

## Research Article

# Prophylactic Potentials of Dietary *Syzygium malaccense* Aqueous leaves Extracts on the Metabolite Activities and Organosomatic Indices of *Clarias gariepinus* Exposed to *Staphylococcus aureus*

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### Abstract

The potency of *Syzygium malaccense* as an immune stimulant and a prophylactic was accessed. 40% crude protein fish diet was formulated, and five different feeds were produced using varying concentrations of *Syzygium malaccense* leave aqueous extracts. (Five hundred grams per liter (500g/l) of water) as follows: 0ml/kg (Do), 25ml/kg (D1), 50ml/kg (D2), 75ml/kg (D3) and 100ml/kg (D4). One hundred and eighty (180) *Clarias gariepinus* was divided into six groups of ten each in triplicate and were fed the five diets and a known commercial diet (Blue crown) so as to compare the results with that of the known commercial diet. After eight weeks feeding, the experimental fish were exposed to *staphylococcus aureus* via intraperitoneal injection and observed for one week. Blood samples were collected at the end of weeks 4 and 8 of feeding, and at the end of the post infection period for metabolite [Urea (UR), creatinine (CR), Total protein (TP), Albumin (ALB), Globulin (GLB) and Albumin/Globulin ratio (A/G)] analysis. Organs were also harvested at the end of the post infection period for the determination of the following organosomatic indices: Hepatosomatic index (HSI), Viscerosomatic index (VSI) cistosomatic index (CSI), gonadosomatic index (GSI), Gilosomatic index (GI-SI); spleenosomatic index (SSI)

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and interperitoneal fat (IF). The result reveals that the diets D1-D4 maintained the functionality of the fish kidney better than the diets Do and BC that negatively altered the functions of the kidney and gills by releasing more UR and CR to the fish blood, at the end of the eight (8) weeks feeding. D1 – D4 unlike Do and BC also prevented the virulence of the *S. aureus* on the reproductive organs, gills and liver of the fish as seen in the improved GSI, SI, UR and CR.

**Keywords:** Metabolites; Organosomatic indices *Clarias gariepinus*; Prophylactic; *Syzygium malaccense*

### Introduction

Fish is a less expensive and significant source of protein, vitamins and other essential nutrients for the body [1], most part of the world especially Africa depend on fish as their source of animal protein [2]. There is serious decline in fish presence in our waters as a result of over fishing arising from high demand for fish and fisheries product, and other anthropogenic activities. Over 65% of our fish stock are either fully exploited or over exploited [3]. *Clarias gariepinus* is the cheapest source of protein for the middle class citizens in the developing countries especially Nigeria [4].

Aquaculture has attracted investors to meet up demand for fish products [5], and it stands out as the only alternative to meet-up the high demand of fish and fisheries products. Chemicals are often used by aquaculturist to enhance growth and improve immunostimulant in fish [6], but the use of chemicals is seriously criticized due to bacteria resistance, environmental pollution and pollution of fish flesh [7]. Ukwe and Gabriel [8] stated that the use of herbs and herbal supplement is the best alternative to the use of chemicals because it is cheap, readily available, non-pollutant and achieve same positive results and even more. Most of the used herbs in aquaculture practice include: *chromolaena odorata* [9], *citrus medica* [10] and *Persea americana* [11]. Herbs and herbal products contains primary and secondary phytochemicals [12] and each of these phytochemicals plays one or several roles in sustainable and productive aquaculture [13].

Though fish is a major source of animal protein to man, it's consumption sometimes lead to contamination and infection as a result of bacterial infections and zoonotic parasites arising from feeds, area of culture and sales point [14,15]. Infected fish most times shows no symptoms, but manifest as zoonotic diseases [16], but they can be identified via haematological and biochemical analysis of the fish blood [17-19] and examination of the fish organs [20].

Metabolite alterations in the blood of fish is an indication of disease presence [21] or an improved innate immune response in fish [22]. *Syzygium malaccense* has high phenolic and flavonoid contents in its parts that are antimicrobial with strong antioxidant activities [23,24].

The purpose of this research is to access the potency of aqueous extract of *Syzygium malaccense* leaves in improving the innate immune response in *Clarias gariepinus* and it's prophylactic efficiency

in preventing the alteration of the organosomatic indices and metabolites activities in *Clarias gariepinus* exposed to *Staphylococcus aureus*.

## Materials and Methods

### Study location

The project work was carried out in the laboratory of the Department of Fisheries and Aquatic Environment, Faculty of Agriculture, Rivers State University, Nkpulu-Oroworukwo, Port Harcourt, Rivers State, Nigeria.

### Experimental fish and acclimatization

One hundred and eighty (180) healthy *C. gariepinus* of mean weight 130-150kg was purchased from Idi-onyana Farms along Abua-Ahoada road in Abua/Odual Local Government Area, Rivers State. The fish was taken to the project site, acclimatization and observation were carried out on the fish for a period of two (2) weeks to access disease present or bruises. During this period the fish was fed to satisfaction with blue crown commercial feed twice daily.

### Source of pathogen

The pathogen *staphylococcus aureus* was procured from the Department of Microbiology of the Rivers State University, Nkpulu Oroworukwo, Port Harcourt.

### Preparation of *syzgium malaccense* leave extract

*Syzgium malaccense* leaves were harvested within Port Harcourt, Rivers State, Nigeria. The leaves were prepared using the method of Ukwe and Jamabo [25]. *S. malaccense* leaves were harvested, washed clean, pounded to paste, soaked in tap water at the concentration of five hundred grams/litre (500g/L) for twelve hours. It was filtered and the filtrate was used immediately.

### Experimental diet

40% crude protein diet was formulated using the following ingredient: Wheat-brand, corn, soyabeans, blood meat, fish meal, lysine, methionine, P. oil, starch and vitamins. Five different diets were produced from the formulated diet using varying concentration of *S. malaccense* leave aqueous extract at 0ml/g, 25ml/g, 50ml/g, 75ml/g and 100ml/g, and labeled Do, D1, D2, D3 and D4 respectively.

### Blue crown feed

The blue crown feed was bought from SAMMIANI CONSULT NIG. LTD, KMI Eneka Junction Igbo Etche Road, Rumukrushu, Obio/Akpor Local Government Area, Rivers State, Nigeria.

### Experimental design

A complete randomized design was used with a total of eighteen (18) experimental tanks.

### Experimental procedure

One hundred and eighty (180) *C. gariepinus* were distributed into six group of ten in triplicates, and were fed accordingly with Do, D1, D2, D3 and D4 diets, and Blue crown feed (BC). Blood samples were collected at the end of week four (4) and week eight (8) of feeding. After eight weeks of feeding, the experimental fishes were exposed through intraperitoneal injection to overnight grown *staphylococcus*

*aureus* using an injection syringe and 21- gauge hypodermic needle at day one (1) and two (2) (twenty four hours interval), and were observed for seven days. Blood samples were taken from the various groups at the end of 4 and 8 weeks feeding and after seven (7) days post infection period and taken to the laboratory for metabolites activities analysis, and organs were harvested for the determination of organosomatic indices, to determine the potency of the experimental diets as prophylactics, and the results were compared to the one obtained in the blue crown feed (known commercial feed).

### Blood Extraction

The fish was blind folded 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.

### Metabolite Analysis

Spectrophotometric analysis was performed on the blood plasma using a spectrophotometer model "SURGISPEC SM – 230" manufactured by surgifield medical in England and was used according to manufacturers instruction.

The following Metabolites: total protein (TP); urea (UR); creatinine (CR); albumin (ALB); globulin (GLB) and albumin-globulin ratio (A/G) were determined according to their required wave lengths and reagents as follows:

- The spectrophotometer was adjusted through a control till the reading on the screen shows zero (0) transmittance.
- A blank solution was prepared with the reagents except the samples it was put into a cuvette and insert into the sample compartment of the spectrophotometer.
- The spectrophotometer was set to 100% transmittance using the absorbent control and the cuvette with the blank solution was removed.
- The sample cuvette was wiped clean and insert into the sample compartment of the spectrophotometer, and its absorbance and transmitting value was on the screen.
- The sample cuvette was removed and the transmittance reading went back to zero.
- The process were repeated for all the sample adjusting wave lengths accordingly and using the recommended reagents to determine the required parameters.

- The metabolites were calculated using the formular
- $$X = \frac{AT}{AS} \times \text{concentration of standard}$$

Where X = Calculated parameter

AT = Absorbance of test

AS = Absorbance of standard.

### Organosomatic Indices (OSI)

Organs such as liver, spleen, heart, visceral organs, gonads, gill and interperitoneal fats were harvested from the various treatments at the end of the one week post infection period and their organosomatic indices (OSI) were calculated as follows:

Experimental Diets (ml/Kg)	HSI	VSI	SSI	CSI	GSI	GI-SI	IF
Do	0.75±0.9 <sup>a</sup>	3.54±2.43 <sup>a</sup>	0.07±0.07 <sup>a</sup>	0.08±0.05 <sup>a</sup>	0.20±0.20 <sup>a</sup>	2.08±1.04 <sup>a</sup>	1.97±0.47 <sup>b</sup>
D1	0.80±0.9 <sup>a</sup>	4.60±2.14 <sup>a</sup>	0.09±0.03 <sup>a</sup>	0.09±0.04 <sup>a</sup>	1.89±0.59 <sup>b</sup>	1.87±1.75 <sup>a</sup>	0.69±0.37 <sup>a</sup>
D2	0.85±0.61 <sup>a</sup>	4.18±0.96 <sup>a</sup>	0.09±0.05 <sup>a</sup>	0.07±0.05 <sup>a</sup>	2.72±0.06 <sup>c</sup>	1.56±1.96 <sup>a</sup>	0.48±0.18 <sup>a</sup>
D3	0.99±0.22 <sup>a</sup>	4.63±2.18 <sup>a</sup>	0.09±0.08 <sup>a</sup>	0.07±0.50 <sup>a</sup>	2.49±1.78 <sup>c</sup>	1.49±2.04 <sup>a</sup>	0.56±0.38 <sup>a</sup>
D4	0.80±0.57 <sup>a</sup>	5.72±5.33 <sup>b</sup>	0.09±0.06 <sup>a</sup>	0.09±0.53 <sup>a</sup>	3.03±3.71 <sup>d</sup>	1.63±1.27 <sup>a</sup>	0.42±0.24 <sup>a</sup>
BC	0.94±0.66 <sup>a</sup>	3.81±4.95 <sup>a</sup>	0.09±0.03 <sup>a</sup>	0.08±0.57 <sup>a</sup>	0.99±0.85 <sup>a</sup>	2.05±1.40 <sup>b</sup>	1.64±0.37 <sup>b</sup>

**Table 1:** Organosomatic indices of the experimental fish fed dietary *S.malaccense* for eight (8) weeks and infected with *S. aureus* for seven (7) days.

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

**Key:** HSI – Hepatosomatic index, VSI – Viscerosomatic index, GSI – Gonadosomatic index, GI – SI – Gilotosomatic index, IF – Interperitoneal fat, CSI – Cistosomatic index SSI – Spleenosomatic index

$$OSI = \frac{\text{Weight of organ}}{\text{Weight of fish}} \times 100 \quad [26]$$

### Data analysis

SPSS statistic software 17.0 for windows was used for the analysis, followed by one way analysis of variance to determine deviation in variables among treatments. Comparison among treatments was done using Turkey multiple comparison [27].

## Results

### Organosomatic indices of *Clarias gariepinus* fed with experimental diets and exposed to *S. aureus*

The Organosomatic indices: hepatosomatic index (HSI); viserosomatic index (VSI); gonadosomatic index (GSI); Gilotosomatic index (GI-SI); interperitoneal fat (IF); cistosomatic index (CSI) and spleenosomatic index (SSI) of *Clarias gariepinus* fed the experimental diets for eight (8) weeks and infected with *S. aureus* for seven days are presented in (Table 1). The results indicates that after infection, there was no significant difference in HSI across the groups. There was significant difference in VSI, significantly lower value was recorded in fish fed Do (3.54 ±2.43) and BC (3.81 ± 4.95) while fish fed D1-D3 were high and similar in values, but fish fed D4 had significantly higher 5.72±5.33 value. The SSI and CSI were similar across the diets. The value for the GSI was significantly lower in Do fed fish 0.20±0.20 and BC (0.99±0.85) followed by D1 (1.89±0.59), D2 (2.72±0.06) and D3 (2.49±1.78), but was significantly higher in D4 (3.03±3.71). The GI-SI was significantly higher in fish fed Do (2.08 ± 1.04) and BC (2.05 ± 1.40), but significantly lower and similar in fish fed D1 – D4, while IF was low across the diets but significantly higher in D1 (1.97±0.47), and BC (1.64 ±0.37).

### Metabolites activities in the plasma of the experimental fish after four (4) and eight (8) weeks of feeding with the experimental diets

The Metabolites activities in the plasma of *Clarias gariepinus* after four (4) and eight (8) weeks of feeding with the experimental diets are shown in (Tables 2&3) respectively.

After four (4) weeks of feeding there was no significant difference in TP, the values were within the same range (46.33±3.21 – 49.00 ±9.54) across the diets. The value for UR in fish fed D2 was higher (2.00±0.36) while the rest were within the same range (1.67±0.25 – 1.83 ± 0.12), CR was significantly lower in the fish fed D1 (39.00±6.56) while the rest were higher and within the same range

(40.33±44.33±8.33). There was no significant difference in the ALB, it was similar across the diets (25.68 ±2.08 ±30.33 ±2.08), the GLB was significantly higher in fish fed Do (20.67±1.15), D3 (20.33±0.58), and D4 (20.67±4.73), while fish fed D1, D2 and BC were lower and within the same range, there was no significant difference in the A/G ratio across the experimental diets.

After eight (8) weeks of feeding, the value for the TP was significantly lower in fish fed Do, D1 and D3 but significantly higher in fish fed BC (50.33±6.81), D2 (51.00±3.60), and D4 (52.33±3.06). The UR was significantly higher in fish fed BC (2.07±0.71), while fish fed Do – D4 were lower and similar 1.07± 0.12±1.60±0.46. The CR was significantly higher in fish fed BC (51.67±7.23) and Do (50.67 ± 10.06), but similar in the rest diets. In ALB there were no significant difference across the experimental diets (25.35±2.31 – 30.08±4.04) also there was no significant difference in GLB (21.00±0.00 – 22.67±2.52), and in A/G there were no significant difference they were within the same range in Do – D4 (1.21±0.55 – 1.34±0.12, but was significantly lower in fish fed BC (0.91±0.12).

### Metabolites activities in plasma of experimental fish after eight (8) weeks of feeding with dietary *S. malaccense* and infected with *S. aureus*

The metabolites activities in the plasma of *Clarias gariepinus* after eight (8) weeks of feeding with dietary *S. malaccense* and infected with *S. aureus* are shown in (Table 4). There were no significant difference in the TP across diets (40.33±1.15 – 43.33±1.53), meanwhile there were significant difference in the values of UR, fish fed Do and BC had significantly higher values 2.20±0.46 and 2.23±0.42 respectively, while fish fed D1 – D4 were significantly lower but similar. The values of CR were significantly high in the fish fed Do (53.33±10.21) and (54.67±5.51), but were similar in fish fed D1 – D4 (41.67±18.93 – 47.00±14.73).

The values of ALB were significantly lower in the fish fed Do and BC, 16.33±0.53 and 16.33 ±0.53 respectively, while fish fed D1 – D4 were higher and similar. The value for GLB was significantly higher in fish fed BC, but similar in fish fed Do – D4. The values for AG were higher in fish fed D2 and D4 compared to the rest, but were in the same range.

Experimental Diets	T.P(g/l)	UR (muol/L)	CR (muol/L)	ALB(g/l)	GLB(g/l)	A/G
Do	46.33±3.2 <sup>a</sup>	1.70±0.20 <sup>a</sup>	42.00±7.00 <sup>b</sup>	25.68±2.08 <sup>a</sup>	20.67±1.15 <sup>a</sup>	1.24±0.12 <sup>a</sup>
D1	47.33±1.53 <sup>a</sup>	1.67±0.25 <sup>a</sup>	39.00±6.56 <sup>a</sup>	28.00±2.89 <sup>a</sup>	19.33±2.89 <sup>a</sup>	1.45±0.35 <sup>a</sup>
D2	48.67±8.50 <sup>a</sup>	2.00±0.36 <sup>b</sup>	43.33±9.72 <sup>b</sup>	29.00±7.02 <sup>a</sup>	19.67±2.31 <sup>a</sup>	1.47±0.23 <sup>a</sup>
D3	48.33±4.72 <sup>a</sup>	1.83±0.35 <sup>a</sup>	46.33±7.57 <sup>b</sup>	28.00±0.58 <sup>a</sup>	20.33±0.58 <sup>a</sup>	1.37±0.17 <sup>a</sup>
D4	47.00±9.44 <sup>a</sup>	1.57±0.12 <sup>a</sup>	40.33±8.74 <sup>b</sup>	26.13±4.73 <sup>a</sup>	20.67±4.73 <sup>b</sup>	1.27±0.12 <sup>a</sup>
BC	49.00±9.54 <sup>a</sup>	1.83±0.12 <sup>a</sup>	44.33±8.33 <sup>b</sup>	30.33±2.08 <sup>a</sup>	18.67±2.08 <sup>a</sup>	1.62±0.1 <sup>a</sup>

**Table 2 :** Metabolites Activities in Plasma Biochemistry of the experimental fish after four (4) weeks of feeding with dietary *S.malaccense*.

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

Key: T.P – Total protein, UR – Urea, CR – Creatinine, ALB – Albumin, GLB – Globulin, A/G – Albumin Glabulin

Experimental Diets	T.P(g/l)	UR (muol/L)	CR (muol/L)	ALB(g/l)	GLB(g/l)	A/G
Do	49.67±3.06 <sup>a</sup>	1.60±0.30 <sup>b</sup>	50.67±10.06 <sup>b</sup>	27.67±1.53 <sup>a</sup>	22.00±1.00 <sup>a</sup>	1.26±0.06 <sup>a</sup>
D1	45.33±2.52 <sup>a</sup>	1.47±0.21 <sup>a</sup>	44.00±14.42 <sup>a</sup>	25.33±2.31 <sup>a</sup>	20.00±2.00 <sup>a</sup>	1.27±0.06 <sup>a</sup>
D2	51.00±3.60 <sup>b</sup>	1.40±0.17 <sup>a</sup>	44.67±17.79 <sup>a</sup>	30.00±4.51 <sup>a</sup>	21.00±0.00 <sup>a</sup>	1.21±0.15 <sup>a</sup>
D3	49.00±3.60 <sup>a</sup>	1.07±0.12 <sup>a</sup>	42.67±8.62 <sup>a</sup>	26.33±2.63 <sup>a</sup>	22.67±2.52 <sup>a</sup>	1.16±0.21 <sup>a</sup>
D4	52.33±3.06 <sup>b</sup>	1.60±0.46 <sup>a</sup>	43.67±6.51 <sup>a</sup>	30.08±4.04 <sup>a</sup>	22.33±1.15 <sup>a</sup>	1.34±0.12 <sup>a</sup>
BC	50.33±6.81 <sup>b</sup>	2.07±0.71 <sup>b</sup>	51.67±7.23 <sup>b</sup>	28.66±2.65 <sup>a</sup>	21.67±4.72 <sup>a</sup>	0.91±0.12 <sup>a</sup>

**Table 3:** Metabolites Activities in Plasma Biochemistry of the experimental fish after Eight (8) weeks of feeding with dietary *S.malaccense*.

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

Key: T.P – Total protein, UR – Urea, CR – Creatinine, ALB – Albumin, GLB – Globulin, A/G – Albumin Glabulin

Experimental Diets	T.P(g/l)	UR (muol/L)	CR (muol/L)	ALB(g/l)	GLB(g/l)	A/G
Do	40.33±1.15 <sup>a</sup>	2.20±0.46 <sup>b</sup>	53.33±10.21 <sup>b</sup>	16.33±0.58 <sup>a</sup>	24.67±0.58 <sup>a</sup>	0.66±0.03 <sup>a</sup>
D1	47.33±4.73 <sup>a</sup>	1.43±0.32 <sup>a</sup>	47.00±14.73 <sup>a</sup>	23.33±3.06 <sup>b</sup>	24.00±0.58 <sup>a</sup>	0.97±0.15 <sup>a</sup>
D2	47.67±4.51 <sup>a</sup>	1.37±0.15 <sup>a</sup>	45.33±18.93 <sup>a</sup>	25.33±5.51 <sup>b</sup>	22.34±5.51 <sup>a</sup>	1.13±0.23 <sup>b</sup>
D3	48.33±1.53 <sup>a</sup>	1.23±1.12 <sup>a</sup>	42.67±10.79 <sup>a</sup>	23.67±1.53 <sup>b</sup>	24.66±1.15 <sup>a</sup>	0.96±0.16 <sup>a</sup>
D4	48.00±8.00 <sup>a</sup>	1.87±0.87 <sup>a</sup>	41.67±18.93 <sup>a</sup>	24.00±4.00 <sup>b</sup>	24.00±4.04 <sup>a</sup>	1.00±0.11 <sup>a</sup>
BC	45.67±4.93 <sup>a</sup>	2.23±0.42 <sup>b</sup>	54.67±5.51 <sup>b</sup>	16.33±1.53 <sup>a</sup>	29.94±2.00 <sup>a</sup>	0.86±0.02 <sup>a</sup>

**Table 4:** Metabolites Activities in the Plasma of the experimental fish after Eight weeks feeding with dietary *S.malaccense* and infected with *S. aureus*.

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

Key: T.P – Total protein, UR – Urea, CR – Creatinine, ALB – Albumin, GLB – Globulin, A/G – Albumin Glabulin

## Discussion

### Organosomatic indices of *C. gariepinus* fed the experimental diets and infected with *S. aureus*

The Organosomatic indices are often used to evaluate the health status of fish [28,29]. In this research, there were no significant difference in the HSI, SSI and CSI, after the period of infection with the experimental pathogen. This result is similar to the one of Ukwe and Jamabo [25] who reported no significant difference in the HSI, SSI and CSI when *C. gariepinus* was fed dietary *Mangnifera indica* back extract and exposed to *P.aeruginosa*. Gupta *et al*, [30] also reported no significant difference in the CSI and SSI when *Rattus rattus* was parasitized. This result could be attributed to the fact that the experimental pathogen was not virulence on the liver, spleen and heart of the experimental fish. The VSI was higher in the fish fed D4 followed by the fish fed D1, D2 and D3, this could be as a result of the enhance

health in the fish organs, probably due to the phytochemicals presence in *S. malaccense*, which are not only antibacterial against the pathogen, but also growth promoters [31,32], but it was lower in the fish fed Do and BC. The interperetoneal fat was significantly higher in the fish fed Do and BC, and significantly lower and similar in the fish fed D1-D4. The result of the interperetoneal fat is in agreement with the report of Ukwe and Entire [28] who reported increase in interperetoneal fat (visceral fat) when *C. gariepinus* was exposed to *P. aeruginosa*. The increase in the interperetoneal fat in the fish fed Do and BC could be as a result of innate immune response of the experimental fish against the experimental pathogen, this argument is supported by Jenab *et al* [33] and Ukwe and Etire [28]. The absence or reduction of the interperetoneal fat in the fish fed D1-D4 and exposed to the pathogen could be due to the presence of the antimicrobial phytochemicals in *S. malaccense* that have prevented the virulence of the *S. aureus* against the fish [34]. Contrary to the interperetoneal



fat, the gonadosomatic index (GSI) was significantly lower in the fish fed Do and BC, and significantly higher in the fish fed D1-D4. This could be as a result of the presence of the excess fat in the abdominal cavity of the fish fed Do and BC, due to the presence of the pathogen (*S. aureus*), since increase in interperitoneal fat has been observed as an innate immune response in fish [28,33].

### Effect of the Experimental diets on the Metabolites of *C. gariepinus*

At the end of the four weeks feeding with the experimental diets Do-D4, and BC, there was no significant difference in the analysed metabolites apart from Creatinine (CR). All the metabolites plays a unique role in the metabolism of the fish. The total protein (TP) indicates the state of the fish innate immune response [22], the albumin (ALB) and globulin (GLB) are associated with improved non-specific immune response, and plays a role as plasma protein carriers [35], the urea (UR) is a major product of the breakdown of complex compound (protein) in the body [36], while Creatinine, a major metabolites in the body is said to be a good assessor for the proper functioning of the kidney [37]. However, after eight (8) weeks feeding there was still no significant difference in the UR, CR, ALB, GLB and A/G in the fish fed D1-D4 indicating that the administered diets did not alter the composition of these metabolites in the fish plasma. But there were significant increase in the TP of the fish fed D2, D4 and BC. This results is similar to the observations of Binali *et al* [38] when *Huso huso* juveniles were fed dietary *Urtica dioica*. This result depicts that the dietary *S. malaccense* at the administered dose has the capacity to improve innate immune response in *C. gariepinus* [22,39]. However the value of the UR and CR in the fish fed Do and BC increased. The increase in UR and CR in fish plasma indicates the inability of the fish kidney to discharge them [40]. The implication of this is that the dietary *S. malaccense* at the used concentrations has the capacity to maintain the functionality of the kidney, even at prolong usage.

### Prophylactic effect of the Experimental diets on the Metabolites of *S. aureus* infected *C. gariepinus*

After seven (7) days post infection the TP was significantly lower in the fish fed Do and BC, and increased significantly in the fish fed D1 – D4. This result is similar to the report of Mohamad and Abasali [41] who reported the effect of *A. hydrophila* on common carp fed plants extracts supplemented diets, and Dos Santos *et al*, [42], who reported effects of *A. hydrophila* on silver catfish fed supplemented *A. triphyla* diets. The improved TP in the fish fed D1-D4 depicts non-specific immune response of the fish [35] arising from the phytochemicals contained in the *S. malaccense* diets, resulting to improve energy due to high protein metabolism [43]. There was significant increase in the UR and CR in the plasma of fish fed Do and BC after seven (7) days post infection period. Increase in UR and CR have been associated with kidney malfunction [44], and Murray *et al* [45] attributed increase UR in fish plasma as gill dysfunction and this could be as a result of the pathogen infection [40], and can lead to alterations in the production of white blood cells, since white blood cells are produced in the kidney [46]. The observation of Murray *et al* [45] could be the reason why there was inflammation (increase in size) in the GI – SI of the infected fish fed Do and BC compared to the fish fed D1-D4. The uniform GI-SI, UR and CR presence in the plasma of the fish fed D1-D4 could be as a result of the presence of the phytochemicals contained in *S. malaccense* that are believe to be bactericidal to the experimental pathogen [34]. ALB was significantly

lower in fish fed Do and BC, but increase significantly in the fish fed D1-D4, this result is similar to the result of Abasali [41] who reported the effect of *A. hydrophila* on common carp fed plants extract supplemented diets, and Dos Santos *et al*, [42] who reported the effects of *A. hydrophila* on silver catfish fed supplemented *A. triphyla* diets. The decrease in ALB can be attributed to the virulence of the pathogen (*S. aureus*) on the *C. gariepinus*. Charlie-Silva *et al* [47] reported decrease in ALB as a way of responding to the negative effect of bacterial presence in fish, when Tilapia was inoculated with *A. hydrophila*. Phytochemicals such as the ones present in *S. malaccense* have been reported to be bactericidal and bacteriostatic to pathogens such as *S. aureus* [34]. The higher value of ALB in the *C. gariepinus* fed D1 – D4 after infection could be as a result of the presence of the phytochemicals in the diets which may have hindered the virulence of the *S. aureus* on the *C. gariepinus* [9,25]. Though values of GLB was higher after infection in all the treatments compared to their values before infections, it was significantly higher in the fish fed BC. The increase in GLB in fish plasma have been associated with the presence of bacteria in fish [47].

### Conclusion

The present results indicates that there was no significant difference in the HSI, SSI and CSI of the experimental fish after seven (7) days post infection, this can be attributed to the fact that the experimental pathogen was not virulence on the liver, spleen and heart of the experimental fish. The presence of interperitoneal fat was significantly higher in the fish fed Do and BC compared to the fish fed D1-D4 and this can be attributed to the virulence of the pathogen on the fish fed Do and BC. It can be concluded that the presence of disease or pathogen such as *S. aureus* in fish especially catfish could lead to increase in interperitoneal fat, with the resultant effect of reducing the fish gonads, indicating negative effect on the fish reproductive capacity. The presence of the pathogen also causes increase in Urea, Globulin and Creatinine in fish, which is an indication of infringement on the fish kidney and gills, and this can alter the production of red blood cells and the fish respiratory system. The increase in GLB and the reduction in ALB was also observed as one of the consequence of the presence of the bacteria (*S. aureus*). The phytochemicals present in *S. malaccense* posses antimicrobial effects that are either bactericidal or bacteriostatic to the tested pathogen (*S. aureus*), as observed in the fish fed D1-D4. *S. malaccense* at the used concentrations can be used to prepare medicinal diets for fish especially *C. gariepinus* as a prophylactic.

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