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

Manuscript Info

……..

Manuscript History
Received: xxxxxxxxxxxxxxx
Final Accepted: xxxxxxxxxxxx
Published: xxxxxxxxxxxxxxx

Key words:-
Natural product,*Albizia procera* (Roxb.) Benth., bark extract, fisheries, fishes,toxicity, LC_{50},mortality.

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 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 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. rerio*fisheswere 243.05 mg/L and 169.78 mg/L. Also, the LC_{50} 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.

……

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 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 activity[25]. 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 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. procera*is known to have a potential source of antioxidant property,[28] bark of *A. procera*has 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 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 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 *A.procera*(Roxb.) Benth. aqueous bark extract on *C. auratus* (Gold fish) and *D. rerio* (Zebra fish) in an aquarium settingto find the median lethal concentrations (LC_{50}) 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 andthe extract was stored in an airtight container and used for the dose-dependantpiscicidal 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 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. auratus*fish species were similar in size with a weight of 20±1 gand length of 10±1 cm. Similarly, *D. rerio*fish 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 *C. auratus* were transferred into the experimental aquarium, and another ten fishes of *C. auratus* were also transferred into the control aquarium with no*A. procera* 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 *A. procera*aqueous 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 *A. procera* aqueousbark extract was utilised for *C. auratus* fishes at concentrations ranging from 5 mg/L to 350 mg/L. *D. rerio* was treated in the same way and the concentration of *A. procera* bark aqueous extract started from5 mg/L to 300 mg/L(Table 1.1). Same methodology was followed for the *A. procera*bark methanol extract against *C. auratus*(Table 1.2).

Phytochemical screening

The *A. procera*bark methanol extract was carried out for phytochemical screening. The test was conducted using standard procedures[54-58].

Saponins

5 mg of the *A. procera*methanolbark 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 the*A. procera*methanolbark 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 the*A. procera*methanolbark extract, reddish-brown precipitate formation indicates that terpenoids are present.

Alkaloids

After mixing 3 mgof *A. procera*methanolbark 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 of*A. procera*methanolbark extract. Brownish blue or black colour formation indicates that tannins are present.

Phenols

3 mg of*A. procera*methanolbark 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. procera*methanolbark 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. procera*methanolbark extract. Brown ring appearance at the interface determines that glycosides are present.

Lethal concentration

The LC_{50} of the aqueous bark extract of *A. procera* 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.

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

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

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

LC_{50} of *A. procera*bark extract for *C. auratus* and *D. rerio* 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 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. procera*methanol bark extract

The probit value calculates mortality of *C. auratus* which was plotted against the log concentration of *A. procera*methanol 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 of *A. procera*bark aqueous extract with different concentrations on *C. auratus* and *D. rerio* fishes 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 of *A. procera*bark methanol extract with different concentrations on *C. auratus* fishes 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. 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*

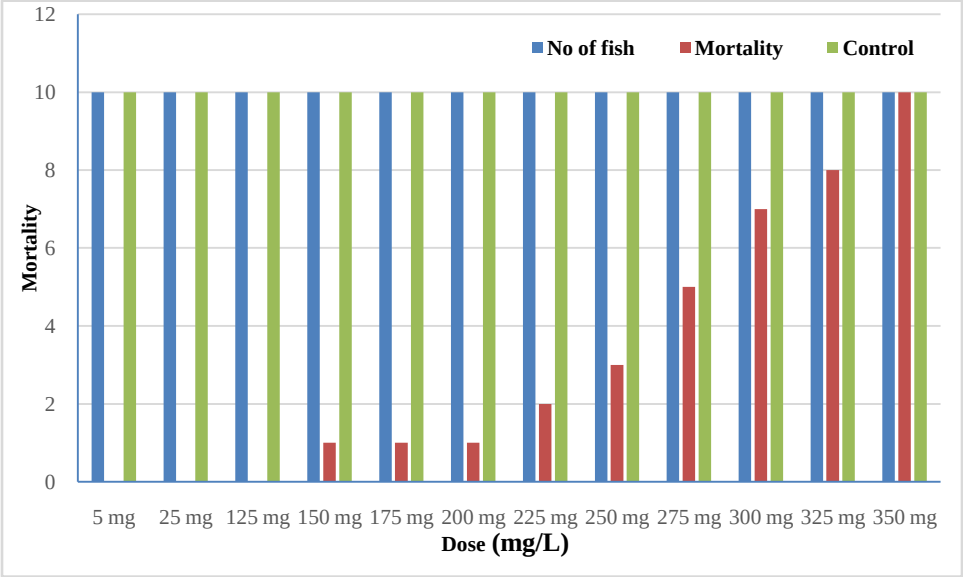
Sl. No.	Solvent	Observation
1	Water (H_2O)	Soluble
2	Methanol (CH_3OH)	Soluble
3	Ethanol (C_2H_6O)	Almost soluble
4	Chloroform ($CHCl_3$)	Almost soluble

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

211

212

213



214

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

216

217

218

219 **Figure 1.2. The mortality rate of *D. rerio* at different concentrations of *A. procera*aqueous bark extractfor 24-hours**

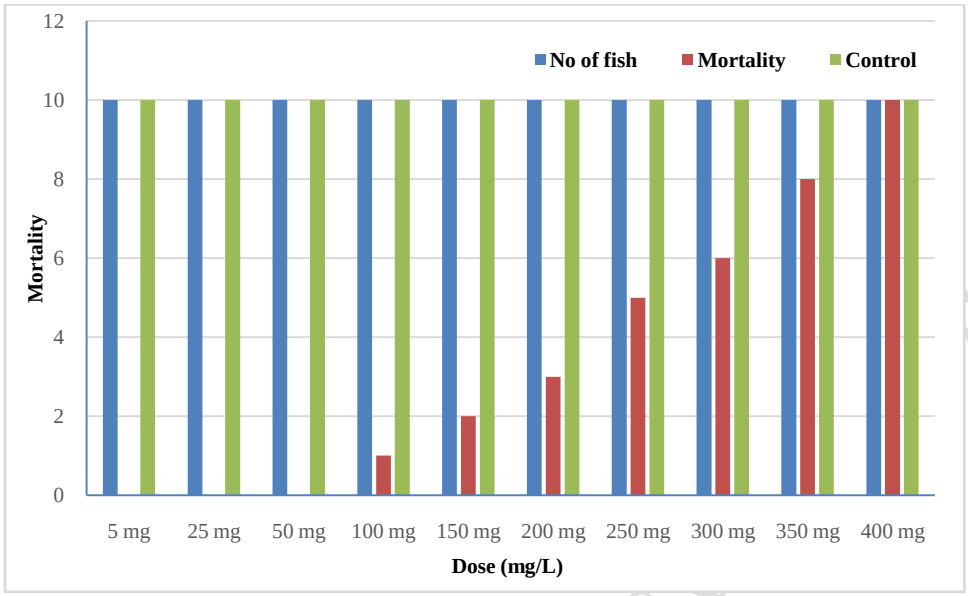


Figure 1.3. The mortality rate of *C. auratus* at different concentrations of *A. procera*methanol bark extractfor 24-hours

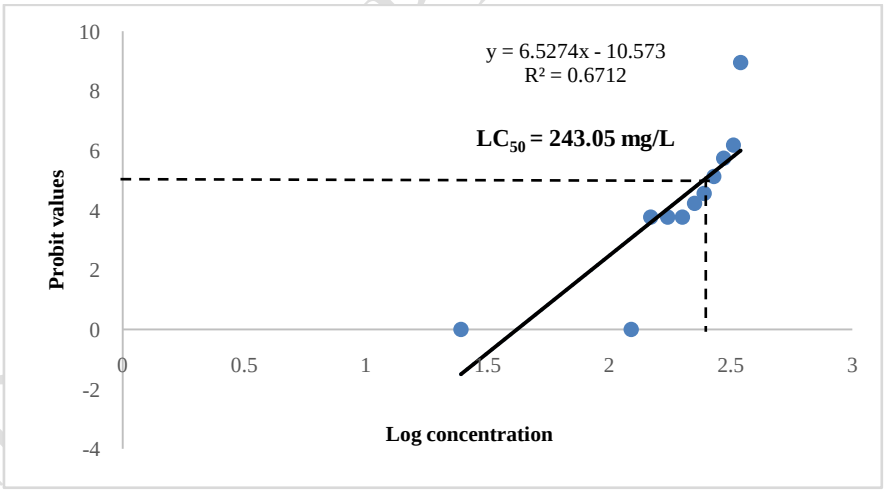


Figure 1.4. 24-hours LC_{50} of *A. procera*freshaqueous bark extract on *C. auratus*fishes

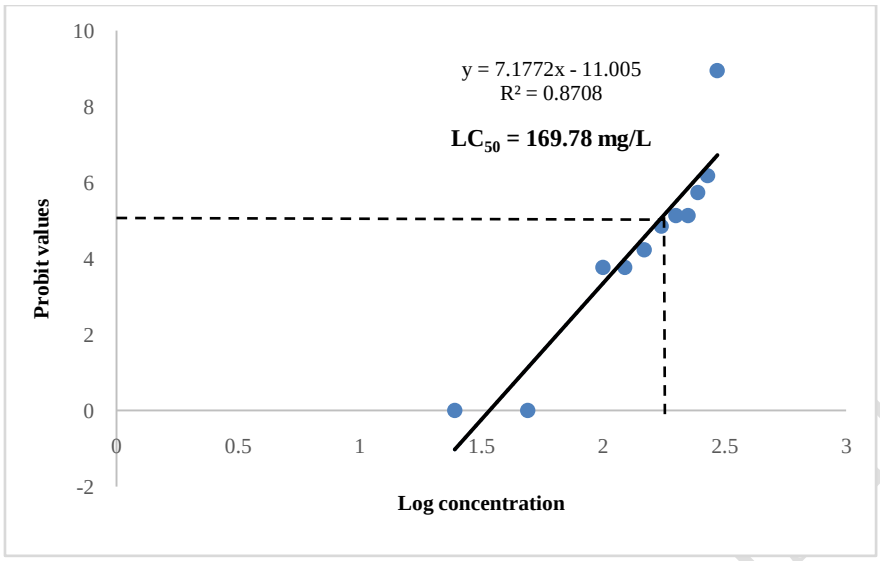


Figure 1.5. 24-hours LC_{50} of *A. procera*freshaqueous bark extract on *D. rerio*fishes

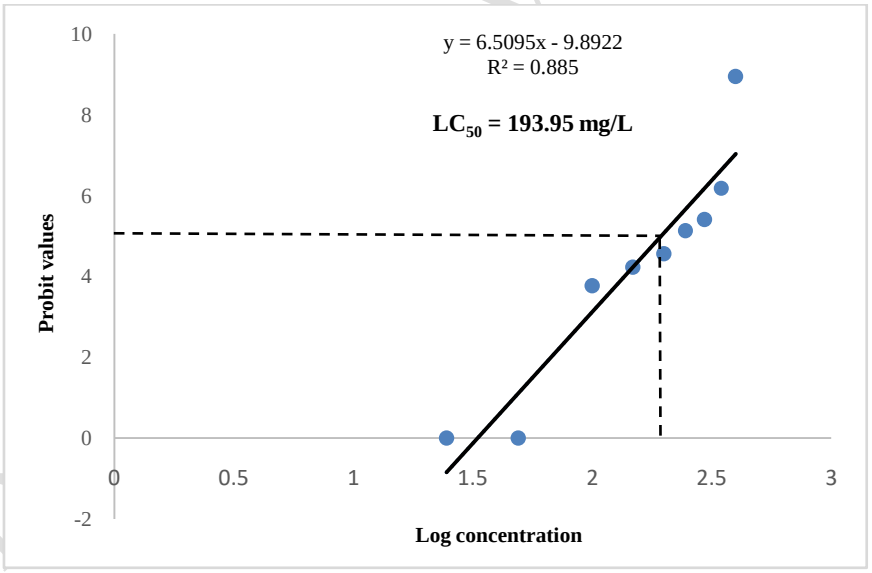


Figure 1.6. 24-hours LC_{50} of *A. procera*freshmethanol bark extract on *C. auratus*fishes

DISCUSSION:-

In this experiment, the aqueous bark extract of *A.procera* was testedfor 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. Thefishes 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 rateswere closely monitored anddocumented.

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 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. 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 subsidedto 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 abasic effectiveconcentration 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 thispiscicidal action of methanol bark extractof *A. procera*, various abnormal behaviours of *C. auratus* 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. 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 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 extractsof*A. procera*was 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. 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 methanolbark extracts of *A. procera*as 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. 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 LC_{50} 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, LC_{50} of aqueous bark extract of*A. procera* for *C. auratus* and *D. rerio*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. auratus*was found to be 193.95 mg/L as shown in Figure 1.6. In this study, the LC_{50} 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* fishesas 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 phytocompounds 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 (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 *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 LC_{50} values of aqueous bark extract of *A. procera*were 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. procera*wasrecorded at 193.95 mg/L for *C. auratus*. Complete mortality of the fishes was observed atconc.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 glycosidescompounds. 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**.**

REFERENCES:-

1. Yuan H, Ma Q, Ye L, Piao G. The traditional medicine and modern medicine from natural products. Molecules. 2016; 21(5). https://doi.org/10.3390/molecules21050559.
2. WHO report on traditional medicine. Who global report on traditional and complementary medicine. Luxembourg: World Health Organization. 2019.
3. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol. 2014; (4) 177.https://doi.org/10.3389/fphar.2013.00177.
4. Singh PK, Singh J, Medhi T, Kumar A. Phytochemical screening, quantification, FT-IR analysis, and in silico characterization of potential bio-active compounds identified in HR-LC/MS analysis of the polyherbal formulation from Northeast India. ACS Omega. 2022; 7 (37), 33067–33078. https://doi.org/10.1021/acsomega.2c03117.
5. Salmeron-Manzano E, Garrido-Cardenas JA, Manzano-Agugliaro F. Worldwide research trends on medicinal plants. Int. J. Environ. Res. Public Health. 2020; 17 (10). https://doi.org/10.3390/ijerph17103376.

6. Dai J, Mumper RJ. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules. 2010; 15 (10), 7313–7352. https://doi.org/10.3390/molecules15107313.
7. Omara T, Kiprop AK, Ramkat RC, Cherutoi J, Kagoya S, Moraa Nyangena D. Medicinal plants used in traditional management of cancer in Uganda: A review of ethnobotanical surveys, phytochemistry, and anticancer studies. Evidence-based complement. Altern. Med. 2020; 1–26. doi:10.1155/2020/3529081.
8. Schultz F, Anywar G, Wack B, Quave CL, and Garbe LA. Ethnobotanical study of selected medicinal plants traditionally used in the rural greater Mpigi region of Uganda. J. Ethnopharmacol. 2020; 256, 112742. doi: 10.1016/j.jep.2020.112742.
9. Richardo G, Caio P, Samantha S, Amy L, Holger C, Daniel GP, Miek J. A review of the WHO strategy on traditional, complementary, and integrative medicine from the perspective of academic consortia for integrative medicine and health. Front. Med. (Lausanne). 2024; *11*, 1395698. doi: 10.3389/fmed.2024.1395698.
10. Richard J Ko. A U.S. Perspective on the adverse reactions from traditional Chinese medicines. J. Chin. Med. Association: JCMA. 2004; 67 (3), 109–116.
11. Obakiro SB, Bunalema L, Gakunga-Nd J, Nyatia E, and Waako JP. Ulcerogenic potential of Eucalyptus Globulus L. leaf extract in Wistar Albino rats. J. Pharmacol. Toxicol. 2017; 13 (1), 45–51. doi:10.3923/jpt.2018.45.51
12. Anywar G, Kakudidi E, Byamukama R, Mukonzo J, Schubert A, Oryem-Origa, H, et al. A Review of the toxicity and phytochemistry of medicinal plant species used by herbalists in treating people living with HIV/AIDS in Uganda. Front. Pharmacol. 2021; 1–10. doi:10.3389/fphar.2021.615147.
13. Selamoglu M. Importance of the cold chain logistics in the marketing process of aquatic products. An Update Study. J. Surv. Fish. Sci. (2021) 8 (1), 25–29. doi:10.18331/SFS2021.8.1.2.
14. ModarresiChahardehi A, Arsad H and Lim V. Zebrafish as a successful animal model for screening toxicity of medicinalnplants. Plants.2020; 9 (10), 1345. https://doi.org/10.3390/plants9101345.
15. Hambrey J. The 2030 agenda and the sustainable development goals: the challenge for aquaculture development and management. FAO Fisheries and Aquaculture Circular. 2017; (C1141).
16. FAO. Meeting the sustainable development goals. 2018.
17. Adesina BT. "Toxicity of Moringa oleifera (lam.) extract to Oreochromis niloticus fingerlings and juveniles", PhD Thesis, University of Ibadan. 2008; Nigeria.
18. Adeyemo O. "Food safety and environmental health concerns: threats to sustainable aquaculture development in Nigeria", World Aquaculture Conference. 2012.
19. Wang, GS, Han, J. Zhao, LW, Jiang, DX, Liu, YT, Liu, XL. Anthelmintic Activity of steroidal saponins from *Paris polyphylla*. *Phytomedicine*. 2010; 17 1102-1105. https://doi.org/10.1016/j.phymed.2010.04.012.
20. Son CRIM, Mohiseni M. "Medicinal herbs, strong source of antioxidant in aquaculture: a mini review", Modern Applications in Pharmacy and Pharmacology. 2017; Vol. 1 No. 1, pp. 1-5. doi: 10.31031/MAPP.2017.01.000504.
21. Cruz ASP. Anthelmintic effect of *Solanum lycocarpum*in mice infected with *Aspiculuris tetraptera*. The journal of American science. 2008; 4(3): 75-79.
22. Vidyadhar S, Saidulu M, Gopal TK, Chamundeeswari D, Rao U, Banji D. In vitro anthelmintic activity of the whole plant of *Enicostemma littorale* by using various extracts. International journal of applied biology and pharmaceutical technology. 2010; 1(3): 1119-1125.
23. Madhuri S, Mandloi AK, Govind P, Sahni YP. "Antimicrobial activity of some medicinal plants against fish pathogens", International Research Journal of Pharmacy. 2012; Vol. 3, pp. 28-30.
24. Mohammad HUR, Abu BI. Biomimetic and synthetic advances in natural pesticides: balancing efficiency and environmental safety. Journal of Chemistry. 2025;1-23. https://doi.org/10.1155/joch/1510186.
25. Anon. The useful plants of India. Publications & Information Directorate, CSIR, New Delhi, India. 1986.
26. Bulusu Sitaram, Chunekar K.C. Bhavaprakasa of Bhavamisra. 2012; 1:354.
27. Little EL Jr and Wadsworth FH. Common trees of Puerto Rico and the Virgin Islands. USDA, Washington, DC,
28. Sivakrishnan S, Kavitha J, Kottai Muthu A. Antioxidant potential, total phenolic and flavonoids content of aerial parts of ethanolic extract of *Albizia procera*(Family: Mimosoideae). Asian Journal of pharmaceutical and Clinical Research. 2013; 6 Suppl 1:108-110.
29. Praveen Kumar P, Ramesh A, Prasad K. Assessment of antihyperglycemic fractions isolated from *Albizia procera*stem.doi: 10.5530/pj.2014.3.5

30. Sivakrishnan S, Kottai Muthu A. *In vivo* Antioxidant activity of ethanolic extract of aerial parts of *Albizia procerarоxb (benth.)* against paracetamol induced liver toxicity on Wistar rats. Journal of Pharmaceutical Science and Research. 2013; 5Suppl 9:174 – 177.
31. Ekambaram Gayathiri, Palanisamy Prakash, Thangaraj Pratheep, Somdatta Y. Chaudhari, Subramanian Deepika Priyadharshini. Computational exploration of bioactive compounds from *Albizia procera*: Molecular docking, dynamics, and pharmacokinetics for AchE and BchE inhibition in Alzheimer's disease treatment. The Microbe. 4 2024; 1-10.https://doi.org/10.1016/j.microb.2024.100150.
32. Ambika K, Jegadeesan M. Comparative Pharmacognostical studies On *Albizia Lebbeck (L.) Wild* and *Albizia Procera (Roxb.) Benth.* leaves. International Journal of Innovative Research in Science, Engineering and Technology. 2017; 6 (9), 19483-19493. doi:10.15680/IJIRSET.2017.0609148 19483.
33. Mahfuza khatun, EramulIsalam, Rafikul Islam and Aziz abdurrahman, khurshidalam, promakhodkar, Mamunur Rashid, Shahnjaparvin. Estimation of total phenol and in vitro antioxidant activity of *Albizia -procera*leaves. *BMC Research Notes.* 2013; 6:121. http://www.biomedcentral.com/1756-0500/6/121.
34. Xavier J, Reddy J. Acute toxicity study of ethanolic extracts of leaf and fruit of two different varieties of M. Charantia in Danio rerio. Journal of Pharmaceutical, Chemical and Biological Sciences. 2019; 7(2):102–109.
35. Ologe IAD and Sogbesan OA. Piscicidal potential of dried *Euphorbia heterophylla* (L.) stem water extract on Barbus occidentalis (Pisces: Cyprinidae) (Boulenger, 1920) fingerlings. 2007. doi:10.3923/rjet.2007.191.197.
36. Omoniyi I, Agbon AO and Sodunke SA. Effect of lethal and sub-lethal concentrations of Tobacco (*Nicotiana tobaccum*) leaf dust extract on weight and hematological changes in Clarias gariepinus (Burchell). *Journal of Applied Sciences and Environmental Management*. 2002; 6(2), 37-41. doi:10.4314/jasem.v6i2.17174.
37. Ashraf M, Ayub M, Sajjad T, Elahi N, Ali I, Ahmed Z. Replacement of Rotenone by locally grown herbal extracts. International Journal of Agriculture and Biology. 2010; 12:77-80.
38. Chowdhury AKA, Latifa GA, Ara S, Raisuddin, R. Potentiality of indigenous *Derris* root in cleaning predatory and weed fishes from nursery ponds. Dhaka University Studies, B. 1981; 29(2):47-73.
39. Latifa GA, Begum S, Akhter A, Ahmed MS. Piscicidal properties of the dry barks of *Azadirachta indica* (A. Juss*) on Heteropneustesfossilis*(Bloch*).* Bangladesh Journal of Life Sciences. 1997; 9(2):31-36.
40. Latifa GA, Begum A. Piscicidal activity of the dry stem of Euphorbia nerifolia (Linn 1753) on *Heteropneustesfossilis*(Bloch) and *Channa punctatus* (Bloch). Bangladesh Journal of Scientific Research. 1993; 11(2):217-225.
41. Nasiruddin M, Azadi MA, Chowdhury R. Piscicidal effects of seed and seed kernel extracts of four indigeneous plants on *Heteropnustesfossilis*(Bloch) and *Anabas testudineus*(Bloch). The Chittagong University Journal of Science. 1998; 22:1-10.
42. Nasiruddin M, Azadi MA, Jahan A, Chowdhury, R. Haemolytic effects of *Cassia tora*(L.) Benth and *Albizzia lebbeck*(L.) Benth seeds on *Heteropneustesfossilis*Bloch and *Channa punctatus* Bloch. Bangladesh Journal of Zoology. 2002; 30(1):11-19.
43. Jothivel N and Paul VI. Evaluation of the acute toxicity of the seed of *Anamirta cocculus* (Linn.) and its piscicidal effect on three species of freshwater fish. Theinternet Journal of Toxicology. 2008; 5:1.
44. Abalaka SE and Auta J. Toxic effects of aqueous and ethanol extracts of *Parkia biglobosa*pods on *Clarias gariepinus*adults. World Journal of Biological Research. 2010; 3, 9-17.
45. Orji OU, Ibiam UA and Aja PM. Acute toxicity studies of the lyophilized aqueous extract of *Psychotriamicrophylla*leaf on *Clarias gariepinus*juveniles. International Journal of Biology and Biological Sciences. 2014; 3(4), 038-044
46. Cagauan AG, Galaites MC and Fajardo LJ. Evaluation of botanical piscicides on Nile Tilapia *(Oreochromis niloticus* L.*)* and mosquito fish (*Gambusia affinis*Baird and Girard). Proceedings of 6thInternational Symposium on Tilapia in Aquaculture. (2004) 179–87.
47. Konar SK. Pesticides and aquatic ecosystem. Indian Journal of Fisheries. 1975; 22, 1-2. https://epubs.icar.org.in/index.php/IJF/article/view/12216.
48. Adewole AM, Faturoti EO, Oladeinde OF and Ayelaagbe OO. A survey of some indigenous fish phytotoxic plants in Ibadan, South Western Nigeria. Book of abstract of the 1st Annual conference of the Zoology Society of Nigeria. 2002.
49. Akinwande AA, Sogbesan AO, Moody FO and Ugwumba AAA. Piscicidal potential of mesocarp of neem plant (*Azadirachta indica L.*) fruit on hybrid, *Heteroclarias*". Journal of Environmental Biology. 2007; 28 (3):533-536.
50. Fafioye OO. Acute and sub-acute toxicities of five plant extracts on white tilapia, *Oreochromis niloticus* (*Trewavas*). *Irjas.* 2012; 2(13):525-530.

51. Ufodike EBC and Omoregie E. Acute toxicity of water extracts of barks of *Balanites aegyptica* and *Kigeliaafricana* to *Oreoechromisnitoticus*(L). *Aquaculture and Fisheries.* 1994; 25, 873-879.https://doi.org/10.1111/j.1365-2109.1994.tb01349.x.
52. Tiwari S, Singh A. Piscicidal activity of active compound extracted from Euphorbia royleana latex through different organic solvents. Proc. First National Interactive Meet on Med. Aro. Plants (AK Mathur, S. Dwivedi, DD Patra, GD Bagchi, NS Sangwan, A. Sharma and SPS Khanuja Eds.), CIMAP, Lucknow, India. 2003; 330-36.
53. Ologe IAD, Sogbesan OA. Piscicidal potential of dried *Euphorbia heterophylla* (L.) Stem water extract on *Barbus Occidentalis* (Pisces: Cyprinidae) (Boulenger, 1920) Fingerlings. Research Journal of Environmental Toxicology. 2007; 1 (4), 191-197. doi: 10.3923/rjet.2007.191.197.
54. Brain KR, Turner TD. The practical evaluation of phytopharmaceuticals. 1975.
55. Sachin C, Arvind N, Vinesh D. The study of in vitro antimicrobial activity and phytochemical analysis of some medicinal plants in Chamoli Garhwal Region. Pharmacognosy Journal. 2010; 2(12) 481-485.https://doi.org/10.1016/S0975-3575(10)80035-5.
56. Evans WC. Trease and Evans' pharmacognosy. General Pharmacology. 1997; 2(29) 291.
57. Evans WC. Trease and Evans' pharmacognosy. Elsevier Health Sciences. 2009.
58. Gibbs RD. Chemotaxonomy of Flowering Plants. TAXON. 1974; 23(1) 220-220.https://doi.org/10.1002/j.1996-8175.1974.tb04032.x.
59. APHA, AWWA, and WEF (2005). Standard Methods for the Examination of Waste and Waste water 21st ed. American Public Health Association, Washington, D.C.