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Phytochemical and Cytotoxicity of "Opey", *Derris elliptica Wall (Benth)* in Stunning Freshwater Fish

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Abstract — This study investigated on "Opey" stem and leaf phytochemical constituents and cytotoxicity in stunning freshwater fish. Phytochemical screening revealed that "Opey" stem contained various secondary metabolites such as triterpenes, alkaloids, saponins, glycosides and tannins and the leaf too contained several secondary metabolites such as sterols, triterpenes, alkaloids, saponins, glycosides and tannins. Likewise, the study conducted the cytotoxicity assay done in vitro to determine the cytotoxicity of the crude extracts. The toxicity of formulation was calculated using the data in terms of lethal concentration, LC50 standard value of the active component, concentration of the treatment and percent mortality. Findings showed that "Opey" stem crude extracts are moderately toxic and non-toxic similarly to "Opey" leaf crude extracts which are moderately toxic and non-toxic. In addition, results implied that stem concentrations exhibited faster time in stunning fish than leaf concentrations. It means that stem extracts were more effective than the leaf extracts in stunning fish.

Keywords: Phytochemical, Cytotoxicity, Indigenous Species, Stunning, Freshwater fish

I. INTRODUCTION

Tropical communities in the Cordillera, like in Mankayan, Benguet and Sadsadan, Mt. Province are abundant in natural and herbal plants which contain phytochemical constituents used by local people in stunning fresh water mudfish. This plant had been used for a long time but was not documented so the researcher was interested to determine the phytochemical content of the stem and leaf. Also, to record the laboratory procedures of this method of fishing. Besides, to regulate such fishing activity if found not feasible especially on its health effects to the consumers.

The power of the plant's ability to stun mudfish lies in the phytochemical constituents present in the plant leaf and stem.

Derris elliptica (Wall.) Benth. belongs to the family Fabaceae under the species of leguminous plant from Southeast Asia and the Southwest Pacific islands, including New Guinea. In the Philippines, the Tagalogs call it tubli and tugling-pula, lapak in Bicol, opei in Bontoc and tuva among the Ivatans. This plant is popularly known as derris powder and tuba root in Indonesia. This climbing vine is commonly found on forest edges, roadside, and along rivers up to 1500 m altitude. Also, it is abundant in thickets, along streams, and in secondary forests at low and medium altitudes from northern Luzon to Mindanao (Godofredo, 2019).

Derris roots contain a bioactive component known as rotenone, a strong insecticide, and fish poison when crushed. Its roots are used widely in the horticulture industry. In South East Asia, rotenone is considered the strongest fish poison because it is toxic to aquatic organisms. Since early times, the root has been used as fish poison in the Philippines. Rotenone is isolated from the alkaloids which have selectively bioactivity, low environmental hazard and relative non-toxicity to plants and mammals (Science and Bioengineering, 2018).

Chaudhary, M. (2017) revealed that herbal fish stunning agents are proven means of fishing that do not kill fish like chemical poisons according to Blackwell (2014). The stem is chopped into small pieces and the saponins released from the bark and wood kill the smaller fish by asphyxiation (Blackwell, 2014). Besides the fish poisons in these plants are not toxic to humans who are the main consumers. Many of these plants have been used for a long time by local people, and have been tested and found to have medicinal properties, such as *Careya arborea*, which is used as an analgesic and antidiarrheal (Khaliq, 2016).

Herbal plants with pharmacological effects may be related to their phytochemical constituents namely alkaloids, flavonoids, saponins, triterpenes, sterols, phenols, tannins and many more. The cyclic nitrogen-containing compounds that are present in over 20 percent of plant species are the alkaloids (Kennedy, 2011). This is an ingredient to majority of the poisons, neurotoxins, social drugs, cocaine and opiates occasionally consumed by humans as pointed by Zenc (2007). Phenols and flavonoids are the source of anthocyanins and polyphenols which can be a brain barrier through the nerves (Winter, 2009). Thus, consumption of phenolic-rich food

should be done in moderation. Phenolic compounds function as defense compounds, have antimicrobial activity, antiulcer, anti-inflammatory, antioxidant, cytotoxic, antitumor, antispasmodic, and antidepressant. They mediate in disease process by protecting the plant from free radicals (Saxena et al, 2013). Flavonoids contain antibacterial, antimicrobial, anti-inflammatory, antitumor, anti-allergic compounds. They inhibit enzymes, protect the human body from free radicals and reactive oxygen species as cited by Saxena et. al. (2013). In addition, tannins are used as astringents against diarrhea, diuretics, anti-inflammatory, antiseptic, and antioxidant (Ashok, 2012).

The need for screening specific plant parts and cytotoxicity test (Li, 2015) is a global re-awakening. Toxicity evaluation that uses tissue cells in vitro is an important indicator on determining the amount of plant poison ingested by the consumer in order to save animals from toxicity. A laboratory procedure to measure and analyze proliferation, viability and cytotoxicity are the assays and commonly used to monitor the response to healthy cells. Approximately, 79 percent of the medicinal plants showed some cytotoxicity while 75 percent of the nonmedical plants showed bioactivity (Booth, 2012). The cytotoxicity of therapeutic and medicinal plant extracts could be determined from their lethal dose or lethal concentration level.

This study determined and analyzed the phytochemical and cytotoxicity of "Opey", *Derris elliptica (Wall) Benth* in stunning mudfish and determine its effectiveness in the laboratory and simulated experimental set-ups. In addition, to document these biological and chemical properties of "Opey" is the main purpose of this study.

II. METHODOLOGY

The study used the experimental design which covers the conducted experiments such as phytochemical for phase I, and then cytotoxicity, and simulated set-ups for phase II as shown by the flowchart of the procedure shown in figure 1. The three principles of experimental research were applied in this study: randomization, replication, and control. Test animals were distributed employing the Experimental Research Design specifically the Completely Randomized Design (CRD). Random assignment assured that groups receiving different treatments are reasonably

equal or similar in a way that could possibly impact the outcome or dependent variable (Cai, 2014). Control variables guaranteed that the independent variable caused the dependent variable e.g. environment and other related factors.

A. Phytochemical Analyses. One thousand grams (1000 g) of air-dried "Opey" *Derris elliptica* (Wall.) Benth. stems and one thousand grams (1000 g) of leaves were chop into small pieces about two millimeters in size, then was powdered using the blender at home. The powdered samples were sealed in a zip loc to avoid air that may add moisture that may lead to contamination.

The powdered plant samples were submitted to the Department of Science and Technology (DOST) Laboratory, Taguig for phytochemical analysis employing the standard methods based from Ghalem (2017). The powdered sample stems and leaves of "Opey" were soaked individually in 95% ethanol for 48 hours. The mixture was filtered to obtain filtrate. The filtrate was transferred in the rotary evaporator to obtain the crude extract. The crude extracts were used in the following phytochemical processes;

Preliminary qualitative phytochemical analysis was carried out to identify the secondary metabolites present in the various plant extracts of "Opey" leaf and stem parts (Mumtaz, 2014).

Phytochemical Screening. The phytochemical screening was conducted at the Department of Science and Technology, Industrial Technology Development Institute- Standards and Testing Division (DOST-ITDI-STD) Bio-Chemistry Laboratory, Taguig, Metro, Manila.

B. Cytotoxicity Analyses. Cytotoxicity assays are useful in determining the potential toxicity of a test substance including plant extracts or biologically active compounds, and toxins isolated from plants (McGaw, 2014). Cytotoxicity is the quality of being toxic to normal cells because it contains the substance that kills living cells. Cytotoxicity test would determine and prescribe the concentration feasible for human consumption. Laboratory test results will reveal which among the treatments is safe and tolerable in the human body.

B.1. Lymphocyte Isolation. Blood from a healthy individual was collected in a green-top vacutainer tube. Then, the whole blood was mixed with an equal volume of Dulbecco's PBS. About 4.8 ml of the diluted blood were carefully overlaid onto 4 ml of Ficoll-Paque™ in each of

two centrifuge tubes are centrifuged at 2000 rpm for 30 minutes at room temperature. The clear plasma top layer from each tube was discarded and the cloudly buffy coat layers were transferred to a fresh centrifuge tube. Three volumes of Dulbecco's PBS were added to the cells and the tube was inverted several times to wash the lymphocytes. The tube was centrifuged at 1000 rpm for 15 minutes at room temperature. The supernatant was discarded and the pellet was resuspended in 8 ml of Dulbecco's PBS. The tube was inverted several times to wash the cells then centrifuged at 1000 rpm for 10 minutes at room temperature. The supernatant was removed and supplemented RPMI medium (RPMI with fetal bovine serum Penicillin-Sreptomycin, Amphotericin) was added to the resuspended pellet such that the final cell density was about 1-2 x 10^6 cells/ml. Lastly, the culture was incubated at 37 ºC for 1 hour.

B.2. Cytotoxicity Assay. "Opey", *Derris elliptica* (Wall.) Benth. leaf extract [25%] and stem extract [50%] were each filtered through a 0.1 um PES syringe filter disc into a fresh microcentrifuge tubes each: with the negative control was supplemented RPMI, positive control was the Triton X-100 (0.1%), 25 % "Opey" leaf extract and 50% "Opey" stem extract. 270 ul of lymphocyte culture were added to the microcentrifuge tubes containing the treatments and finally, treated cultures were mixed and incubated with 5% CO_2 at 37 ºC for about 24+ 2 hours.

B.3. Cell Count. After incubation, treated cultures were obtained for cell counting. Seven microliters of incubated culture were added to 7 ul of trypan blue, and 7 ul of the mixed solution were placed in a hemocytometer. The number of live lymphocytes were counted in all 25 squares within the 1 mm center grid. Lastly, cell density (number of cells per ml) is computed using the following formula: #cells/ml= # of cells total/ # of 1mm^2 squares x 10^4 original dilution, where the dilution factor = 2 (GE Healthcare, 2007).

To determine the cytotoxicity effects of the leaf and stem crude extracts, the cytotoxicity assay was conducted using the prepared treatments. The cytotoxicity of the crude extract concentration was expressed in terms of the toxicity of formulation using the lethality 50 (LC50) standard value of the active ingredient (Oudejans, 1982). Besides, it was also based on the positive control which is commonly used.

The cytotoxicity test of the indigenous plant leaf and stem using the cytotoxicity assay was conducted at the Natural Sciences Research Institute (NSRI)- Biological Research and Services Laboratory, UP Diliman, Quezon City.

The study was conducted from December 2017 to April 2019. Then revised from November, 2023 to May, 2025.

The experimental and control groups for **cytotoxicity assay** are as follows:

Treatment Group

Derris elliptica (Wall.) Benth.“Opey” Leaf

T_1 - 25%, diluted 2.5 ml leaf crude extract and 7.5 ml of tap water.

T_2 - 50%, diluted 5.0 ml leaf crude extract and 5.0 ml of tap water

T_3 - 75%, diluted 7.5 ml leaf crude extract and 2.5 ml of tap water

Derris elliptica (Wall.) Benth. “Opey” Stem

T_1 – 25 %, diluted 2.5 ml stem crude extract and 7.5 ml tap water

T_2 - 50%, diluted 5.0 ml stem crude extract and 5.0 ml tap water

T_3 – 75, diluted 7.5 ml stem crude extract and 2.5 ml tap water

Control Group

Supplemented RPMI, Roswell Park Memorial Institute Medium (negative)

Triton X- 100, 0.1% (positive)

C. Simulated Experimental Set-up

For simulated experiments, three (3) different treatment concentrations were prepared from the crude extracts: 25 percent, 50 percent and 75 percent in three replicates. The simulated was conducted in the rice field. The experimental and control groups used for the **simulated experiments** are as follows:

Treatment Group

Derris elliptica (Wall.) Benth. "Opey" leaf

T_1- 25%, diluted 250 ml crude leaf extract and 750 ml distilled water

T_2- 50%, diluted 500 ml crude leaf extract and 500 ml distilled water

T_3- 75%, diluted 750 ml crude leaf extract and 250 ml distilled water

Derris elliptica (Wall.) Benth. "Opey" stem

T_1- 25%, diluted 250 ml crude stem extract and 750 ml distilled water

T_2- 50%, diluted 500 ml crude stem extract and 500 ml distilled water

T_3- 75%, diluted 750 ml crude stem extract and 250 ml distilled water

Control Group

T_0 – 0.5 ppm Sodium cyanide (NaCN)

The indigenous plant leaves and stems were collected from the barangays of Colalo, Mankayan, Benguet and Sadsadan, Mt. Province. The specimen was identified based on its local name from the area.

Materials and Procedures

The flow chart of the procedure is shown in figure 1. It involves two phases namely; Phase I which covers the phytochemical analysis of "Opey", powdered stems and leaves. The samples were brought to the laboratory analyst for phytochemical screening. Phase II covers cytotoxicity, laboratory and simulated experiments.

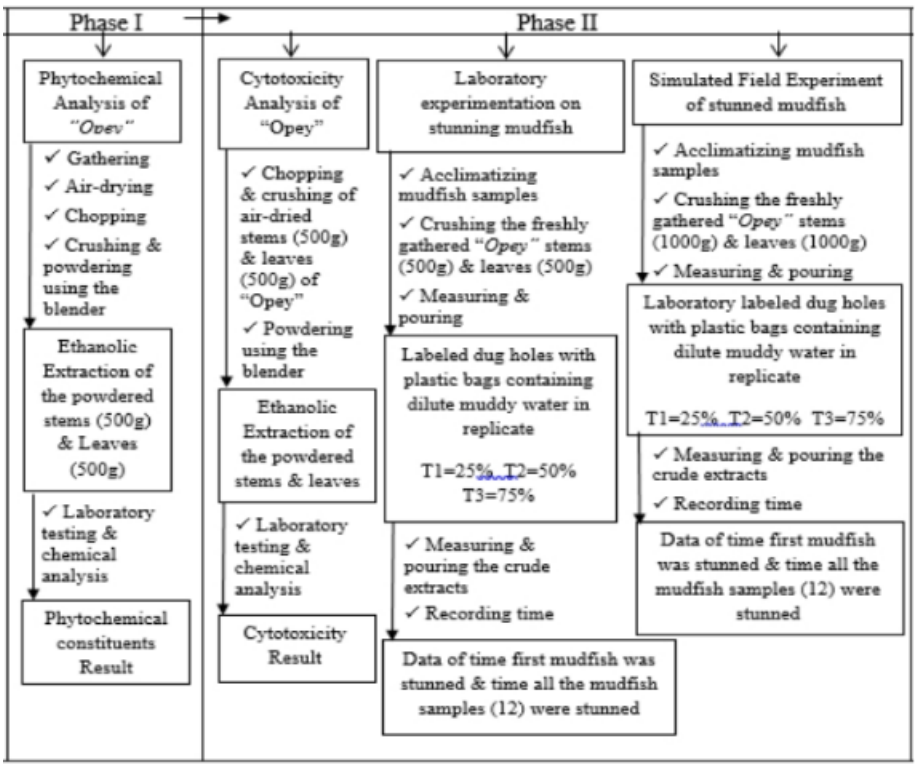

FIGURE 1
FLOWCHART OF PHYTOCHEMICAL, CYTOTOXICITY, LAB AND SIMULATED EXPERIMENTS

An electron microscope was used for counting the number of live cells, the number of dead cells, and the total number of cells. Also, the laboratory and simulated experiments.

Procurement of Mudfish. Mudfish samples were procured from residents of Bontoc and Sabangan, Mt. Province. Only the big-sized mudfish about five to six months old were used as samples. Each big-sized mudfish was weighed using the analytical balance. The 144 mudfish samples were acclimatized in a large aluminum basin for two weeks.

Data Gathered. The phytochemical constituents of the plant leaf and plant stem samples were determined. The cytotoxicity effects of the crude extract samples were determined. To assess the cytotoxicity of the "Opey" crude extracts, an approximate indication of the toxicity of the formulation was obtained and modified from applying the formula as cited by Oudejans (1982);

$$\text{Toxicity of formulation} = \frac{\text{LC50 standard value of active ingredient x 100\%}}{\text{\% active ingredient formulation}}$$

As a general guide, the probable lethal concentration for an average adult human may be derived from the acute oral or dermal LC50 (technical grade) for test animals cells and consumers. Moreover, to describe the cytotoxicity of the crude extracts, the following Oudejan's toxicity criterion was used (Oudejans, 1982).

Toxicity of Formulation (in mg/kg)	Description
Less than 5	Highly Toxic
5-50	Toxic
50 – 500	Moderately Toxic
500 – 5000	None Toxic

To classify the cytotoxicity formulation of the crude extracts, the following toxicity criterion for assessment of plant poisons was used (Oudejans, 1982). The acute toxicity of rotenone is believed to be moderately toxic to humans with an oral lethal concentration LC50 estimated from 300 mg/kg to 500 mg/kg (Goldman and Dj Monte, 2017).

Analysis of Data. In the data analysis, means, percentages, and differences were used, to check, test and analyze all the data. Qualitative analyses for the presence and absence of the phytochemical constituents were used for the secondary metabolites in "Opey" stems and leaves. Meanwhile, cytotoxicity assay for the toxic components.

The Completely Randomized Design was used when all variables were compared among groups using one-way ANOVA. This was used to determine the significant differences if any among the treatment means, if effective in the time of stunning mudfish performed in the laboratory and simulated set-ups compared with the positive control group (sodium cyanide). T-test for two independent means which was a comparison between the leaf and stem. Post hoc by Tukey's was used for the least significant difference.

III. RESULTS AND DISCUSSION

Phytochemical Analysis of "Opey" Leaf and Stem Crude Extracts, *Derris elliptica* (Wall.) Benth.

The phytochemical components present in the crude extracts of "Opey" leaf and stem; *Derris elliptica* (Wall.) Benth. are shown in Table 1.

The findings reflected on Table 1 imply the cytotoxic chemical compositions of the indigenous plant stem and leaf. The phytochemical constituents present in the plant stem and leaf are significant in the sense that some of them are proven to contain the rotenone, an organic fish poison that can be isolated from the bioactive component alkaloid (Qi, 2014). Plants that contain alkaloids are generally considered a feeding deterrent because of their bitter taste primarily

TABLE 1
QUALITATIVE ANALYSES OF THE PHYTOCHEMICAL CONSTITUENTS OF "OPEY" LEAF AND STEM CRUDE EXTRACTS, *DERRIS ELLIPTICA* (WALL.) BENTH.

Phytochemicals	Stem	Leaves
Sterols	-	+
Triterpenes	++	++
Flavonoids	-	+
Alkaloids	+++	++
Saponins	++	+
Glycosides	+	+
Tannins	+	+

Legend: + = traces ++=moderate +++ = abundant - = absence of constituents

due to its nitrogen content (Facchimi et. al., 2008). Alkaloids in plants have versatile heterocyclic nitrogen compounds with reported antimicrobial activity against fungal or bacterial

phytopathogens (Zotchev, 2013). Alkaloids at lower doses are pharmacologically useful and poison agents at lower doses (Glencross et.al., 2006) to fish. According to Staden et.al. (2019), triterpenes in plants have protective property against pathogens and pests. They also possess a vital role in the production of melanin and the biological content of ginseng tea which is known as an herb of great value. Alkaloids are abundant in the stem and moderate in the leaves which display a diverse and important physiological effects to humans and other animals. These are the well-known alkaloids which are in a way useful to humans, morphine, nicotine and ephedrine (Encyclopedia Britannica, 2019).

The presence of saponins in plants possess important bioactive properties such as expectorant, anti-inflammatory, vasoprotective, hypocholesterolemic, immunomodulatory, hypoglycemic, cytotoxic, molluscicidal, antifungal and antiparasitic activities (Singh, 2012). Besides, flavonoids were predicted as a potential drug in preventing chronic diseases because their effects attributed to their anti-oxidative, anti-inflammatory, anti-mutagenic, and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme function (Chandra, 2016). Meanwhile, glycocides are natural sugars found in several plants manufactured as medicines for treating heart failure. Cardiac glycocide drugs work by acting directly on the cell membranes of heart fibers. They increase the contraction and pumping strength of the heart (Morsy, 2017). Saponins are moderate in the stems while traces in the leaves which indicate a moderate anti-inflammatory, antimicrobial and cytotoxic properties as pointed by Chen (2010).

Moreover, tannins are the naturally occurring phenolic compounds in plants which are widely distributed in plant parts but present in high amounts in the bark of certain trees and bushes. Tannin is responsible for the astringency, color, and the tea flavor properties in plants and has been used to treat tonsillitis, pharyngitis, hemorrhoids and skin eruptions (Walker.et. al., 2014). Triterpenes are both moderate in “Opey” stems and leaves as they are found to treat inflammation and has a defense mechanism to the human body (Boleti, 2015).

Fishes stunned by alkaloids at low concentrations can be eaten by humans without adverse reaction. Rotenone incorporated in alkaloids has advantage that is unstable to heat and light loosing almost its toxicity after two to three days (Diaz, 2015).

TABLE 2
CYTOTOXICITY MEAN RESULT OF "OPEY" STEM CELL COUNT & CONTROL VARIABLES

Treatments in Replicate *"Opey"* Stem Crude Extracts	Mean Number Live Cells	Mean Number Dead Cells	Mean Number Total Cells	Mean Percentage Live Cells
25%	5.00	144.00	149.00	3.1
50%	1.22	156.78	158.00	0.8
75%	0.00	139.00	139.00	0.00
Supplemented RPMI (Negative Control)	149.00	6.67	155.67	95.7
Triton X – 100 0.1% (Positive Control)	0.00	147.67	147.67	0.00

Further in table 2, the test results for the 50 percent concentration are 0.8 percent of live cells was calculated and more than 50% mean number of dead cells. Using the result and the formula for formulation of toxicity gives the concentration of 50 percent which is 264 mg/kg with a descriptive equivalent of moderately toxic (Oudejans, 1982). Again, the main factor that affected the calculated concentration was the time of exposure which was quite long.

Likewise, table 2 presents the results for the cytotoxicity test of the 75 percent "Opey" stem concentration with 0 percent of live cell. The calculated concentration of the 75 percent "Opey" stem is 176 mg/kg which is considered as moderately toxic. Furthermore, the factors that might have affected the calculated concentrations was the time of exposure.

The positive control, Triton X- 100, only 0.1% percent of live cells. It is similar to the 75 percent 'Opey" stem treatment; however, the positive control is more toxic because it is synthetic

and commercially prepared. Supplemented RPMI which was the positive control is friendly because it killed 6.67 cells out of the 156 total cells.

As emphasized by Rakesh (2009) that toxicity of the prepared treatments has the capacity to cause injury to a certain specific test organism. To determine the effectiveness of the toxicity of the "Opey" stem, crude extracts were prepared in various concentrations and consider the time of exposure (Cahill, 2006). The 25% "Opey" stem crude extracts had the following results shown on table 2; the mean percent of live cells is 3.1and more than 50% mean number of dead cells. The test results that were used in the calculation were the percent mortality and number of total cells, the constant value for the LC50 of rotenone is 132mg/kg for adults and the percent concentration is 25 percent (Oudejans, 1982). Therefore, the toxicity of the 25 percent concentration is 550mg/kg. This indicates a non-toxic for the 25 percent "Opey" stem concentration.

TABLE 3
CYTOTOXICITY MEAN RESULT OF "OPEY" LEAF CELL COUNT & CONTROL VARIABLES

Treatments in Replicate *"Opey"* Leaf Crude Extracts	Mean Number Live Cells	Mean Number Dead Cells	Mean Number Total Cells	Mean % Live Cells
25%	132.00	13.00	144.00	91.2
50%	124.00	30.00	154.00	80.3
75%	32.00	128.00	160.00	20.2
Supplemented RPMI (Negative Control)	140.00	6.00	146.00	96.1
Triton X – 100 0.1% (Positive Control)	0.33	119.00	119.33	0.3

Also, table 3 shows the test results for the 50 percent concentration with 80.3 percent of live cells. Using this result and the formula for formulation of toxicity gives the concentration of 50 percent which is 1,389 mg/kg with a descriptive equivalent of non-toxic (Oudejans, 1982). It takes longer time for "Opey" leaf crude extract piscicides to take effect probably due to the moderate to traces of its phytochemical constituents. Another factor that might have attributed was the extraction process where the phytochemical constituents were not completely extracted.

The results for the cytotoxicity test of the 75 percent "Opey" leaf concentration with 20.2 percent of live cells is shown in table 3. The calculated concentration of the 75 percent "Opey" leaf is 220 mg/kg which is considered as moderately toxic. Furthermore, the factors that might have affected the calculated concentration were the longer time of exposure and the ethanol solvent which was not totally removed during the extraction process.

The positive control, Triton X- 100, 0.1% shows 0.3 percent live cells. It is similar to the 75 percent "Opey" leaf; however, the positive control is more toxic because it is synthetic and commercially prepared. Supplemented RPMI which was the positive control is friendly because it killed 6 cells out of the 146 total cells. Thus, the plant stem and leaf sample extracts reveal that there are cytotoxic phytochemicals present in the crude extracts.

Table 3 also presents that the finding is congruent with the results of the phytochemical analyses as all crude extracts tested are positive for the presence of alkaloids which are the nitrogenous compounds but have medicinal uses in humans. Alkaloids contain morphine, it is used as pain reliever and quinine used to treat malaria (Perviz et.al. 2016). The percent mortality from the 50 percent and 75 percent stem crude extracts likewise from the 75 percent leaf crude extract were evident to the alkaloid content which has the capacity to paralyze the nervous system (Diaz, 2015). Also, tested positive for flavonoids which are found in the roots, stem bark and leaves and possess a wide range of biological activities such as antimicrobial activity (Singh, 2014). The leaf is positive for flavonoids and are imparted by their ability to inhibit cellular DNA in a concentration-dependent manner (Asaduzzaman et. al., 2015). Another bioactive component is the saponin which has a haemolytic toxin in blood because of its tested effectiveness as cited by Das, 2018. As supported by Derongtin (2016), that phenolic compounds have anti-proliferative activities on the other hand tannins will biochemically target digestive enzymes and inhibit digestion (Brillouet et.al., 2014). Lastly, glycoside is an important chinese folk medicine that have

a well proven cardioprotective and cytotoxic effect (Mohamed, E. T..et.al, 2016). The stunned mudfish are safe to eat because the computed toxicity of formulation which indicated a less fatal result.

Table 4 presents the comparison on the rate of time the first mudfish sample was stunned by comparing the leaf and stem extracts simulated results. Also, the comparison on the rate of time all the mudfish samples were stunned from the application of the leaf and stem extracts. For the rate of time in stunning all the fish samples, T_3 from the stem extract has the shortest time with an average time of 8.22 minutes than the leaf extract which shows an average time of 10.23 minutes.

TABLE 4
COMPARISON BETWEEN THE EFFECTS OF "OPEY" LEAF AND STEM CRUDE EXTRACTS (SIMULATED)

Areas	Leaf	Stem	t_{VAL}	P_{VAL}
Rate of time the first fish was stunned				
T0	0.38	0.42	-1.852^{ns}	0.138
T1	3.08	2.17	13.242**	0.000
T2	4.36	3.63	2.953*	0.042
T3	4.46	4.06	11.433**	0.000
Rate of time all the fish was stunned				
T0	3.84	3.86	-0.069^{ns}	0.948
T1	6.30	5.04	16.815**	0.000
T2	8.33	7.38	10.254**	0.001
T3	10.23	8.22	17.153**	0.000

Legend: ** - *highly significant* * - *significant* *ns – no significant difference*

The findings on Table 4 suggest that there is highly significant difference on the average time it took the first mudfish to be stunned as well as the time all the mudfish were stunned as indicated by the p- value which is higher than .01, in comparing the effectiveness of the leaf and stem extracts. Specifically, the stem extract had shorter time to stun the mudfish than the leaf extract. This finding shows that the stem extract has a better stunning effect to mudfish than the

leaf extract. Roots and stem contain much alkaloids and these phytochemicals are toxic in nature but if taken in small amounts exert useful beneficial benefits in animals and humans (Matsuura, 2015). Foods are consumed with limitations.

IV. CONCLUSION

Based on the results of the study, the following conclusions were drawn:

"Opey", *Derris elliptica* (Wall.) Benth. stem contains various secondary metabolites such as triterpenes, alkaloids, saponins, glycosides and tannins. The leaf contains also various secondary metabolites such as sterols, triterpenes, alkaloids, saponins, glycosides and tannins. These secondary metabolites that are present in the stem and leaf can stun freshwater fish.

Crude extracts from the 25 percent "Opey" stem have non-toxic toxicity of formulation while the 50 percent and 75 percent have moderate toxicity. The "Opey" leaf 25 percent and 50 percent indicated non-toxicity while the 75 percent have moderate toxicity. The toxicity of formulation indicates the presence of cytotoxic phytochemicals in the "Opey" stem and leaf which proves their property to stun freshwater fish.

It is inferred that higher concentrations of the "Opey" stem extracts may result to shorter time for stunning fish. Also, it was found out that higher concentrations of "Opey' leaves showed shorter time for stunning more fish. Results imply that stem concentrations exhibited faster rate of time than leaf concentrations. It means that stem extracts were more effective than the leaf extracts in stunning fish, both in the laboratory and simulated experiments.

V. RECOMMENDATION

Explore other plants which have toxic contents to plants and animals but not to humans. Consider these plants which exhibit toxic effects but also have medicinal properties. Many herbs which are toxic have already been used traditionally to treat various ailments. The use of toxic herbs is acceptable or even desirable and preferred in the treatment of certain critical conditions such as cancer. Moreover, confirm the toxin in plant extracts through experiments and laboratory assays before describing as edible or not.

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