

**Toxicological evaluation of plant crude extracts on helminth parasites of *Clarias gariepinus* using host low observed effect concentration (LOEC).**

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### ABSTRACT

The potency of seven medicinal plant crude extracts against helminth parasites of *Clarias gariepinus*; *Wenyonia* sp. and *Procamallanus* sp. with less toxicological effect on host fingerlings were investigated. The activities of crude extracts of *Allium sativum*, *Cymbopogon citrates*, *Ocimum sanctum*, *Thymus vulgaris*, *Vernonia amygdalina*, *Zingiber officinale*, and *Zanthoxylum zanthoxylones* were investigated at 96 hours exposure. Relative Toxicity Factors (RTFs) of the extracts increase at host fingerlings' Low Observed Effect Concentration (LOEC) - 0.1 LC<sub>50</sub> g/L in the trend; *A. sativum* (RTF, 1.0) < *C. citrates* (RTF, 2.0) < *O. sanctum* (RTF, 2.0) < *T. vulgaris* (RTF, 3.0) < *V. amygdalina* (RTF, 4.0) < *Z. officinale* (RTF, 5.0) < *Z. zanthoxylones* (RTF, 10.0). As the concentration/response increases in the intervals; 1.0 LC<sub>50</sub> g/L and (1.5) LC<sub>50</sub> g/L, there were no change in relative toxicities except for *Z. officinale*, that showed decreased RTFs, while *Z. zanthoxylones* showed tremendous increase in RTFs (RTFs, 10.0, 16.0 and 27.0). Average Survival Time (AST) and Percentage Reduction in Survival Time (%RST) relative to saline solution for the intestinal parasites exposed to the extracts at the least interval; 0.1 LC<sub>50</sub> g/L host fingerling, showed the similliar trend in susceptibility. *Z. zanthoxylones* had shown the highest potency against helminth parasites compared to the other extracts. The effects of plant extracts on parasites and the sensitive lifestage of their fish host is dose-dependent and to avoid overdosage, the need for determination of suitable concentration is of great importance.

**Keywords:** Plant crude extracts, Reduction in Survival Time, Intestinal parasites, Relative Toxicity Factors, fingerlings.

### INTRODUCTION

Fish and fishery products represent a very valuable source of protein and essential micronutrients for balanced nutrition and good health. Factors such as overcrowding, sudden changes in temperature, poor water quality and nutritional status could cause changes in fish physiology: stress tolerance, immunological status and thus, susceptibility to infestation. Moreover, diseases outbreak, are frequently associated with fish fitness and health, most pathogens being opportunistic and taking advantage of immunocompromised or stressed fish, thus alternative solutions should maximize fish immunity and fitness to avoid and face pathogen infections (Ruane *et al.*, 1999; Davis *et al.*, 2002; Iguchi *et al.*, 2003; Ashley 2007). Fish diseases do not occur as a single caused event but are the end result of interactions of the disease, the fish and the environment. Fish diseases constitute one of the most important problems and challenges confronting fish culturists.

Avoidance of economic losses associated with this problem, several drugs like trichlorforms or parziquantel in bath treatments for ectoparasites has numerous disadvantages like development of resistance (Umeda *et al.*, 2006), being hazardous

for animal health (Kiemer and Black, 1997; Forwood *et al.*, 2013) and environmental disadvantages. Considering the potential harm of veterinary drug treatments on the environment and fish health and in some cases their limited efficacy, disease management should concentrate on harmless, preventive and lasting methods. Due to the limitation of chemical products in aquaculture, the use of natural treatments could enhance the consumption of aquaculture products (Makkar *et al.*, 2007; Panigrahi and Azad, 2007; Lee *et al.*, 2009; Citarasu, 2010; Mohapatra *et al.*, 2013).

Toxicity is the capacity of a chemical to cause harmful effects on tissues of living organisms. The effects are usually referred to as being acute or chronic depending mainly on the time interval between exposure to the chemical and the manifestation of the effects. A number of general cellular targets exists which can mediate cytotoxicity in host cells but also in parasites (Wink, 2007). Major targets include: DNA, RNA, proteins of the cytoskeleton and enzymes, biomembranes and the nervous system. Typical DNA intercalating compounds are berberine and sanguinarine (Wink and Schimmer, 2010). Many of the plants which produce such alkaloids (families of Papaveraceae, Berberidaceae, Menispermaceae, Ranunculaceae) are known for their antiparasitic, antimicrobial, and antiviral properties. Small lipophilic secondary metabolites, such as terpenoids or phenylpropanoids as found in the essential oil of many plants (especially in Lamiaceae, Myrtaceae, Rubiaceae, Apiaceae, Asteraceae, Lauraceae, Rutaceae, Burseraceae, Verbenaceae, Pinaceae, Cupressaceae), can dissolve in biomembranes and disturb their fluidity and the function of membrane proteins, which can lead to cell death by apoptosis (Wink, 2008). Alkaloids, a class with more than 21,000 compounds which occur in almost all plant families, are infamous for their neurotoxic properties (Wink, 2000). Many of them are agonists or antagonists at neuroreceptors and/or ion channels (Wink, 2000). They provide interesting candidates for anthelmintic drugs.

This study is aimed to formulate some of the possible solutions to the cure of fish parasitic helminth infections using medicinal plant extracts. It would establish the potencies of the medicinal plants against helminths with less toxicological adverse effect on host and its sensitive life forms. It would also provide answers to the pharmacokinetics of the extract and its possible side effects. The results obtained can possibly be used as a basis to compose new framework for the therapy and prevention of helminths. It can also be used for the basis of developing new anthelmintic drug.

## MATERIALS AND METHODS

### Test Animals

One thousand (1000) specimens of life *Clarias gariepinus* (*Fingerlings*) which were 6 weeks old were bought from a local pond and acclimatized to the laboratory condition in a well aerated dechlorinated tap water for a period of ten days. Test animals were of similar size, weight and age. They were categorized into treated and control groups (20 animals in each group in two replicates).

### Collection of Plants

Plant with medicinal potential were carefully selected and bought from Oyingbo, Lagos market early in the morning for fresh specimen. Seven medicinal plants were collected for the experiments. The leafy ones were rinsed in a running tap water and blotted with filter paper, spread over newspaper for air drying under shade 2-3 weeks. The specimens collected are: garlic bulbs (*Allium sativum*), lemongrass leaves (*Cymbopogon citrates*), basil leaves (*Ocimum sanctum*), thyme leaves (*Thymus*

*vulgaris*), bitter leaf (*Vernonia amygdalina*), ginger rhizomes (*Zingiber officinale*), and stem of candlewood (*Zanthoxylum zanthoxyloides*).

### Extraction of Phytochemicals

The succulent and woody plant parts were pulverised using mortar and pestle while the dried leaves were blended with a grinder after complete dryness. A known quantity of leaf powder of each plant and other plant parts was put in a 250 ml conical flask and added with 200 ml of Methanol (95%). The Methanol-leaf powder mixtures were kept at room temperature for 72 hours and rapidly stirred using glass rod every 8 hours. After 72 hours, the extract of each plant was filtered through Whatman No. 1 filter paper to exclude the plant tissue debris. The filtrate was concentrated under reduced pressure using rotary evaporator. Concentrated extracts were transferred in a beaker and dried at 40.5°C in a water bath. Crude extract, obtained from each plant parts, was transferred to screw cap bottles, labelled and store under refrigerator (4°C) condition till use. Their yields and other physical properties were noted and recorded.

### Bioassay technique

The test media was made by adding 1.0 litre of fresh water to the glass containers. The plant extracts were then added to the assay using different calibrations depends on the activeness of the phytochemical, after a range test was carried out. Acute testing lasted for 96 hours. This includes the controls. Fish was added to test chambers within 30 minutes of addition of test material to dilution water. 20 fingerlings of *C. gariepinus* in each group, a minimum of ten fish per replicate and two replicate per test concentration to provide statistical baseline. Construction materials and equipments for testing followed standard procedure. Gentle aeration of test vessels was used in the static-renewal. A minimum of five test concentrations was employed. Mortality observations would be recorded at 6, 24, 48, 72 and 96 hour, additional observations would be made every 24 hours until termination. Constant conditions were maintained throughout the test period. The test fish were fed with commercial fish pellets (coppen zeigler) 1mm, twice daily. However, the fish were not fed for 24 hrs before test initiation. In addition to these, abnormal behavior would be recorded, such as, erratic swimming, loss of reflex, increased excitability, lethargy, and changes in appearance or physiology such as discoloration, excessive mucous production, hyperventilation, opaque eyes, curved spine, or hemorrhaging. They were taken to be dead if they show no body movement even when probed with a pointed glass rod and they also appear whitish.

### Toxicity Factor

Relative Toxicity factor (RTF) for each plant extract at 96 hours exposure to 0.1 LC<sub>50</sub>, 1.0 LC<sub>50</sub>, and 1.5 LC<sub>50</sub> concentrations were estimated using the formulae below;

$$\text{Relative Toxicity factor (RTF)} = \frac{\text{The highest 96h LC}_{50} \text{ value}}{96\text{h LC}_{50} \text{ value for each plant extract}}$$

### Determination of Average Survival Time (AST) for the parasites

Intestinal parasites was extracted from sixty (60) wild adult each of *Parachanna obscura* (African Snakehead) weighing 41g to 65g and length 17.2 cm to 20.0 cm and *Clarias gariepinus* (African Cat fish) weighing 60g to 90g and length 16.0 cm to 18.0 cm were collected in Lekki lagoon, 4°00' and 4°15'E; between 6°25' and 6°33'N. *Clarias gariepinus* and *Parachanna obscura* caught from the wild after two day laboratory acclimatization. AST of the parasites in direct exposure to the sub-lethal concen-

trations of each plant extract was estimated using low observed concentration level (LOEC) i.e 0.1 LC<sub>50</sub> for 96 hour - host fry determined from the acute toxicity. AST for *Wenyonia sp.*, a cestode parasite and *Procamallanus longus*, a nematode of *Clarias gariepinus* and *Parachanna obsura* in saline solution (0.0085g/ml, NaCl) were estimated. Percentage Reduction in Survival Time (%RST) in each plant extract was estimated using the formulae below;

$$\%RST = 100\% - \frac{(\text{AST in each plant extract}) \times 100}{\text{AST in Saline solution}}$$

Toxicological endpoint for the parasites in the various exposures is death, this exposure and observation would be performed under stereo-microscope and poking with a needle for body movement.

### STATISTICAL ANALYSIS

Toxicological dose response involve mortality was analysed using probit analysis. This was done by converting concentration to logarithm and percentage response was converted to probit (Regression Analysis). Number of organism exposed in each assay was also taken into consideration. The probit values were then plotted against logarithm of concentration in order to determine the lethal concentration (LC<sub>50</sub>). Statistical analysis was executed using the Sigma Stat software (SPSS), IBM Statistic Package.

## RESULTS

### Crude plant extracts from seven medicinal plants

Table 1 shows a list of crude extract from plants; *Allium sativum*, *Cymbopogon citrates*, *Ocimum sanctum*, *Thymus vulgaris*, *Vernomia amygdalina*, *Zingiber officinale*, and *Zanthoxylum zanthoxyloenes*. Some were prepared dried; *Cymbopogon citrates*, *Thymus vulgaris*, *Vernomia amygdalina*, and *Zanthoxylum zanthoxyloenes* while others were obtained fresh; *Allium sativum*, *Ocimum sanctum*, and *Zingiber officinale*. *Allium sativum*, *Zingiber officinale*, and *Zanthoxylum zanthoxyloenes* were highly soluble in water with pH range of 5.50 – 6.13, Total Dissolved Solid; 581 – 887 mg/L, Temperature of 26.4 – 29.0<sup>0</sup>C, while others were either moderately soluble or partially soluble with pH range of 5.60 – 7.10, Total Dissolved Solid; 563 – 938 mg/L, Temperature of 26.1 – 28.1<sup>0</sup>C with varying coloration.

Table 1: Crude plant extracts from seven medicinal plants

S/N	Plant	Status	Solubility of plant extract in aqueous	Solution Colouration	Mass of plant part (g)	Mass of crude plant extract (g)	Percentage of extract (%)	Conc. in 50ml distil water (g/ml)
1	<i>Allium sativum</i>	Fresh	Highly Soluble	Orange	50	2.9	5.8	0.058
2	<i>Cymbopogon citratus</i>	Dry	Moderately Soluble	Light green	20	0.8	4.0	0.016
3	<i>Ocimum sanctum</i>	Fresh	Moderately Soluble	Slightly Black	20	1.1	5.5	0.022
4	<i>Thymus vulgaris</i>	Dry	Partially Soluble	yellow	20	2.7	13.5	0.054
5	<i>Vernonia amygdalina</i>	Dry	Partially Soluble	Dark green	20	1.1	5.5	0.022
6	<i>Zingiber officinale</i>	Fresh	Highly Soluble	yellow	50	1.8	3.6	0.036
7	<i>Zanthoxylum zanthoxyloenes</i>	Dry	Highly Soluble	Golden yellow	20	1.6	8	0.032

**Lethal Concentrations (LC<sub>50</sub>) of plant crude extracts on fingerlings of *Clarias gariepinus*.**

There were decreases in lethal concentrations with exposure time, from 24h LC<sub>50</sub> to 96h LC<sub>50</sub> in all the plant extracts. But, this rate varies among the individual extracts. *Allium sativum* decreased in lethal concentration with increased exposure time from 24h LC<sub>50</sub> to 96h LC<sub>50</sub> (5.751 g/L to 0.416 g/L), *Cymbopogon citrates* (5.339g/L to 0.239g/L), *Ocimum sanctum* (0.793g/L to 0.246g/L), *Thymus vulgaris* (1.009g/L to 0.179g/L), *Vernonia amygdalina* (1.964g/L to 0.107g/L), *Zingiber officinale* (0.741g/L to 0.169g/L), *Zanthoxylum zanthoxylones* (0.134g/L to 0.026 g/L).

**Relative Toxicity Factors (RTF) of Plant Extracts at increasing toxicities on fingerlings of *Clarias gariepinus* (96h LC<sub>50</sub> Toxicity) Using Probit.**

Table 2 shows relative toxicities of the plant crude extracts on fingerlings of *Clarias gariepinus* at 96 hour exposure, using probit analysis. *Allium sativum* was the least toxic with RTF of 1. RTFs of the extracts increase at Low Observed Effect Concentration (LOEC) at 96 h 0.1 LC<sub>50</sub> g/L in the trend; *Allium sativum* (RTF, 1.0) < *Cymbopogon citrates* (RTF, 2.0) < *Ocimum sanctum* (RTF, 2.0) < *Thymus vulgaris* (RTF, 3.0) < *Vernonia amygdalina* (RTF, 4.0) < *Zingiber officinale* (RTF, 5.0) < *Zanthoxylum zanthoxylones* (RTF, 10.0). As the lethal concentration increases at 96 h 1.0 LC<sub>50</sub> g/L and 1.5 LC<sub>50</sub> g/L, there were no change in relative toxicities except for *Zingiber officinale*, which showed decreased in RTFs while *Zanthoxylum zanthoxylones* showed tremendous increase in RTFs (10.0, 16.0 and 27.0).

Table 2: Relative Toxicity Factors (RTF) of plant extracts at increasing toxicities on fingerlings of *Clarias gariepinus* at 96h (LC<sub>50</sub> Toxicity) using probit.

Plant Extract	0.1 LC <sub>50</sub>		1.0 LC <sub>50</sub>		1.5 LC <sub>50</sub>	
	g/L	RTF	g/L	RTF	g/L	RTF
<i>Allium sativum</i>	0.084	1.0	0.416	1.0	0.800	1.0
<i>Cymbopogon citratus</i>	0.050	2.0	0.239	2.0	1.128	1.0
<i>Ocimum sanctum</i>	0.051	2.0	0.246	2.0	0.470	2.0
<i>Thymus vulgaris</i>	0.033	3.0	0.179	2.0	0.358	3.0
<i>Vernonia amygdalina</i>	0.019	4.0	0.107	4.0	0.215	5.0
<i>Zingiber officinale</i>	0.017	5.0	0.169	3.0	0.435	3.0
<i>Zanthoxylum zanthoxylones</i>	0.009	10.0	0.026	16.0	0.042	27.0

**Percentage Reduction in Survival Time of Intestinal parasites of *Clarias gariepinus* Exposed to LOEC - 0.1 h LC<sub>50</sub>**

The average survival time of intestinal parasites; *Wenyonia sp*, a cestode and *Procamallanus sp*, a nematode in saline solution (0.0085g/ml NaCl) were 40.00±0.69 and 42.50±0.96 hr respectively with statistical significance at 0.01 level. Table 3 shows Average survival time and percentage reduction in survival time relative to saline solution of the intestinal parasites exposed to *Allium sativum* (*Wenyonia sp*; AST, 36.30±1.56, p < 0.01, %RST, 9.25, *Procamallanus sp*; AST, 40.48±2.68, p < 0.01, %RST, 4.75). *Cymbopogon citratus* (*Wenyonia sp*; AST, 31.60±0.31, p < 0.01, %RST, 21.00, *Procamallanus sp*; AST, 37.28±1.25, p < 0.01, %RST, 12.28), *Zanthoxylum zanthoxylones* (*Wenyonia sp*; AST, 17.87±0.96, p < 0.01, %RST, 57.95, *Procamallanus sp*; AST, 16.13±0.85, p < 0.01, %RST, 59.68). The parasites had shown least suseptibility to *Allium sativum* just like their host fingerlings while extract of *Vernonia amygdalina*, *Zingiber officinale* and *Zanthoxylum zanthoxylones* high toxicity. *Z. zanthoxylones* had shown the highest potency against helminth parasites compared to the other extracts.

Table 3: Percentage reduction in survival time of intestinal parasites of *Clarias gariepinus* exposed to 0.1 LC<sub>50</sub> host fingerlings for 96 h.

Plant Extract/Saline Solution	Parasites (n = 5)	Average Survival Time (AST) Mean $\pm$ SD (Hr)	Percentage Reduction in Survival Time (%RST)
Saline Solution (0.0085g/ml, Nacl)	<i>Wenyonia sp</i>	40.00 $\pm$ 0.69**	0.00
	<i>Procamallanus sp</i>	42.50 $\pm$ 0.96**	0.00
<i>Allium sativum</i>	<i>Wenyonia sp</i>	36.30 $\pm$ 1.56**	9.25
	<i>Procamallanus sp</i>	40.48 $\pm$ 2.68**	4.75
<i>Cymbopogon citratus</i>	<i>Wenyonia sp</i>	31.60 $\pm$ 0.31**	21.00
	<i>Procamallanus sp</i>	37.28 $\pm$ 1.25**	12.28
<i>Ocimum sanctum</i>	<i>Wenyonia sp</i>	33.63 $\pm$ 0.89**	15.93
	<i>Procamallanus sp</i>	39.24 $\pm$ 7.95*	7.67
<i>Thymus vulgaris</i>	<i>Wenyonia sp</i>	30.23 $\pm$ 0.69**	24.43
	<i>Procamallanus sp</i>	33.76 $\pm$ 5.65*	20.56
<i>Vernonia amygdalina</i>	<i>Wenyonia sp</i>	18.97 $\pm$ 6.61*	51.26
	<i>Procamallanus sp</i>	27.95 $\pm$ 1.26**	34.24
<i>Zingiber officinale</i>	<i>Wenyonia sp</i>	20.03 $\pm$ 0.79**	49.93
	<i>Procamallanus sp</i>	29.67 $\pm$ 0.59**	30.88
<i>Zanthoxylum zanthoxylones</i>	<i>Wenyonia sp</i>	17.87 $\pm$ 0.96**	57.95
	<i>Procamallanus sp</i>	16.13 $\pm$ 0.85**	59.68

\*\* means values significant at P < 0.01 level

\* means values significant at P < 0.05 level

## DISCUSSION

The field of aquaculture and capture fisheries is receiving a lot of attention in recent times in Nigeria because demand for fish far exceeds supply and fish protein is preferred over other animal protein. However, parasitic diseases of fish seem to be one of the major problems confronting fish culturists. Application of aquatic medicine in treatment and prevention of diseases in fisheries and aquaculture is also gaining great momentum in recent years. The organism is classified at a given time as having responded after treatment. Quantal response is employed to obtain quantitative results because the percentage responding in randomly chosen group of organisms in general increases with concentration. In this study, there were decrease in lethal concentrations with increase in exposure time, from LC<sub>50</sub> of 24 h exposure to LC<sub>50</sub> of 96 h exposure in all the plant extracts.

It is important to have suitable dosing to obtain desired effects on parasites of the fishes at safe concentration. One extract appears to be more toxic than the other at higher concentrations, this is not true at lower concentration. For example, *Vernonia amygdalina* appears less toxic than *Zingiber officinale* at low concentration, 0.1 LC<sub>50</sub> g/L for 96 h, but at higher concentrations, 1.0 LC<sub>50</sub> g/L and 1.5 LC<sub>50</sub> g/L, there were steady increase in toxicity (RTF, 4.0 to 5.0) compared with decrease in toxicity with *Zingiber officinale* (RTF 5.0 to 3.0). *Zingiber officinale* has a lower threshold and actually begins to cause adverse effects at lower concentrations than *Vernonia amygdalina*. Actually *Vernonia amygdalina* is of greater concentration not necessarily because of its steeper concentration-response curve. Once individuals become overexposed (exceeded the threshold concentration) the increase in response occurs with much smaller increases in concentration and more individuals are affected with subsequent increase in concentration. In other words, once the toxic level is reached, the margin of error for *Vernonia amygdalina* decreases more rapidly than that of *Zingiber officinale*, because each incremental increase in exposure greatly increases in percentage of individuals affected. *Allium sativum* was the least toxic with Relative Toxicity Factor (RTF) of 1. RTFs of the extracts increase at host fingerlings' Low

Observed Effect Concentration (LOEC) - 0.1 LC<sub>50</sub> g/L for 96 h as shown in table 2. *Ocimum sanctum* having RTF of 2.0 implies that it is twice as toxic as the least toxic plant extract i.e *Allium sativum* (RTF, 1.0). At lethal concentrations; 1.0 LC<sub>50</sub> g/L and 1.5 LC<sub>50</sub> g/L for 96 h, there were no change in relative toxicities except for *Zingiber officinale*, which showed decreased in RTFs while *Zanthoxylum zanthoxylones* showed tremendous increase in RTFs (RTFs, 10.0, 16.0 and 27.0).

Many direct measures of effects are not necessarily related to the mechanism by which a substance produce harm to an organism but have the advantage of permitting a casual relation to be drawn between the agent and its action. The customary starting point in toxicological evaluation utilizes lethality as an index. Determination of lethality is precise, quantal, and unequivocal and is therefore useful in its own right, if only to suggest the level and magnitude of the potency of the substance. Lethality provides a measure of comparison among many substances whose mechanism and sites of action may be markedly different (Don-Pedro, 2010). Fish host and parasites as regard to susceptibility to the plant crude extracts showed similar trend (as shown in Tables 2 and 3). The use of plant extracts could reduce costs of treatment and more environmentally friendly as they are less likely to produce drug resistance in parasites due to high diversity of plant extract molecules (Blumenthal *et al.*, 2000; Logambal *et al.*, 2000; Olusola *et al.*, 2013).

The cestode, *Wenyonia* sp. showed greater susceptibility to most extracts compared to the nematode, *Procamallanus* sp. This could be due to differences between them; morphologically, *Procamallanus* sp. has protective cuticle when compared with absorptive integument of the cestode, since route of exposure affects toxicity of most substances. Genetic makeup is another important factor that could be a reason for this difference. Studies have reported that gastrointestinal nematodes of fish and mammals are capable of producing superoxide dismutase (SOD) in order to reduce oxygen radical formation during stress in their host (Dzik, 2006). Effective protection of an invading parasite from host produced reactive oxygen species (ROS) depends on levels of scavenger enzymes in the parasites (Dzik, 2006). Both parasites had shown least susceptibility to *Allium sativum* just like their host fingerlings but they were highly susceptible to *Vernonia amygdalina*, *Zingiber officinale* and *Zanthoxylum zanthoxylones*. Despite the wide safety margin of herb extracts, there are also scant reports on their negative impacts in fish culture. Plants such as garlic (*Allium sativum*) have been described in numerous research to have benefits where they were effective in controlling fish diseases, enhancing the cultured performance and immune response of farmed fish. However, garlic has been shown to cause a harmful and even lethal effect on certain larviculture farming. In this study, *Allium sativum* compared with other extracts, showed increased mortality with time from 24h LC<sub>50</sub> to 96h LC<sub>50</sub> (5.751 g/L to 0.416 g/L) and maintained a relative toxicity of 1 on fingerlings of *Clarias gariepinus* in intervals of 1/10<sup>th</sup> of LC<sub>50</sub> (g/L), a unit LC<sub>50</sub> (g/L) and a unit and half of the LC<sub>50</sub> (g/L), at 96 hour exposure. At 1/10<sup>th</sup> LC<sub>50</sub> (g/L), Average survival time and percentage reduction in survival time relative to saline solution of the intestinal parasites exposed to *Allium sativum* were; *Wenyonia* sp, a cestode; AST, 36.30±1.56, p < 0.01, %RST, 9.25 and *Procamallanus* sp, a nematode; AST, 40.48±2.68, p < 0.01, %RST, 4.75. A number of studies have demonstrated the chemopreventive activity of garlic by using different garlic preparations including fresh garlic extract, aged garlic, garlic oil and a number of organosulfur compounds derived from garlic (Chan *et al.*, 2013; Capasso, 2013). Most recent efforts have been made in identifying potential activities on different plant species. However, identifying the active molecules responsible for the observed bioactivities would

allow optimization of extraction procedure, estimate appropriate dosage and understand mechanism of actions (Reverter *et al.*, 2014). Enriched diets with plant extracts will have beneficial effects on fish health and enhance the immune system and hence play importance role in preventing disease outbreak (Reverter *et al.*, 2014).

## CONCLUSION

The parasites showed similar susceptibility to the extracts as their host fingerlings. *Z. zanthoxylones* had shown the highest potency against helminth parasites compared to the other extracts. The beneficial properties and efficacy of plant extracts on health of fish depend on the active ingredients, method of extraction and the extract concentration. Studies have reported multiple activities and potential application of plant extracts in aquaculture, there has been little effort to homogenize the extraction concentration and the administration way. Besides the effects of plant extracts on parasites and their fish host is dose-dependent and there is a potential for overdosing, so determining suitable concentration is of great importance.

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