AQUEOUS ALMOND (*TERMINALIA CATAPPA*) LEAF EXTRACT ENHANCED GROWTH PERFORMANCE, IMMUNOCOMPETENCE AND RESISTANCE OF *HETEROBRANCHUS LONGIFILIS* TO *PSEUDOMONAS AERUGINOSA*

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ABSTRACT
The growth, survival, oxidative stress and resistance of Heterobranchus longifilis fingerlings to Pseudomonas aeruginosa after receiving dietary Terminalia catappa extract for 10 weeks were investigated. Five diets; AL1 (no extract, control), AL2 (0.5% ethanolic extract), AL3 (1.0% ethanolic extract), AL4 (0.5% aqueous extract) and AL5 (1.0% aqueous extract) of T. catappa were allotted triplicate groups of H. longifilis (3.82± 0.05 g) for 70 days. Results shows higher concentrations of total phenolics, tannins, alkaloids and flavonoids were observed in ethanol extracts than aqueous extracts. Feed intake significantly (P<0.05) reduced in fish fed ethanol extract, with mean weight gain significantly (P<0.05) high in AL1 (22.06 g), AL5 (21.87 g) and AL4 (20.20 g), while the least value was recorded in AL2 (14.13 g). Hematocrit values significantly reduced in AL2 and AL3, while the lowest value of haemoglobin was recorded in AL3. Higher values of glutathione S-transferase (GST) and superoxide dismutase (SOD) were observed in AL4 and AL5. Survival ranged from 83.67% in AL3 to 95% in AL5. The relative percentage survival (RPS) ranged from 20% in AL1 to 90% in AL5 after the challenge with Pseudomonas aeruginosa. This study shows that extraction using water as solvent of almond leaves (T. catappa) conferred more benefits in terms of growth, improved immune system and resistance to virulent A. aeruginosa.
Keywords: Indian almond, African Catfish, growth, solvents, immune system.

INTRODUCTION

he search for organic substances as alternatives to synthetic chemicals for the sustainable control of stress and diseases, and improved performance and overall enhancement of fish productivity under the intensive aquaculture systems increased in recent times (Abdel-Tawwab *et al*, 2018; Omitoyin *et al*., 2019; Ajani *et al*., 2020). Several plants extract rich in polyphenolic compounds are considered feed additives in this regard, haven shown high potential to enhance weight gain, feed efficiency and/or disease resistance in fish (Reverter *et al.,* 2014). Extracts of tropical almond (*Terminalia catappa*); one of the numerous herbal plants identified for its antifungal, antimicrobial, antiparasitic, growth promoting, immune-stimulating and stress reducing properties, is rich in flavonoids, phenolic acids, and tannins (Ahmed *et al.,* 2005). However, the methods of extraction, solvent used and the plant parts are significant to the quality of extract (Bae *et al.,* 2012). *Pseudomonas aeruginosa* is a part of the microbiota found in fish, but with strong tendency to become opportunistic under conditions of stress in *Heterobranchus longifilis;* one of the most prominent cultured species (Abubakar *et al*, 2015). Attack of *P. aeruginosa* results in a systemic infection known as *Pseudomonas* septicemia, manifested by haemorrhagic ulceration of skin and peeling, distention of abdomen, exophthalmia and high mortality (Raj *et al*., 2019; El-Bahar *et al*., 2019). This study evaluates the growth performance, gut morphometry, haematology of *Heterobranchus longifilis* fed diets supplemented with ethanoic and aqueous extracts of *T. catappa* leaf and its ability to resist *P. aeruginosa* infection.

MATERIALS AND METHODS

Air-dried Indian almond leaves (96 hours at a temperature of 30ºC) were reduced to fine particles and cold maceration method was used for extraction using ethanol and aqueous solvents. The extracts were subjected to quantitative and qualitative screening (Manjulika *et al.,* 2014). Five experimental diets containing 40% crude protein, with diet AL1 containing no extract (control), AL2 (0.5% ethanol extract), AL3 (1.0% ethanol extract), AL4 (0.5% aqueous extract and AL5 (1.0% aqueous extract) were produced. Diets were randomly assigned to 300 *Heterobranchus longifilis* fingerlings (3.82±

0.05g) in 15 rectangular plastic tanks of 0.42m x 0.29m x 0.25m, for 12 weeks at 3% of body weight. After 70 days of feeding, growth in fish and nutrient utilization parameters were determined (Castel and Tiews, 1980). For haematological indices, 6 fish randomly selected per treatment, were serially bled (Omitoyin *et al.,* 2019) into 2 bottles each (one with anticoagulant and the other without for blood and serum biochemical analysis respectively). Liver samples from 6 fish per treatment weighing 0.5 g were macerated with physiological saline and centrifuged (3,000 rpm) for 10 min (Ilavazhahan *et al*., 2012). Supernatants from the samples were put in plain bottles at -20°C for superoxide dismutase (SOD), glutathione S-transferase (GST), malondialdehyde (MDA), total protein and glutathione peroxide (GPx) determination using standard procedures.

A virulent *P. aeruginosa* strain was used to challenge the fish after the feeding trial. The bacterium was inoculated in cetrimide agar plate and incubated at 37°C for 16-24 hours (Brown and Lowbury, 1965). Twenty fish were randomly collected from each experimental group and divided into two groups each of 10 fish. After fasting for 24 hours, the first group was intraperitoneally injected with 0.2 ml PBS containing virulent *P. aeruginosa* (1×10^7 CFU/ml) (Misra *et al*., 2006) and the second group with 0.2 ml of saline solution. All fish were kept under observation for 14 days to record the daily mortalities. Data were subjected to one-way analysis of variance and differences in mean separated using Duncan Multiple Range Test at a significant level of $p<0.05$ (Duncan, 1955).

RESULTS AND DISCUSSION

The quantitative assessment of the extracts from the two solvents shows higher concentrations of total phenolics, tannins, alkaloids and flavonoids in ethanol extracts than aqueous extracts, except for saponin (Table 1). This can be attributed to the varying polarity in the solvents. According to Archundia *et al,* (2019), the polarity in ethanol be described as medium while it is high in water. The effect of extracts on growth and survival of *H. longifilis* is presented in Table 2. Feed intake significantly (*P<0.05*) reduced in fish fed ethanol extracted *T. catappa* based diets. This is also reflected in the mean weight gain in experimental fish, as the groups of fish fed aqueous extracts recorded significantly higher values when compared with those fed ethanol extracts-based diets. Similarly, superior feed conversion ratio (FCR), specific growth rate (SGR) and protein efficiency ratio (PER) values were observed in fish fed aqueous extracts-based diets compared to the group fed ethanol extract-based diets. Growth indices showed statistical similarity between the aqueous groups and the control group. Survival ranged from 83.67% in AL3 to 95% in AL5, indicating poor performance in the ethanol group.

Table 1: Result of the quantitative screening of ethanoic and aqueous extracts of *T. catappa*

Phytochemical	Ethanoic extract concentration	Aqueous extract concentration
Total Phenolics (mg/g GAE)	89.139±0.01	42.462±0.00
Tannins (m/g GAE)	5.862±0.01	2.100±0.00
Alkaloids (mg/g)	13.66±0.03	10.30±0.03
Flavonoids (mg/g RE)	97.259±0.26	2.741±0.02
Saponins (%)	1.34±0.01	2.04±0.04

Table 2: Growth performance and survival of *H. longifilis* fed experimental diet for 70 days.

Parameter	AL1	AL2	AL3	AL4	AL5
Initial weight (g)	3.70±0.12	3.87±0.07	3.90±0.06	3.80±0.05	3.83±0.03
Final weight (g)	25.76±1.33^{a}	18.00±0.58^{b}	19.33±0.88^{b}	24.00±3.46^{a}	25.67±1.76^{a}
Feed intake (g)	33.96±0.64^{a}	28.20±0.69^{b}	29.41±3.76^{b}	31.43±2.41^{a}	33.61±1.45^{a}
Weight gain (g)	22.06±1.42^{a}	14.13±0.52^{b}	15.46±0.85^{b}	20.20±3.46^{a}	21.87±1.51^{a}
SGR (%g/day)	2.77±0.04^{a}	2.19±0.04^{b}	2.28±0.04^{b}	2.63±0.08^{a}	2.72±0.03^{a}
FCR	1.53±0.05^{c}	1.99±0.43^{a}	1.90±0.48^{b}	1.55±0.22^{c}	1.51±0.04^{c}
PER (%)	1.62±0.00^{a}	1.25±0.01^{b}	1.32±0.01^{b}	1.60±0.02^{a}	1.64±0.00^{a}
Survival rate (%)	90.00±7.64^{a}	85.00±2.89^{ab}	83.67±21.28^{b}	90.33±4.41^{a}	95.00±2.89^{a}

^{abc}Means on the same row with different superscripts are significantly ($P<0.05$) different *FCR, feed*

conversion ratio, PER, protein efficiency ratio What of SGR

The poor utilization of nutrients which resulted in reduced growth in groups fed ethanol extract-based diets can be attributed to the possible high concentration of bioactive compounds including phenols, flavonoids and tannins in the diets, as pointed to by the quantitative phyto-screening results. According to Thakur *et al.* (2019), phenols and other anti-nutrients present in plants, although exert beneficial health effects at low concentrations, becomes anti-nutrients responsible for deleterious effects related to absorption of nutrients and micronutrients. Hematocrit values significantly reduced in AL 2 and AL3. Also, the lowest value of haemoglobin was recorded in AL3. While values for white blood cells and total protein did not vary significantly (*P>0.05*). Higher values of glutathione S transferase (GST) and superoxide dismutase (SOD) were observed in fish fed aqueous extract-based diets. At the end of the challenge with *A. aeruginosa*, the relative percentage survival ranged from 20% in the control group to 90% in AL5, indicating increased survival in fish fed extracts of *T. catappa*. Superoxide dismutase and glutathione S-transferase are antioxidant and sensitive indicators of oxidative stress (Carvalho *et al*., 2012). The higher value in the aqueous groups is indicative of antioxidant protection activation, which is required in fish during the normal feeding cycle. This study also reveals that extract of *T. catappa* increased the resistance of *H. longifilis* to virulent *Pseudomonas aeruginosa*.

Table 3: Haematological indices of *H. longifilis* fed experimental diet for 70 days

Parameters	AL1	AL2	AL3	AL4	AL5
PVC	30.00 ± 1.55^{a}	27.20 ± 0.58^{b}	22.00 ± 0.58^{c}	31.00 ± 0.58^{a}	29.00 ± 0.58^{a}
Hb	9.83 ± 0.27^{a}	9.57 ± 0.22^{a}	7.80 ± 0.44^{b}	10.03 ± 0.26^{a}	9.57 ± 0.22^{a}
RBC	2.86 ± 0.08^{a}	2.91 ± 0.04^{a}	1.78 ± 0.08^{b}	3.00 ± 0.09^{a}	1.76 ± 0.06^{b}
WBC	13.90±0.18	13.56±0.47	13.35±0.05	14.60±0.19	14.30±0.08
Total protein	$4.47^{a} \pm 0.08$	4.63± 0.09	4.63± 0.03	4.57 ± 0.09	4.80± 0.17

[abc]Means on the same row with different superscripts are significantly (P<0.05) different. Please, define all abbreviations

Use the standard deviations as used in table 3 and re-write the values in table4. Avoid pasting of values and their Standard deviations

Table 4: Mean values for liver biomarkers of *H. longifilis* fed experimental diet for 70 days and Relative percentage survival after challenge with *Pseudomonas aeruginosa*.

Parameters	AL1	AL2	AL3	AL4	AL5
TP (µg/dL)	12.33 ± 0.08^{d}	$15.43 \pm 0.16_{c}$	$15.31 \pm 0.10_{c}$	$16.19 \pm 0.09_{b}$	$17.24 \pm 0.38_{a}$
GPx (units/mg protein)	$10.81 \pm 0.35_{b}$	$11.51 \pm 0.57_{ab}$	$11.79 \pm 0.37_{ab}$	$11.03 \pm 0.16_{ab}$	$12.26 \pm 0.54_{a}$
GST (unit/mg protein)	$42.74 \pm 1.39_{bc}$	$46.8 \pm 6.05_{bc}$	$39.24 \pm 0.55_{c}$	$56.29 \pm 0.06_{a}$	$51.41 \pm 3.04_{ab}$
MDA (µmol/mg protein)	$1.94 \pm 0.30_{bc}$	$2.39 \pm 0.28_{b}$	$5.61 \pm 1.38_{a}$	$2.03 \pm 0.310_{b}$	$1.35 \pm 0.12_{c}$
SOD (units/mg protein)	$5.56 \pm 0.18_{ab}$	$3.60 \pm 0.67_{c}$	$1.73 \pm 0.26_{d}$	$4.01 \pm 1.60_{bc}$	$6.60 \pm 0.59_{a}$
Relative percentage survival (%)	20	60	50	80	90

[abcd]Means on the same row with different superscripts are significantly (P<0.05) different

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