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EFFECTS OF *PERSEA AMAERICAN* LEAVES EXTRACTS ON ANTIOXIDANT ACTIVITIES AND DISEASES RESISTANCE OF *PSEUDOMONAS AERUGINOSA* INFECTED *CLARIAS GARIEPINUS*

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1. ABSTRACT
Disease resistance and antioxidant activities in *Pseudomonas* infected *clarias gariepinus* exposed to aqueous extracts of *Persea amaericana* leaves were assessed. One hundred and twenty (120) *C. gariepinus* of mean weight 120±13g were infected with 1.0ml of $4.1x10^4$cfu, using 2ml injection syringe. After diseases presence/sign, the infected fish were exposed to four different levels of aqueous *P. americana* leave extracts (0.0ml, 1.0ml, 1.5ml and 2.0ml) in triplicate, for a period of seven (7) days. Blood samples were collected from the experimental fish via kidney puncture before infection, after diseases presence and day 2,5 and 7 of exposure to *P. americana* leaves extracts and taken to the laboratory and the following antioxidants were accessed: Catalase (CAT), Glutathione (GSH), Glutathione-S-Transferase (GST), superoxide dismutase (SOD) and Melondialdehyde (MDA) to determine the level of stress imposed on the fish by the *P. aeruginosa*. All the assessed antioxidants were higher in the fish after the infection with *P. aerugionsa* and the therapeutic effect of the *P. americana* aqueous leaves extract on the infected *C. gariepinus*. At the end of the 7 days exposure (treatment) with the extracts, the antioxidant activities were lower in the treated groups (1.0ml – 2.0ml) compared to the untreated group (0.0ml), and the disease resistance ability were: 0.00 ± 0.00, 100.00±0.01, 91.67±9.71 and 66.67±7.51 in 0.0ml, 1.0ml, 1.5ml and 2.0ml aqueous *P. americana* leaves extract respectively.

KEYWORDS: Aquaculture, *Clarias gariepinus*, antioxidants, Disease resistance, *P. aeruginosa*.

2. INTRODUCTION
Aquaculture as the rearing of aquatic organisms (fish, molluscs and crustaceans) in a well-defined compartment, controlling the environment and their activities positively to produce a final commercial product (Garner, 2016). It serves different purposes such as; food production, restoration of threatened and endangered species population, wild stock population enhancement, building of aquaria, fish cultures and habitat restoration etc (FAO 2011).Aquaculture products such as fish and other seafood are good sources of protein, with immense nutritional value like natural oil such as omega-3 fatty acids and omega-6 fatty acids (Hasan, 2001, Ukwe *et al* 2018). Aquaculture increases the number of possible jobs in the market as it provides both new products for a market and create job opportunities because of the labor required to maintain the facilities and harvest grown organisms (Jangampalli 2019, Ukwe *et al* 2018).Some of the bottle necks in aquaculture include disease presence, pollution, poor feeds availability etc. (Ugwuba and Chukwuji 2010; Ajmal *et al* 2016).

In Nigeria, most of the fishing ground has been rendered unproductive by oil exploration, dredging of some water bodies and dumping of toxic industrial effluents (Olowosegun *et al* 2005). Successful rearing of fish in an artificial environment requires the administration of feed with the necessary ingredients in the right proportion, these feeds can be live, commercial or locally formulated (Bui *et al.*, 2010, Ukwe *et al*, 2018; Ukwe, 2019). Riche and Garling (2003) reported that fish reared in intensive tank systems require all nutrients in a complete pelleted diet since natural food is limited and fish cannot forage freely for natural foods.

The increase of pathogenic diseases in aquaculture has led to the use of various chemicals in aquaculture to help curb the spread of diseases in the culture system (Ayalew and Fufa 2018; Burridge *et al.*, 2008). Plants are a rich source of bioactive compounds like alkaloids and glycosides which are alternative sources of natural disease control (Ammar *et al* 2017; Ukwe and Gabriel 2019). Medicinal plants have been reported as appetite stimulators, antimicrobial, immnuno-stimulant, anti-inflammatory, bio-pesticides and anti-parasitic, (Ukwe

and Gabriel, 2019; Ukwe and Jamabo, 2020) and their use in traditional medicine is known all over the world (Parmar and Rawat 2012).

The intensive use of synthetic drugs/vaccines in aquaculture to prevent disease outbreak have the disadvantages of depositing in the fish flesh, polluting the environment, causing drug resistance diseases specific and are relatively expensive (Cabello, 2006, Romero-Ormazabal *et al*, 2012; Seyfried *et al* 2010; Ukwe and Gabriel, 2019; Pansik *et al*., 2005).

Practicing good farm management will go a long way to reduce mortality and unproductivity in aquaculture. Medicinal plants can therefore provide a cheaper and more sustainable alternative to chemotherapy in aquaculture, since they have been reported to display numerous bioactive properties such as anti-stress, immunostimulant, growth boaster etc, with no negative effect (Reverter *et al*., 2014, Ukwe and Gabriel, 2019).

Some approaches has been used to control pathogens in aquaculture. They include the following:

- Boosting the natural defense of the fishes by oral application of vaccines or via injections to boost their immune system. But the use of vaccines has the disadvantage of been deposited in the fish flesh, polluting the environment and developing drug resistance (Julia and Phillip 2010).
- Plant product application in aquaculture for disease control, growth etc. is one of promising alternatives to vaccines, they stimulate the immune system of fish, act as antibacterial and antiparasitic agents without the disadvantages associated with the use of vaccines (Ayalew and Fufa 2018).

African catfish, Clarias gariepinus, is the most popularly cultured fish in Nigeria (Sogbesan and Ugwumba, 2006). This species has drawn the attention of aquaculturists because of its biological attributes that include faster growth rate, resistance to disease and possibility of high stocking density (Saad *et al*., 2009; Olukunle, 2013; Megbowon *et al*., 2014).

The level of awareness of the impact of disease to aquaculture is scanty (Adeyemo *et al.*, (2003). Fish is the most parasitized of all vertebrates (Arme and Wakey, 1970). In aquaculture, some diseases are pathogenic and contribute to high fish mortalities and economic loss and may threaten the abundance and diversity of indigenous fish species (Mashego, 2001).

Fish disease are commonly caused by viruses, parasites, bacteria and worms (Madhuri *et al* 2012). An infected fish can be easily detected by its restless behavior or by spots on its skin (Ukwe and Oladapo-Akinfolarin 2019).

Antioxidants are believed to play a very important role in the body defense system against reactive oxygen species (ROS) (Boxin *et al*., 2002, Vivek and Surendra 2006). Halliwell (2007) reported that an antioxidant is "any substance that delays, prevents or removes oxidative damage in the body. Antioxidants are inhibitors of the process of oxidation, even at relatively small concentration and thus have diverse physiological role in the body (Anuj *et al* 2016). Antioxidant constituents of the plant material act as radical scavengers, and helps in converting the radicals to less reactive species (Anuj *et al* 2016). A variety of free radical scavenging antioxidants is found in dietary sources like fruits, vegetables, tea, etc. (Dhan *et al*; 2011). Nema *et al* (2009) reveals some information about the antioxidant presence in herbs and their role in organisms. Several studies have shown that an antioxidant-rich diet has a positive health impact in the body (Sin *et al*., 2013 and Willis *et al*., 2009).

Pseudomonas aeruginosa is a common encapsulated, Gram-negative, rod-shaped bacterium that can cause disease in plants and animal, including humans (Ugur *et al* 2012). Treatment of *P. aeruginosa* infection can be difficult due to its natural resistance to antibiotics (Quinn 2011).

Persea americana is a tree plant known as avocado pear, they are widely cultivated throughout the tropics and subtropics of the world for their edible fruits and for some economic and therapeutic uses (Purseglove 1977). Avocado pears are rich source of soluble phenolic, ascorbic acid and belatins compared to most common fruits and vegetables (Garcia *et al* 2001). It is recommended for gastritis, gastro duodenal ulcer, hypertension, anemia and exhaustion (Pamplona 1999). All parts of the avocado plant including leaves, bark, root, seed and fruit pulp are used for medicinal purposes (Tene *et al* 2016; Owolabi *et al*, 2005). Flavonoids found in avocado leaf can be used in the treatment of oxidative stress because flavonoids can freeze free radicals by donating hydrogen atoms or by single electron transfer (Prochazkova *et al* 2011, Putri *et al* 2013). Dietary powdered avocado pear leaves have been assets as growth enhancers and immunostimulants, and the results were impressive (Ukwe *et al*, 2020a; Ukwe *et al*, 2020b).

3. MATERIALS AND METHODS

Experimental Fish: One hundred and twenty (120) healthy *C. gariepinus* of mean weight 120-130kg was purchased from Rivers State University Aquaculture Center Rivers State, Nigeria.

Observation was carried out on the fish for a period of two weeks to access disease presence or bruises during this period and was fed to satiation with blue crown commercial feed twice daily.

Source of Pathogen: *Pseudomonas arueginosa* was ordered from the National Veterinary Institute, Vom in Jos, Plateau State, Nigeria and transferred to the Microbiology department of the Rivers State University for preservation.

Preparation of Experimental Herb: The *Persea americana* leaves aqueous extract was prepared using the methods (Ukwe and Jamabo 2020*). Persea.americana* leaves were washed clean, pounded to paste, soaked in tap water (50^0c) at the concentration of one hundred grams/liter (100g/l) for twenty four (24) hours. It was filtered and the filtrate was used immediately.

Experimental Design: A complete randomized method (CRD) was used. There were four treatment in triplicates.

Experimental Procedure: One hundred and forty (140) *C. gariepinus* were infected via intra muscular with 1.0ml of 4.1 x 10^4 cfu/ml of overnight grown *P. aeruginosa* using 2ml injection syringe and 21-guage hypodermic needle at day 1, 2, 4 and 5 and observed for disease presence. After disease presence, the infected fish was distributed into four (4) groups of ten (10) in triplicate and was treated via immersion with *P. americana* leaves aqueous extracts at 0.00ml/L, 1ml/L, 1.5ml/L and 2ml/L, for eight (8) hours daily.

Blood samples were collected before infection, after disease presence and at day 2, 5 and 7 and taken to the laboratory to ascertain the therapeutic effect of *P. americana* leaves extracts on the antioxidants (Catalase, Gluthathine, Superoxide Dismutase and Glutathione-S-Transference, Malondialdehyde) activities of the infected fish (*C. gariepinus*) as a means of treatment.

Blood Extracts: The fish was blindfolded by covering the head with a thick cloth, to attain calmness and blood was extracted via kidney puncture through the genital opening using 5ml injection syringe.

Antioxidant Analysis: The analysis was done using a spectrophotometer mode "SURGISPEC SM-23D" manufactured by Surgifield Medical, England according to manufacturer's instruction. The parameters were determined according to their required wave length and reagents as follows.

1. The spectrophotometer was adjusted through a control till the reading on the screen showed zero (0) transmittance.
2. A blank solution was prepared with the reagents except the sample, it was put into a cuvette and insert into the sample compartment of the spectrophotometer.
3. The spectrophotometer was set to 100% transmittance using the absorbent control and the cuvette with the blank solution was removed
4. The sample cuvette was wiped clean and insert into the sample compartment of the spectrophotometer, and its absorbance and transmitting values were read on the screen.
5. The sample cuvette was removed and the transmittance reading went back to zero.
6. The process was repeated for all the samples, adjusting wavelength accordingly and using the recommended reagents to determine the required parameters
7. The parameters were calculated using the formula:

$$X = \frac{AT}{AS} \; x \; Concentration \; of \; standard$$

Where X = Calculated parameter
AT = Absorbance of test
AS = Absorbance of standard

Disease Resistance: Was determined using the formula.

$$1 - \frac{\%Mortality \; in \; treated \; group}{\%Mortality \; in \; control} \; X \; 100$$

(Harikrishnan *et al*; 2010)

Data Analysis: The collected data was subjected to a one-way analysis of variance to determine if there were differences in the variables among treatments. Turkey's Multiple Comparism test was used to compare the means of the treatments (Wahua, 1999).

4. RESULTS

4.1 Antioxidants Activities in Plasma Biochemistry of the Experimental *C. gariepinus*

The antioxidant activities of the experimental *C. gariepinus* before and after the infection is shown in Table 4.1. All the analysed antioxidants increase in activities after the infection compared to the activities before the infection. Tables 4.2 – 4.4 shows that antioxidant activities of the experimental fish at day 2, 5, and 7 of the treatment respectively. The activities of all the antioxidants were higher in the treated groups compared to the untreated group (0.0ml) in all day 2 and 7, but is day 5, the activities of the GST was higher in the fish treated with 1.0ml of the experimental extracts compared to the rest, but CAT, GHS, SOD and MDA were higher in the treated groups compared to the untreated group.

The comparative activities of the analysed antioxidants within the period of the experiment is shown in Figures 4.1 – 4.5.

4.2 Percentage Survival and Disease Resistance (RSP) of *P. aeruginosa* Infected *C. gariepinus* exposed to different levels of *P. americana* aqueous leaf extracts

Table (4.5), shows the result for the survival rate and disease resistance of *C.gariepinus* after 7 days with *P.aeruginosa.* The treated groups had a higher survival rate and disease registance: 1.0ml (100.00 ± 0.01 and 100.00 ± 0.01), 1.5ml (96.67 ± 8.92 and 91.67 ± 9.71) and 2.0ml (86.67 ± 9.01 and 66.67 ± 7.51) respectively compared to the untreated group: 0.0ml (76.33 ± 9.11 and 0.00 ± 0.00).

Table 4.1: Anti-Oxidants Activities in Plasma Biochemistry of *Clarias gariepinus* before and after Infection (BI and AI) with *P.aeruginosa.*

Antioxidants (µg/ml)	*Before (BI)	Range		*After (AI)	Range	Max
		Min	Max		Min	
CAT	1.49±0.99[a]	0.41	2.36	3.18±1.05[b]	2.10	4.20
GSH	1.15± 0.81 [a]	0.40	2.02	2.19± 0.18 [b]	1.99	2.34
GST	0.13±0.06 [a]	0.08	0.20	0.38±0.14 [a]	0.29	0.55
SOD	0.31±0.06 [a]	0.12	0.46	0.51±0.13 [a]	0.37	0.62
MDA	0.39±0.13 [a]	0.25	0.52.	0.56±0.11 [a]	0.47	0.68

***Means within the same row with different superscripts are significantly different (P<0.05)**
Key: CAT- Catalase; GSH- Glutathione; GST- Glutathione-S-Transferase; SOD- Superoxide dismutase; MDA- Malondialdehyde.

Table 4.2: Antioxidant Activities in Plasma Biochemistry of *Clarias gariepinus Infected with P. aeruginosa* and exposed to different concentration of *P. americana* aqueous leaf extracts for two (2) days

Concentration (ml)	CAT	Antioxidants (µg/ml)		SOD	MDA
		GSH	GST		
0.00	2.39±0.70[b]	1.99±0.74 [b]	0.27±0.02 [a]	0.44±0.31 [a]	0.61±0.17 [a]
1.00	2.07±1.06 [b]	0.93±0.35 [a]	0.21±0.11 [a]	0.32±0.09 [a]	0.50±0.10 [a]
1.50	1.53±0.55 [a]	1.31±0.61 [b]	0.12±0.03 [a]	0.29±0.13 [a]	0.48±0.20 [a]
2.00	1.65±1.05 [a]	1.17±0.69 [b]	0.15±0.08 [a]	0.33±0.16 [a]	0.38±0.04 [a]

Means within the same column with different superscripts are significantly different (P<0.05)
Key: CAT- Catalase; GSH- Glutathione; GST- Glutathione-S-Transferase; SOD- Superoxide dismutase; MDA- Malondialdehyde.

Table 4.3: Antioxidant Activities in Plasma Biochemistry of *Clarias gariepinus Infected with P. aerugunosa* and exposed to different concentration of *P. americana* aqueous leaf extracts for Five (5) days.

Concentration (ml)	CAT	Antioxidants (µg/ml)		SOD	MDA
		GSH	GST		
0.00	2.22±0.29[a]	1.56±0.68 [a]	0.17±0.06 [a]	0.36±0.03 [a]	0.63±0.13 [a]
1.00	1.49±0.50 [a]	0.95±0.33 [a]	0.21±0.13 [a]	0.31±0.11 [a]	0.47±0.11
1.50	1.37±0.99 [a]	1.09±0.80 [a]	0.11±0.09 [a]	0.28±0.20 [a]	0.39±0.19 [a]
2.00	1.49±1.08 [a]	1.07±0.95 [a]	0.13±0.05 [a]	0.33±0.23 [a]	0.38±0.04 [a]

Means within the same column with different superscripts are significantly different (P<0.05)
Key: CAT- Catalase; GSH- Glutathione; GST- Glutathione-S-Transferase; SOD- Superoxide dismutase; MDA- Malondialdehyde.

Table 4.4: Antioxidant Activities in Plasma Biochemistry of *Clarias gariepinus Infected with P. aeruginosa* and exposed to different concentration of *P. americana* aqueous leaf extracts for Seven (7) days.

Concentration (ml)	CAT	Antioxidants (µg/ml)		SOD	MDA
		GSH	GST		
0.00	1.54±0.52[a]	1.06±0.35 [b]	0.14±0.05 [a]	0.44±0.29 [a]	0.56±0.17 [a]
1.00	1.29±0.22 [a]	0.85±0.64 [a]	0.09±0.03 [a]	0.40±0.26 [a]	0.35±0.15 [a]
1.50	1.33±1.31 [a]	0.91±0.12 [a]	0.08±0.06 [a]	0.36±0.26 [a]	0.43±1.21 [a]
2.00	1.27±0.33 [a]	0.87±0.37 [a]	0.08±0.05 [a]	0.37±0.22 [a]	0.51±1.65 [a]

Means within the same column with different superscripts are significantly different (P<0.05)
Key: CAT- Catalase; GSH- Glutathione; GST- Glutathione-S-Transferase; SOD- Superoxide dismutase; MDA- Malondialdehyde.

Table 4.5: Percentage Survival and Diseases Resistance (RSP) of *gariepinus* Infected with *P. aeruginosa* and exposed to different concentration of *P. americana* aqueous leaf extracts.

Concentrations (ml)	Stocking Density	% Mortality	% Survival	RSP
0.00	10.00±0.02[a]	23.37±3.56[c]	76.33±9.11[a]	0.00±0.00[a]
1.00	10.00±0.01[a]	0.00±0.00[a]	100.00±0.01[d]	100.00±0.01[d]
1.50	10.00±0.02[a]	3.33±0.12[a]	96.67±8.92[c]	91.67±9.71[c]
2.00	10.00±0.01[a]	13.33±2.05[b]	86.67±9.01[b]	66.67±7.51[b]

Means within the same column with different superscripts are significantly different (P<0.05)

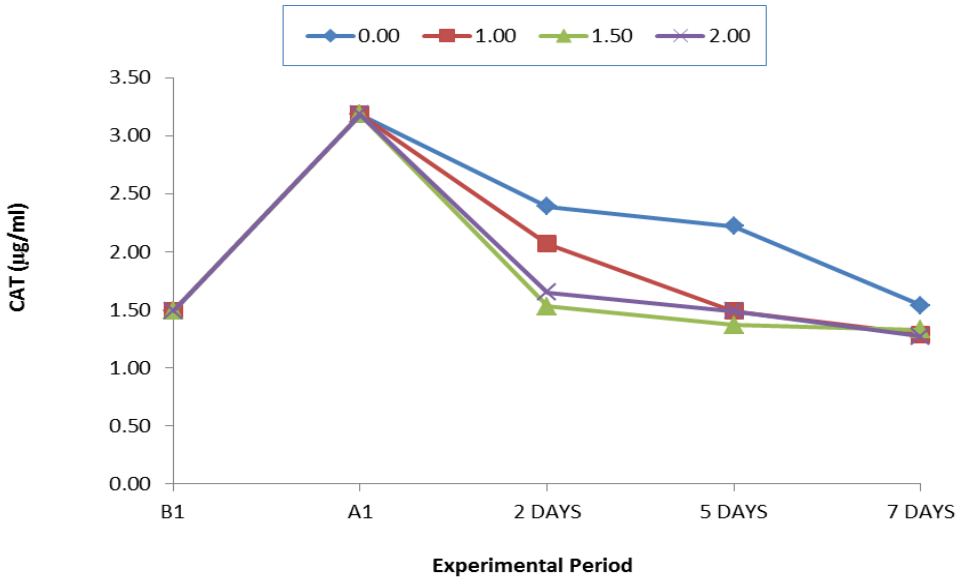

Figure 4.1: Comparative Values of CAT in the Plasma of *C.gariepinus* Infected with *P.aeriginosa* and exposed to *P.americana* Extracts at different Experimental Period

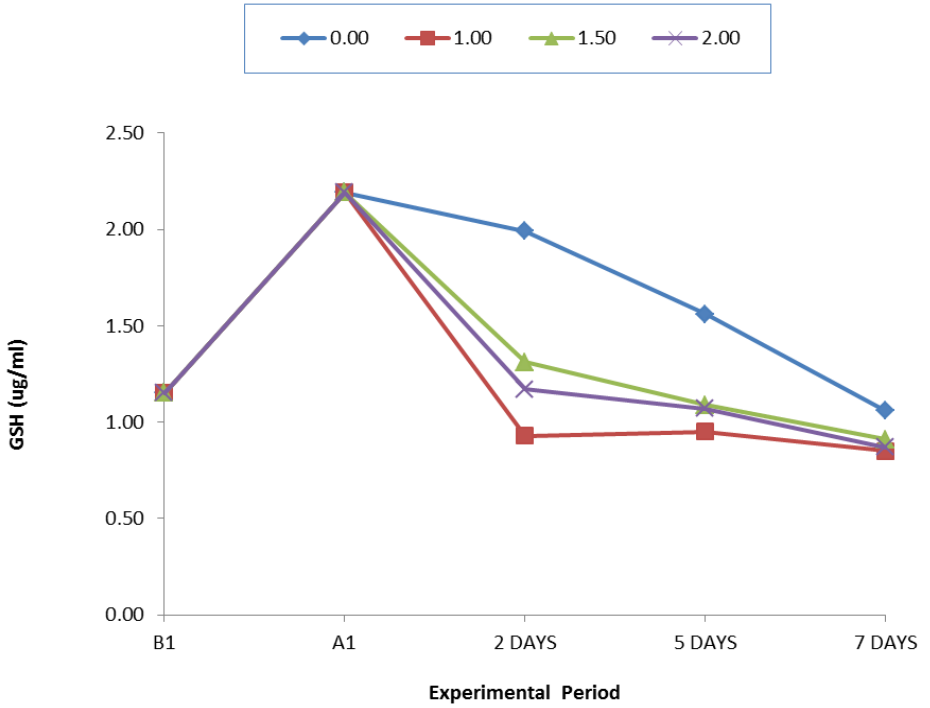

Figure 4.2: Comparative Values of GSH in the Plasma of *C.gariepinus* Infected with *Paeruginosa* and exposed to *P. americana* Extracts at different Experimental Period

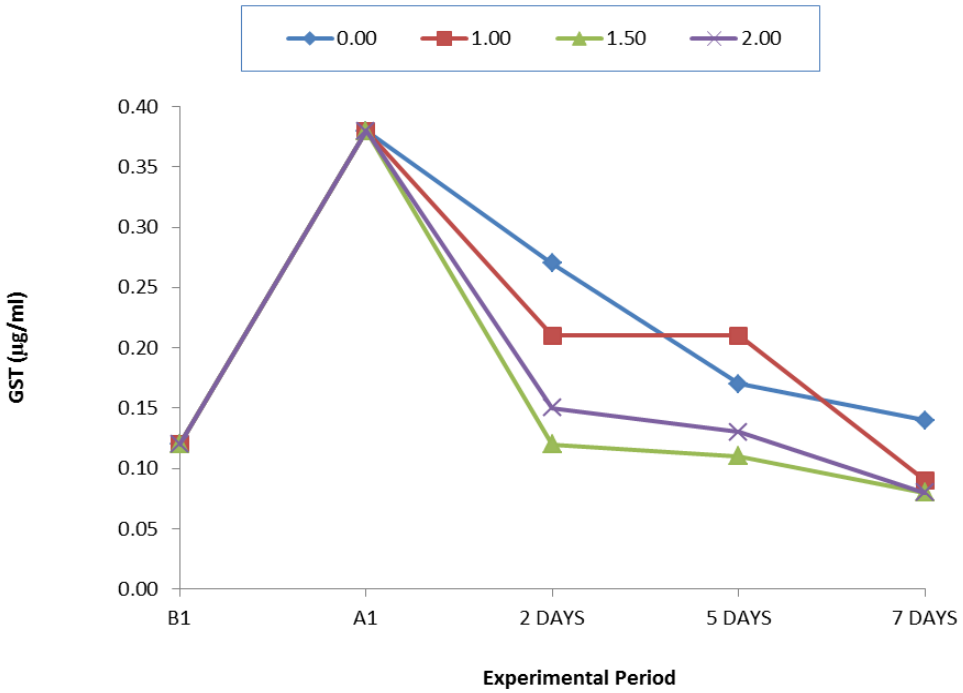

Figure 4.3: Comparative Values of GST in the Plasma of *C.gariepinus* Infected with *P.aeriginosa* and exposed to *P.americana* Extracts at different Experimental Period

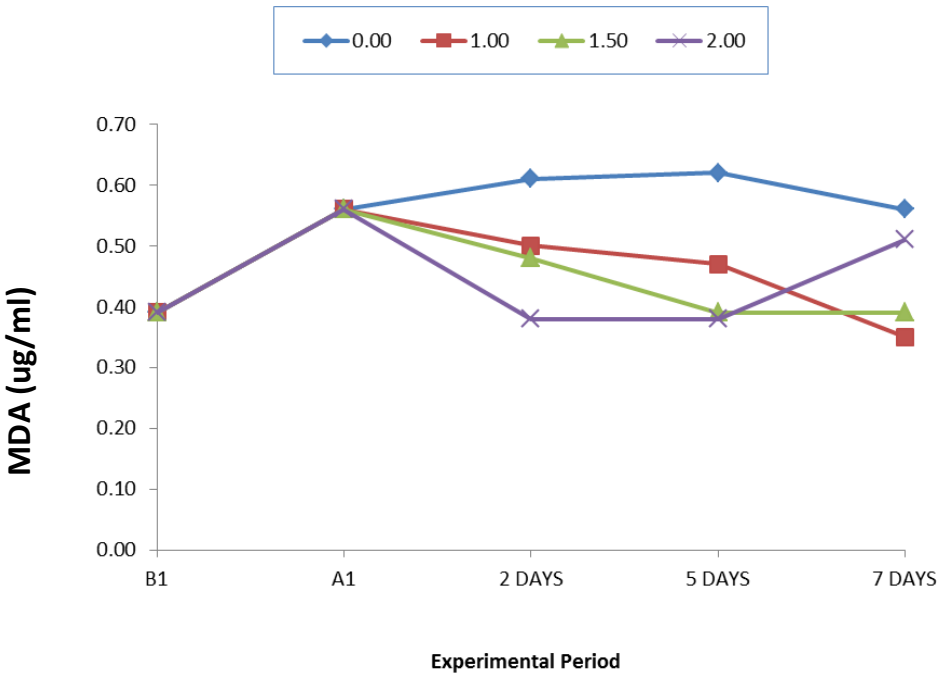

Figure 4.5: Comparative Values of MDA in the Plasma of *C.gariepinus* Infected with *P.aeruginosa* and exposed to *P. americana* Extracts at different Experimental Period

5. DISCUSSION

Antioxidants are necessary nutrient component of the body to prevent and cope with oxidative stress (Nurdin *et al.*, 2018). Antioxidants such as CAT, GSH, GST, SOD and MDA were analyzed to determine the oxidative stress imposed on *C. gariepinus* by *P. aeruginosa*, and the therapeutic effect of *P. americana* leaves extract on the infected fish. Oxidative stress occurs in fish when the reactive oxygen specie (ROS) present in the fish is above the considerable limit (Oaks and Vander Kraak, 2003). Some of the reactive oxygen species includes; Hydrogen peroxide (H_2 0_2), hydroxyl radical ($H0^-$), super oxide radicals (0_2), singlet oxygen ($.0^2$) etc (Sato et al, 2013; Navarro-Yepes *et al*, 2014). Some of the functions of these reactive oxygen species are ageing, iron transportation, including stress, gene expression etc (Lu *et al*, 2002), and they have negative effects on the membranes, lipids, proteins, lipoproteins, and deoxyribonucleic acid (DNA) (Droge, 2002; Wilcox, *et al*, 2004; Young and Woodside, 2001). Some factors responsible for oxidative stress in fish includes: pathogen presence, polluted environment, diet and seasonal variation (Mattos *et a*l., 2017; Ural., 2013). The first line of defense against oxidative stress is the production of antioxidant such as CAT, GSH, GST, SOD and MDA (Ural, 2013).

After infecting the fish (*C. gariepinus*) with *P. aeruginosa*, all the activities of the analyzed antioxidants were higher compared to the activities before infection and they all had a decrease with the application of aqueous avocado pear leaves extracts (1.0ml, 1.5ml and 2.0ml) compared to the untreated group (0.00ml), though the activities in the untreated group were lower than they were before the treatment period as the days of treatment increases. This research is in line with that of Owalabi *et al* (2010), who observed that avocado pear leaves have strong antioxidant activity, which may help in preventing or reducing the effect of various diseases associated with oxidative stress. Dautremepuits *et al* (2003) also reported an increase in antioxidant enzymes in common carp infected with *Phychoborthrius spp*, and Rudneva *et al* (2014) while analyzing blood biomarkers of three species of Black sea elasmobranchus in their works arrived at findings agreeable to this results.

According to Nurdin *et al.*, (2018), avocado pear leaf extract contains flavonoids, saponins, tannins and steroids. Flavonoids found in avocado leaf extracts can be used in the treatment of oxidative stress because flavonoids can freeze free radicals by donating hydrogen atoms or by single electron transfer (Prochazkova *et al*., 2011; Putri *et al*., 2013). Increase in SOD activity after infection may be due to the presence of increasing superoxide radicals (0^{2-}) which stimulates the production of the SOD, for the disputation of 0^{2-} to molecular oxygen (0_2) and hydrogen peroxide (H_20_2) which are less reactive (Weydert and Cullen 2010; Aziz *et al*., 2019), the presence of high superoxide radical (0^{2-}) depicts a pathogen – killing process and a by product of mitochondrial respiration (Aziz *et al*, 2019, Krishnonurthy and Wadhwani, 2012). Increase in superoxide dismurtase (SOD) was observed in *Salmo trutta trutta* with ulcerative dermal necrosis plus infection of *Saprolegnia fungus* (Kurhalyuk *et al*., 2009). The presence of hydrogen peroxide stimulates the organs to produce more CAT to scavenge the free radicals, a process that induce stress if prolonges (Xie et al, 2008). H_20_2 is also produced by the oxidation of fatty acids, respiration and purine catabolism (Krishnomurthy and Wadhwam, 2012), and excess production of H_20_2 can lead to CAT deficiency which can result to abnormalities and several disease conditions (Goth *et al*, 2004).

The MDA was higher after infection compared to the activities before infection. MDA is a mutagenic product of lipid peroxidation (Esterbamer *et al*, 1990), and they are biomarkers of lipid peroxidation of Omega-3 and Omega-6 fatty acids (Esterbamer *et al*, 1990; Pryor, 1989), and lipid oxidation which produces MDA as by product can be use as a biomarker to determine oxidative stress in tissues, and this stress increase daily (Arguelles *et al*, 2007a; Arguelles *et al*, 2007b). The higher level of MDA in the infected fish in this experiment could be as a result of higher perioxidation (Nagasaka *et al*, 2004; Moureute *et al*, 2000). This could lead to accelerated cell and tissue damage (Buege and Aust, 1978), and can also affect flesh quality in fish (Farahi *et al*, 2012). The increase presence of glutathione (GSH) in the infected fish is an indication of the presence of excess free radicals in the cells and tissue (Sun, 2010), and this to an extend explains the higher presence of glutathione – S-Transferase (GST) since it is a cofactor to GSH (Sun, 2010). This could lead to diseases (Homma and Fujii, 2015).

The increase in CAT, GSH, GST, SOD and MDA in the untreated group (0.0ml) compared to the treated groups (1.0ml, 1.5ml and 2.0ml) is an indication that *P. aeruginosa* was virulent on the fish and it was exhibiting its protective tendencies against the pathogen (Adeyemi 2014; Ural, 2013), and prolong stress situations such as this can lead to mortality as a result of cardiovascular diseases (Pacher, 2007), respiratory disorders (Caramori and Papi 2004) and renal disorders (Droge, 2002). The decrease in CAT, GSH, GST, SOD and MDA in the treated groups (1.0ml – 2.0ml) expresses the antibacterial activities of the aqueous *P. americana* leaf extracts which may have reduced the pathogenicity of the *P. aerguinosa* by boosting the fish immune system (Sales *et al* 2013) or saving the fish organs from the stress of releasing these antioxidants, by making them available to eliminate ROS (Goda, 2008), it could also be as a result of vitamin C and other phytochemicals present in the *P. americana* (Nurdin *et al.*, 2018; Ogundare and Oladejo, 2014) which prevents stress (Altemini *et al.*, 2017). Abdelazim *et al.*, (2018), reported that the elevated values of some antioxidants in Nile tilapia exposed to zinc oxide nanoparticles were returned to normal when the infected fish was fed with a mixture of vitamin C and

E, while Tan *et al.*, (2018) also reported the restoration effects of these antioxidants enzymes by Vitamin C and E.

At the end of day 7 of the experiment, the survival rate and disease resistance were higher in the treated groups (1.0ml, 1.5ml and 2.0ml) of aqueous *P. americana* leaves extracts compared to the untreated group (0.0ml). This result is inline with that of Olusola and Nwokike (2018) who observed improvement in RSP and percentage survival when *C. gariepinus* was fed diets of various inclusion of bitter leaves (*Veronia amylgdalina*) and pawpaw (*Carica papaya*) leaves extracts were infected with *A. hydrophila*; and Nasir *et al* (2018) who reported improved performance in RSP and survival percentage when C. gariepinus fed with diets supplemented with spirulina (*Arthrospia platensis*) was challenged with *A. hydrophila*. The low survival and relative survival percentage in the untreated group could be as a result of the stress impose on the fish by the experimental pathogen which may have cause severe cellular damages (Law *et al*, 2017). It could also be as a result of stress related cardiovascular failure in the infected fish (Pacher, 2007). Some medicinal phytochemicals in *P. americana* leaves include flavonoids, tannins and saponins (Sung *et al*, 2012; Tiwari *et al* 2011). These phytochemicals which are antibacterial may have improved the immune system of the fish (Sales *et al.*, 2013) or weaken the potency of the pathogen (*P. aeruginosa*) (Sales *et al.*, 2013). These phytochemicals also play the role of anti-stress to the fish in the treated groups (Walter and Marchesan, 2011).

CONCLUSION AND RECOMMENDATIONS

Stress presence in aquactulture is a major bottle neck to fish culture, as it affects production due to poor growth rate, diseases presence, poor fish quality. The presence of anti-oxidants such as CAT, SOD, MDA, GSH and GST in noticeable quantities in the untreated fish plasma is an indication that the fish was stress as a result of reactive oxygen species, and are susceptible to disease that can cause mortalities if prolonged. This work have shown that avocado pear aqueous leaf extract contains phytochemicals with strong antioxidant activities that can be used to prevent and cope with oxidative stress arising from the presence of pathogens such as *P. aeruginosa*. The use of avocado leaf (*P. americana*) should be considered for the treatment or control of bacteria infections especially *P. aeruginosa* in *Clarias gariepinus.* Avocado pear leaf which has little or no economic value, can be used as a means of controlling or reducing stress associated with *P. aeruginosa* infection in fish. This is cost effective and limits the use of inorganic drugs which are not environmental friendly and deposits in the fish flesh. Planting or growing of avocado pear tree should be encouraged in farms to enhance the availability of the leaves.

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