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Research Article

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Influence of Phytochemical Constituents of Garlic Extract (*Allium Sativum*) on the Treatment of Bacterial and Fungal Infections in *Clarias Gariepinus*.

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Abstract

Bacteria and fungi are among the most common pathogens that infect fish, leading to a significant economic lost in aquaculture. These microbial pathogens have also been reported to be of serious public health concern to humans. This study determined the influence of phytochemical constituent of garlic extract (Allium sativum) on the treatment of bacterial and fungal infection in Clarias gariepinus. Garlic bulbs were purchased from Kure market in Minna Niger State. The bulbs were peeled, washed under running tap water and pulverized using cold maceration method. Quantitative analysis was carried out to determine phytochemical compound (flavonoids, phenol, saponins, alkaloid and tannins) present in the extract. A total of thirty infected Clarias gariepinus were sampled from randomly selected fish farms. The total Bacteria, Coliform and Fungal count were determined and characteristically distinct colonies obtained were subcultured onto fresh agar plates repeatedly to obtain pure cultures which were then stored on appropriate agar slants for identification and further analysis. Bacterial species isolated were identified based on colony morphology and biochemical characteristics. The antimicrobial activity of Allium sativum was carried out using agar well diffusion method. Aqueous extraction of Allium sativum bulbs yielded 37.69g equivalent to 9.4% percentage yield. Quantitative phytochemical analysis revealed the presence of Phenols (205.96 mg/100g), Flavonoids (62.85 mg/100g), Saponin (55.22 mg/100g), Tannins (9.39 mg/100g) and Alkaloids (7.72 mg/100g). The total bacterial count of infected Clarias gariepinus samples range from $6x10^4$ – $37x10^4$ CFU/g with average count of 18.73 x 10^4 CFU/g while the coliform count ranged from $1x10^3$ – $23x10^3$ CFU/g and had an average count of 9.00 x 10^3 CFU/g. No fungal contamination was observed in this study. Bacteria isolated from infected Clarias gariepinus include members of the genus Lactobacillus, Staphylococcus, Bacillus, Micrococcus, Streptococcus, Enterobacter and Escherichia. Data indicated that the aqueous extracts of the Allium sativum bulbs had antimicrobial activity against suspected Clarias gariepinus pathogens. The antimicrobial activity was concentration dependent as inhibition zones increases with increasing concentration of the extract. With significantly highest antibacterial activity observed at 1000mg/ml. The results of this study indicate that garlic extract (Allium sativum) shows antibacterial potential in vitro. These shows that Allium sativum bulbs extracts are potentially effective as natural alternatives for the treatment of infections in Clarias gariepinus

Keywords: *Influence; Phytochemical Constituents; Garlic Extract; Bacterial and Fungal Infection; Clarias Gariepinus*

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Introduction

Clarias gariepinus is a dominant freshwater fish and popular in commercial aquaculture due to its ability to grow rapidly and high tolerance to environmental conditions (Ayele, 2015). However, *C. gariepinus* has been found to be susceptible to both microbial and parasitic infections particularly in intensive culture systems (Sudheesh *et al*., 2012; Opiyo *et al*.,2020). For decades' antibiotics have been used for the treatment of bacterial infection in fish. The adverse effects associated with the use of antibiotics include drug residue, bioaccumulation and resistance of pathogens, which threaten human consumers (Ben *et al*., 2019). Hence, herbs are now being used as probiotics in preventing bacterial infections and are gaining success because they are cost effective, eco-friendly and have minimal side effects (Abd El-Hack *et al*.,2018). Herbs exhibit anti-microbial, anti-stress, appetite stimulation, immune stimulation, and aphrodisiac and antipathogenic effects which facilitate growth and maturation of cultured species (Nwabueze *et al*., 2020). Alluim sativum is a pungent herb and has been reported to inhibits bacterial growth, promote fish growth and enhancement of blood parameters (Metwally, 2009; Alam *et al*., 2016). In aquaculture, A. sativum has been observed to promote growth, enhance immunity, stimulate appetite and strengthens the control of bacteria and fungi pathogens (Harris *et al*., 2001; LeeandGao, 2012). S. aureus is one of the major bacterial agents causing food-borne diseases in humans worldwide and has been found on skin of healthy people and animals including fish (Fetsch and Johler, 2018). Economic Important of *Clarias gariepinus*

Fishes especially, catfish (*C. gariepinus*) are good sources of food for human beings. It is very rich in proteins and vitamins, especially, vitamin A (Retinol). They are source of animal protein. Fishes such as those in the class Clariidae are highly commercialized. Fishes have been known to feed on wide variety of things ranging from Sandy particles, phytoplankton, zooplanktons, leaves, roots, insects, insect larvae, warms, fishes etc. *C. gariepinus* is a bentho-pelagic fish which is known to have wide range of diet. Catfishes (Clarias species) are some of the most important fish species for aquaculture due to its high growth rate, significant tolerance to environment stress, reproduction in captivity, hardy to high density culture and its market demand. *C. gariepinus* especially is widely accepted by Nigerian consumers and was acknowledged that these bigger fish are sold for about twice the price of 30 days old fish. *C. gariepinus* has an average adult length of more than 1-meter long. These fish have slender bodies, a flat bony head, and a broad, terminal mouth with 4 pairs of barbells. They also have a large accessory breathing organ. They can weigh up to 29 kg or more.

Statement of the Research Problem

Fish disease is a substantial source of monetary loss to aqua culturists. Production costs are increased by fish disease outbreaks because it leads to investment lost in fish mortality, disease treatment, and decreased growth during convalescence. Fish are much less crowded in natural systems than in captivity. Parasites and bacteria may be of minimal significance under natural conditions, but can cause substantial problems when animals are crowded and stressed under culture conditions. The adverse effects associated with the use of antibiotics include drug residue, bioaccumulation and resistance of pathogens, which threaten human consumers (Lee and Gao, 2012).

Justification for the Study

Catfish culture has contributed significantly to the growth of aquaculture in Nigeria on the potential effectiveness of some plant derived phyto constituents such as garlic extract that can serve as organic substance in treatment of fish disease. Its phytochemical constituents which are known to have antibacterial, anti-fungal, measure in treatment of *Clarias gariepinus* fingerlings. Alluim sativum is a pungent herb and has been reported to inhibits bacterial growth, promote fish growth and enhancement of blood parameters (Metwally, 2009; Alam *et al*., 2016).

The use of organic product in recent years has been frequently researched. These products are often considered as easy to obtain and safe to use in terms consumption, such as garlic (Bilen *et al*., 2019; Elkordy *et al*., 2021).

Objectives of the Study

The objective of the study is to determine the phytochemical constituent of garlic extract (*Allium sativum*) and its influence on the treatment of bacterial and fungal infection in *Clarias gariepinus*.

Null Hypothesis

Null hypothesis: Garlic do not have significant content of photochemical for treating bacterial or fungal diseases ($p > 0.05$)

Materials and Methods

Study Location

The experiment was conducted at the Microbiology Laboratory Department of Microbiology, Federal University of Technology Minna,

Niger State, Nigeria and the Centre for Genetic Engineering and Biotechnology (CGEB), Federal University of Technology in Bosso Campus.

Processing of Plants Materials

Garlic bulbs were purchased from Kure market in Minna Niger State. One (1) kilogram of garlic bulb was used. The bulbs were peeled, washed under running tap water and pulverized. Using a pestle and mortal so as activate the various sulphur compound in garlic which was indicated by the pungent smell then it was further soak in a beakers containing 800ml of sterile water in a complete submersion.

Aqueous Extraction of Garlic

Garlic was extracted using cold maceration technique (Ahmad et al., 2020). About 400g quantity of the pulverized Allium sativum bulbs were soaked with clean distilled water in a clean steriled airtight container for three days (72 hours) at room temperature, while undergoing vigorous shaking at regular interval. The mixture was then filtered through muslin cloth and the concentrated filtrate was received in a sterile beaker. Using a water bath, the concentrated extract was subsequently transferred into clean sterile airtight glass container and stored in the refrigerator at 4°C until required. The weight of the garlic extract was recorded and the percentage yield was estimated using El-Rokiek et al., (2019) formula:

$$\% \ Yield = \frac{Weight\ of\ dried\ extract}{Weight\ of\ plant\ sample} \ X\ 100 \qquad (1)$$

Quantitative Determination of Phytochemicals

The quantitative phytochemical analysis of the obtained extract was carried out at the center for genetic engineering and biotechnology (CGEB), Federal University of Technology Minna, Bosso campus.

Flavonoids determination

Ibrahim *et al.*, (2020) method was used to determine total flavonoid content of the extracts. of each extract was measured and added to a test tube containing 1.5 mL of absolute methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1% sodium acetate and 2.8 mL of distilled water. The tubes were incubated at ambient temperature for 30 minutes. The absorbance was read at 415 nm with double beam shimadzu UV spectrophotometer, UV-1800. Standard quercetin was used to prepare the calibration curve.

Phenol determination

Total phenol content of the extracts was determined using the method of Chaudhry et al., (2022), 0.01g of each extract was dissolved in 10 mL of distilled water, and 0.5 mL was oxidized by 2.5 mL of 10% Folin-Ciocalteu's reagent which was then neutralized by 2 mL of 7.5% sodium carbonate. Followed by vigorous shaking, the mixture was allowed to stand for 2h. Finally, the absorbance was read at 765 nm using double beam Shimadzu UV spectrophotometer, UV-1800. Standard garlic acid was used to prepare the calibration curve.

Saponin determination

Saponin content of the extract was determined using the method of Adam *et al*., (2023), 0.5g of each extract was weighed and dissolved in 20 mL of 1N HCl and boiled in water bath at 80°C for 4h. The reaction mixture was cooled and filtered, 50 mL of petroleum ether was added and the ether layer was collected and evaporated to dryness. Thereafter, 5 mL of acetone-ethanol (1:1), 6 mL of ferrous sulphate and 2 mL of concentrated sulphuric acid were added and allowed to stand for 10minutes. The absorbance was taken at 490 nm. Standard saponins was used to prepare the calibration curve.

Alkaloid determination

Total alkaloid of the extracts was determined using method of Chaudhry *et al*. (2022), about, 0.5g of each extract was weighed and dissolved in 5 mL of mixture of 96% ethanol:20% H_2SO_4 (1:1) and then filtered.1 mL of the filtrate was then added to a test tube containing 5 mL of 60% H_2SO_4 and allowed to stand for 5 minutes. Thereafter, 5 mL of 0.5% formaldehyde was added and allowed to stand at room temperature for 3h. The absorbance was read at wavelength of 565 nm. Vincristine extinction coefficient (E_{296}, ethanol {ETOH} = 15136$M^{-1}cm^{-1}$) was used as reference alkaloid.

Tannin determination

Tannin content of the extracts was determined using the method of Palacios et al., (2021), 0.2g of each extract was weighed into a 50 mL beaker and 20 mL of 50% methanol was added to it and covered with para film and heated in water bath at 800C for 1 hour. The reaction mixture was shaken thoroughly to ensure uniformity. The extract was then filtered into a 100 mL volumetric flask, and 20 mL of distilled water, 2.5 mL of Folin-Denis' reagent, and 10 mL of sodium carbonate were added and mixed properly. The reaction mixture was then allowed to stand for 20 minutes at room temperature for the development of bluish-green coloration. The

absorbance was taken at 760 nm using double beam shimadzu UV-spectrophotometer, UV-1800. Standard tannic acid was used to prepare the calibration curve.

Data Analysis

Results were analysed statistically by using analysis of variance and Least Significant Difference test (LSD) according to the statistical system (SPSS-23). One-Way ANOVA was used to compare antibacterial activities of Garlic extracts with Chlorophenicol. Excel was used to determine the yield of garlic extract

Results and Discussion

Results

Extract	Weight (g)	Extract weight (g)	Yield (%)
Allium sativum bulb	400	37.69	9.422

Table 1: *Yield of Allium sativum bulb extract.*

Phytochemicals	Composition/Concentration (mg/100g)
Phenols	205.96
Flavonoids	62.85
Alkaloids	7.72
Tannins	9.39
Saponins	55.22

Table 2: *Phytochemicals Composition of Alliumsativum bulb extract.*

Infected Clarias gariepinus	Colony count (CFU/g)	
	TBC(x10^4)	TCC(x10^3)
1	10	23
2	6	9
3	26	1
4	16	8
5	23	6
6	27	1
7	11	12
8	20	4
9	12	7
10	9	1
11	30	16
12	20	1
13	14	6
14	37	12

Table 3: *Total bacterial and coliform countof infected Clarias gariepinus fingerlings.*

CONT'D

Infected Clarias gariepinus	Colony count (CFU/g)	
	TBC(x10^4)	TCC(x10^3)
15	22	9
16	17	2
17	16	7
18	7	7
19	24	18
20	13	5
21	8	11
22	25	9
23	37	14
24	16	7
25	21	9
26	9	19
27	26	7
28	35	3
29	14	20
30	11	16

Keys: TBC: Total bacterial count; TCC: Total coliform count; CFU: Colony forming unit

Table 4.3: *Total bacterial and coliform count of infected Clarias gariepinus fingerlings*

Discussion

Yield of crude extract of Allium sativum bulb

The yield of Allium sativum bulb extract is shown in table 4.1. The aqueous extract obtained weighed 37.69g and the estimated yield from 400g of Allium sativum bulb used for extraction was 9.4%. The Allium sativum bulb that yielded37.69g (9.4%) is similar to the yield observed by Kallel et al. (2014) and Abd Elwahed et al. (2019). Kallel et al., (2014) reported a percentage yield of 26.5% using methanol and 50/50 methanol–water extracts while Abd

Elwahed et al. (2019) reported 20% yield using bioregulators of two bio-regulators, indole acetic acid (IAA) and indole butyric acid (IBA). The observed discrepancy in result could be attributed to various factors majorly, the extraction method and quantity of extraction material used in each study. According to Alara et al. (2021), there are various extraction techniques including maceration, decoction, percolation, infusion, digestion, serial exhaustive extraction, and soxhlet extraction with each having its advantage and demerit on extraction outcome.

Quantitative phytochemicals screening of Allium sativum bulb extract

The phenol, tannin, flavonoid and alkaloid observed in garlic in this study were also observed by Gulfraz *et al*. (2014), Divya *et al*. (2017), Nazirand Chauhan (2019), Azizah *et al*., (2020) and Deepa and Sivakumar, (2023). Quantitatively, alkaloid (7.2%) was found to be the most abundant constituent, followed by tannin (4.80%), saponin (4.3%), flavonoids (2.18%) and phenols (0.80%). Their study also reported other phytochemicals including Glycosides, steroids, terpenoid and anthraquinones. However, Bar et al., (2022) did not observed alkaloids and flavonoid in garlic extract. The disparity observed in the phytochemical constituent of Allium sativum extract could be attributed to difference in solvents used in each study. According to Dzah et al., (2020) and Gil-Martín et al. (2022) the recovery of polyphenols from plant materials is influenced by the solubility of the phytochemical compounds in the solvent used for extraction.

Total bacteria and coliform count

The total bacteria and coliform count of infected Clarias gariepinus fingerlings is shown in Table 4.3. The total bacterial count of infected Clarias gariepinus ranged from 6 x 10^4–37 x 10^4 CFU/g with average count of 18.73 x 10^4 CFU/g the total coliform count ranged from 1 x 10^3–23 x 10^3 CFU/g with an average count of 9.00 x 10^3 CFU/g.

In the current study the total bacterial count of infected Clarias gariepinus range from 6 x 10^4–37 x 10^4 CFU/g with average count of 18.73 x 10^4 CFU/g while the coliform count ranged from 1 x 10^3–23 x 10^3 CFU/g with an average count of 9.00 x 10^3 CFU/g. Similar finding was reported by Afolabi et al. (2020) who assessed the bacterial loads of Clarias gariepinus obtained from cultured and natural habitats. They recorded a total bacterial count of 25.77×10^4 CFU/g. Higher Bacterial and coliform count was observed in the study of Ezeama et al. (2023), who reported a total bacterial count (x10^{12} CFU/g) of 117.33 ± 11.554 while coliform count (x10^{12} CFU/g) observed was66.08 ± 5.51. The observed microbial load in these studies could be as a result of exposure and consumption of bacteria for long time through food and water (Kebbi et al., 2020). The survival of these bacteria is dependent on the conditions prevailing in the aquatic environment and fish are often simply their hosts (Sehnal et al., 2021). High bacterial abundance is not necessarily a disadvantage. If the bacteria are not pathogenic, as high bacterial abundance may indicate healthy organic matter recycling and remineralization (Kasozi et al., 2023).

Identification of bacterial isolates

Bacteria isolated from infected Clarias gariepinus include members of the genus *Lactobacillus, Staphylococcus, Bacillus, Micrococcus, Streptococcus, Enterobacter* and *Escherichia*. Represented by the species *Bacillus subtilis, Escherichia coli, Staphylococcus epidermidis, Micrococcus luteus, Enterobacter aerogenes, Staphylococcus aureus, Streptococcus sp*. and *Lactobacillus bulgaricus. Similar* findings was reported by Deborah et al., (2022) in their study on the occurrence of pathogenic bacteria associated with Clarias gariepinus in selected fish farms of Kumbotso Local Government Area of Kano State, Nigeria. From their study they reportedthe presence of *Escherichia coli, Micrococcus luteus, Enterobacter aerogenes, Pseudomonas aeruginosa, Staphylococcus epidermidis* and *Staphylococcus aureus. Similarly, Ogbukagu et al*., (2021) reported *Vibrio, Aeromonas, Pseudomonas, Lactobacillus, Staphylococcus, Microbacterium, Serratia, Proteus, Bacillus, Streptococcus, Citrobacter* and *Micrococcus*.

Conclusion

Eight bacteria which include; *Staphylococous epidemis. Bacillus subtilis, Streptococcus sp., Entrobacter aerogenes, Staphylococcus aureus. Escherichia coli, Micrococcus luteus, Lactobacillus bulgaricus* were isolated from infected Clarias gariepinus during the practical Phytochemical analysis of the garlic extract reveal the presence of five secondary metabolite which includes phenols 205.96 (mg/100g), flavonoids 62.85 (mg/100g), alkaloids 7.72 (mg g/100g), Tannins 9.39 (mg/100g), saponins 55.22 (mg/100g).

Antibacterial activity of Allium sativum extract against suspected bacteria isolate tested show antibacterial activity. With highest activity observed at 1000mg/ml compared lower concentration of 800mg/ml, 600mg/ml, 400mg/ml respectively.

Garlic extract prove to be highly effective in treatment and control of bacteria disease infection in Clarias gariespinus at 1000mg/ml

Recommendations

- Further studies are needed to elucidate the Pathophysiological mechanism of action of garlic as well as its efficacy and safety in treatment of various diseases.
- The extract mechanism of all ingredient and their long terms effect are not fully understood. There fore studies should be carried out this area.
- Further studies on the isolation and purification of active compounds present in Allium sativum bulbs should be carried out

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