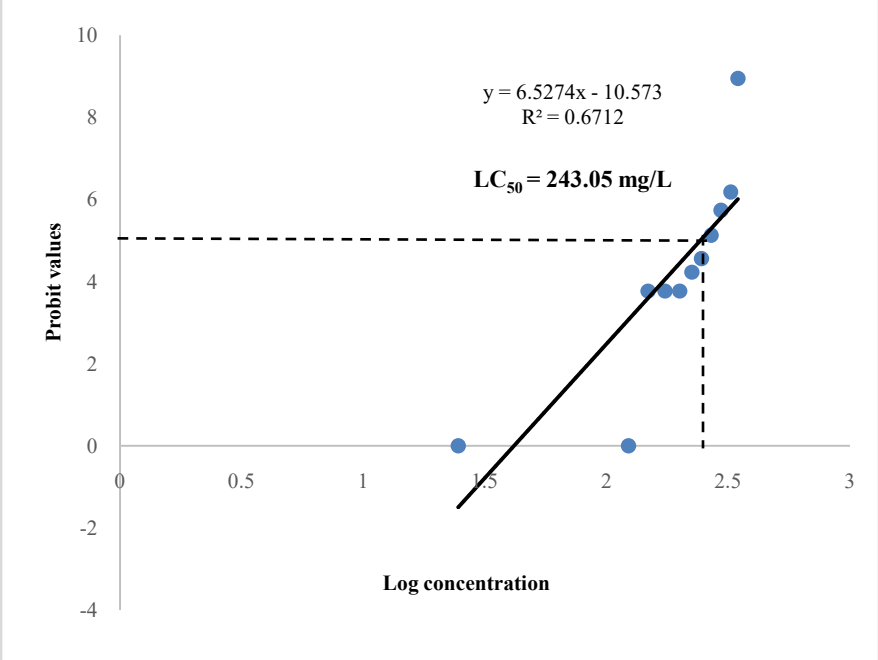

Figure 1.4. 24-hours LC_{50} of A. procerafreshaqueous bark extract on C. auratusfishes

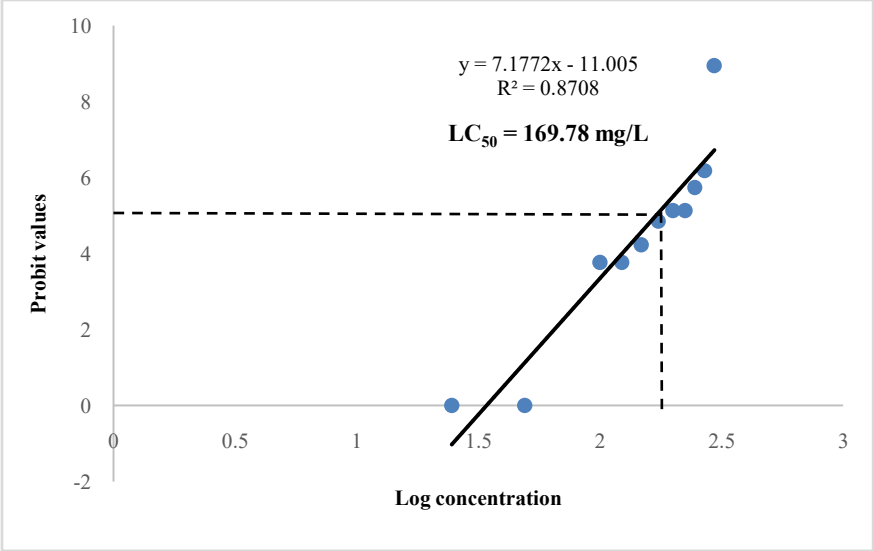

Figure 1.5. 24-hours LC_{50} of A. procerafreshaqueous bark extract on D. reriofishes

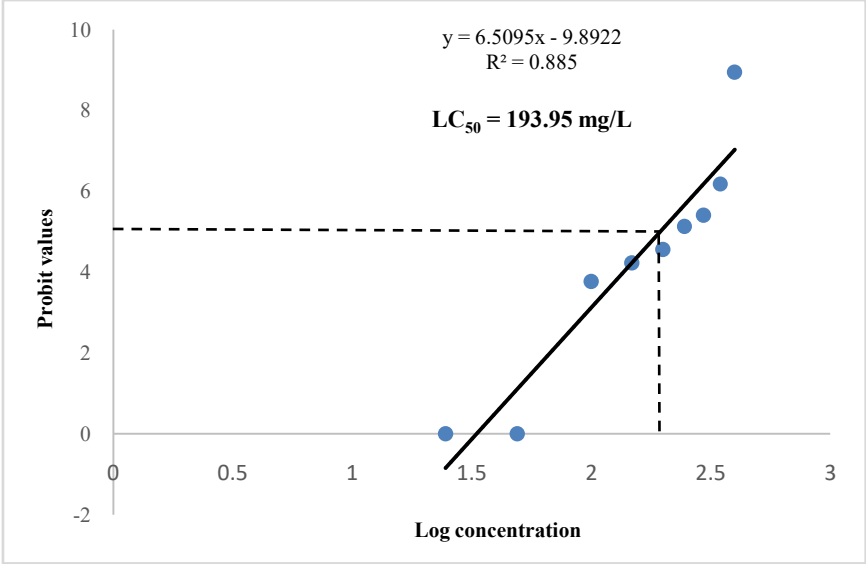

Figure 1.6. 24-hours LC_{50} of A. procerafreshmethanol bark extract on C. auratusfishes

Discussion:-

In this experiment, the aqueous bark extract of Albizia procera was testedfor acute toxicity to two different fish speciesCarassius auratusand Danio rerio. Ten C. auratus fishes were selected randomly from the 60 L rectangular aquarium and transferred into the experimental aquarium (20 L) and another ten more C. auratusfishes 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 fishes.The water was continuously aerated in both the experimental and control aquarium from the starting till the end of the experiment. Thefishes were starved for 24-hours prior to experiment and they were not fed throughout the whole experiment.Both the fishes, C. auratusand D. reriowere stressed progressively with time before death and the mortality rateswere closely monitored anddocumented. Different concentrations of A. proceraaqueous bark extract (5, 25, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350 mg/L) for C. auratus fishes (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 fishes (Table 1.1 and Figure 1.2) were used for the piscicidal experiments, like each one of the concentration was added to the experimental aquarium to check the 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. proceraexposed to C. auratusfishes and D. reriofishes for 24-hours is shown in Table 1.1, and for the methanol bark extract of A. procerais 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. auratuswere 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. auratusfishes started to show response to the aqueous bark extract of A. proceraat a concentration of 150 mg/L onwards and we observed 10 % mortality under 24-hours exposure time. While there were no much fluctuations in the mortality of C. auratusfishes 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 subsidedto some fishes after 10 to 20 minutes**.**Later, at a higher concentration of 275 mg/L we recorded 50 % mortality for C. auratusfishes 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. procerathat 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. procerawhen exposed to D. reriofishes. This signifies that the toxicity of A. proceradiffers 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. reriofishes as compared to C. auratusfishes because D. reriofishes did not show any effect to the aqueous bark extract of A. procerafrom 5 mg/L till 75 mg/L. From then on, D. reriofishes 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. auratusfishes. Likewise, we recorded 50 % mortality of D. reriofishes 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. auratusfishes and D. reriofishes as shown in Table 1.1. By exposing the aqueous bark extract of A. proceraat various effective concentrations, both C. auratusfishes 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 we observed that the fish mortality was 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.procerawhen exposed to C. auratusfishes and D. reriofishes.Gradually, the fishes became inactive at abasic effectiveconcentration and subsequently loses their balance as the concentration of aqueous bark extract of A. proceragoes higher. The affected fishes after exposing to aqueous bark extract of A. procerashows 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. procerabark extracts. Also, the mortality of C. auratusfishes when exposed to methanol bark extract of A. procerais represented in Table 1.2. For methanol bark extract of A. procera, no mortalities were observed from 5 till 50 mg/L for C. auratusfishes; but the mortalities notably increased from 100 to 400 mg/L.C. auratusfishes 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 thispiscicidal action of methanol bark extractof A. procera, various abnormal behaviours of C. auratusfishes were observed againin 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 fishesdisplayed aggressive tendencies after exposure to the aqueous and methanol bark extracts of A. proceraat 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, bothC. auratusfishes and D. reriofishes became lethargic to the aqueous and methanol

bark extracts of A. procera, lost their balanced 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 extractsofA. procerawas the sole cause of mortality to all the fishes used in the piscicidal experiment. These abnormalbehavioural 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 uptake[46].

Fish respiratory functions are known to be lowered by such extensive mucus productions[47].Piscicidal abilities and phytotoxic properties of A.proceraaqueous 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. auratusfishes and D. reriofishes 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 onC. auratusfishes and D. reriofishes after exposing to the aqueous and methanol bark extract of A. procerawere 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 methanolbark extracts of A. proceraas reported byUfodike and Omeregie, 1994[51]. 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. auratusfish had a greater fatal effect than D. reriofish.The mortality probit value is plotted versus each treatment concentration of C. auratusfishes and D. reriofishes 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. proceraagainst C. auratusfishes is given as $y = 6.5095x - 9.8922$.Probit analysis was applied to determine the relative LC_{50} or lethal concentration of aqueous and methanol bark extracts of A. procerathat caused death at 50 % from the tested fishes in the exposure period for 24-hours.

Through this analysis, LC_{50} of aqueous bark extract ofAlbiziaprocera for Carassiusauratusfishes and Danioreriofishes were found to be 243.05 mg/Land 169.78 mg/L as shown in Figure 1.4 and Figure 1.5. Additionally, the LC_{50} of the methanol bark extract of A. procera for C. auratusfishes was found to be 193.95 mg/L as shown in Figure 1.6. In this study, the LC_{50} value was relatively lesser forD. reriofishes as compared to C. auratusfishes. This implies that A. proceraaqueous bark extract is more toxic to D. reriofishesas 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. auratusfishes and D. reriofishes 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. procerawas also done and this analysis reveals the presence of phytocompounds like glycosides, flavonoids, alkaloids, steroids, tannins, phenols, terpenoids and saponins (Table 1.6). Solubility was also done for the methanol bark extract of A. proceraand from this, we found out that the crude extract is soluble in solvents like methanol (MeOH), water (H_2O) and dimethyl sulfoxide (DMSO); almost soluble in solvents like chloroform ($CHCl_3$) and ethanol (C_2H_6O); partially soluble in hexane (C_6H_{14}); and not soluble in dichloromethane (CH_2Cl_2), acetone (C_3H_6O) 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 Albiziaprocerabark extract against the Carassius auratusand Danio 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 fishes andC. auratusfishesrespectively. The LC_{50} values of aqueous bark extract of A. procerawere found at 243.05 mg/L and 169.78 mg/Lfor C. auratus and D. rerio fishes. Whereas, the LC_{50} value of methanol bark extract of A. procerawasrecorded at 193.95 mg/L for C. auratusfishes. Complete mortality of the fishes was observed atconc.300 mg/L forD. rerio fishesand conc. 350 mg/L for C. auratusfishes. Additionally, the phytochemical screening results of A. procera bark methanol extract reveals the presence of saponins, phenols, tannins, alkaloids, flavonoids, steroids, terpenoids, and glycosidescompounds. The results of this scientific study shows that the aqueous fresh bark extract of Albiziaprocerahas piscicidal effect on Carassius auratusand Danio rerio fishes at a very low concentrations. Therefore, these research findings may be useful for aquaculture-industry to use the minimum concentrations of theA. 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.

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Conflict Of Interest:-

The authors declares that there is no conflict of interest**.**

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