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Effect of Garlic Extract in the Treatment and Control of Bacterial and Fungal Diseases in *Clarias Gariepinus*

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ABSTRACT

This study determined the effect of Allium sativum in the treatment of bacterial and fungal infections in Clarias gariepinus fingerlings. Bacteria and fungi are among the most common pathogens that infect fish, leading to a significant economic loss in aquaculture. These microbial pathogens have also been reported to be of serious public health concern to humans. 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 sub-cultured 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 increase 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 show that Allium sativum bulbs extracts can potentially be effective as a natural alternative for the treatment of infections in Clarias garipenus.

Key Words: Investigation, Effect, Garlic Extract, Treatment and Control, Bacterial and Fungal Diseases, *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, andaphrodisiac 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 inhibit 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; Lee and Gao, 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).

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).

Objective of the Study

The objective of the study is to determine and investigate the effect of garlic extract in the treatment and control of bacterial and fungal diseases in *Clarias gariepinus*

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 to activate the various sulphur compound in garlic which was indicated by the pungent smell, then it was further soaked in a beaker 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:

$$\%Yield = \frac{Weight\ of\ dried\ extract}{Weight\ of\ plant\ sample}\ X\ 100 \qquad (3.1)$$

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), 0.01 g 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 2 h. 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), 0.5 g of each extract was weighed and dissolved in 20 mL of 1N HCl and boiled in water bath at 80 $^{\circ}$C for 4 h. 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, 0.5 g 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 3 h. 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 80^0C 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.

Microbiological Analysis

Sample collection

Thirty (30) *Clarias gariepinus* fingerlings were randomly selected from fish farms in Minna, Niger state. The samples of size 3-4 centimetre and average weight 1.6 were properly packaged in containers containing clean water and immediately transported to the laboratory. The presence of bacterial or fungal diseases was checked in each fingerlings transported to the laboratory. The diseased fingerlings were isolated for treatment using garlic extract. Other experiments for this work were carried out **a**t the Microbiology laboratory, Department of Microbiology Federal University of Technology Minna, using standard microbiological methods.

Media preparations

Nutrient Agar: Nutrient agar medium was prepared according to manufacturer's instructions by dissolving 28g of the nutrient agar powder into 1litre of distilled water. The mixture was boiled to allow complete dissolution of the powder and then sterilized by autoclaving at 121°C for 15minutes. The medium was allowed to cool to 45°C before usage.

MacConkey Agar: MacConkey agar is a selective and differential media used for the isolation of gram-negative rods, particularly members of the *Enterobacteriaceae* family. The medium was prepared by dissolving 49.53g of the powder into 1 litre of distilled water according to manufacturer's instructions. The mixture was boiled to allow complete dissolution of the powder and then sterilized by autoclaving at 121°C for 15 minutes. The medium was allowed to cool to 45°C before usage.

Sabouraud Dextrose Agar (SDA): Sabouraud dextrose agar is a growth medium used to cultivate various types of fungi. Sabouraud dextrose agar medium was prepared by dissolving 65g of the Sabouraud dextrose agar powder into 1litre of distilled water according to manufacturer's instructions of chloramphenicol antibiotics was added and the mixture was boiled to allow complete dissolution of the powder and then sterilized by autoclaving at 121°C for 15 minutes. The medium was allowed to cool to 45°C before usage.

Sample preparation

The flesh of each fish sample was weighed (1g) and added into test tube containing 9ml of sterile water and 10-fold serial dilution was carried out until a dilution of 10^{-4}.

Total bacterial count (TBC)

The total bacteria count was a quantitative estimate of the number of bacteria in a sample. Total Bacteria Count was carried out using pour plate technique. One mill (1ml) of the 10^{-4} dilution of the fish was aseptically transferred into sterile petri dishes and molten nutrient agar medium was poured into the respective petri dishes and allowed to solidify before

incubating at 37°C for 24hours. This process was carried out in duplicates for each fish sample. The plates were then observed for the presence of discrete colonies after incubation and the actual numbers of bacteria were estimated in colony forming unit per gram (CFU/g).

Total coliform count (TCC)

Coliforms are often used as indicators of faecal contamination. Quantitative analysis for the presence or absence of specific microorganisms was done by plating on selective media. MacConkey agar was used for enumeration of total coliform bacteria by transferring 1 ml of 10^{-3}dilution of each sample into petri dishes, Molten MacConkey agar medium was poured into respective petri dishes and allowed to solidify before incubating at 37°C for 24 hours. This process was carried out in duplicates for each fish sample. After which the plates were then observed for the presence of discrete colonies after incubation period and the actual numbers of bacteria were estimated in colony forming unit per gram (CFU/g).

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%

RESULTS

Table 1: Yield of *Allium sativum* bulb extract

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

Source: Field Experimentation, 2024

Table 2: Antibacterial Activity of*Allium sativum* bulb extract against suspected *Clarias gariepinus* bacterial pathogens

Test isolates	Garlic extract Concentrations (mg/ml)				
	Control (Chloramphenicol 250)	400	600	800	1000
Staphylococcus epidermidis	30.00±0.00^{a}	0.50±0.50^{d}	10.00±0.50^{c}	10.50±0.50^{c}	16.50±0.50^{b}
Bacillus subtilis	32.00±1.00^{a}	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{c}	7.50±0.50^{b}
Streptococcus sp.	51.33±1.15^{a}	0.00±0.00^{d}	10.50±0.50^{c}	26.50±1.50^{b}	26.50±0.50^{b}
Enterobacter aerogenes	32.33±0.58^{a}	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{c}	3.50±0.50^{b}
Staphylococcus aureus	41.00±0.00^{a}	0.00±0.00^{e}	11.00±1.00^{d}	16.00±1.00^{c}	20.50±0.50^{b}
Escherichia coli	32.33±2.08^{a}	2.50±0.50^{e}	4.50±0.50^{d}	14.50±0.50^{c}	20.50±0.50^{b}
Micrococcus luteus	33.33±1.53^{a}	0.00±0.00^{e}	11.00±1.00^{d}	17.50±0.50^{c}	21.50±0.50^{b}
Lactobacillus bulgaricus	30.67±1.15^{a}	0.00±0.00^{d}	0.00±0.00^{d}	9.50±0.50^{c}	14.50±0.50^{b}

Mean having similar superscript in same row not significantly different (p>0.05)

DISCUSSION

Antibacterial activity of *Allium sativum* bulb extract

Fish are susceptible to numerous diseases, which could lead to partial or complete loss of produce (In Salwany *et al*., 2019). They are exposed to numerous pathogenic microorganisms inhabiting the water environment. In order to prevent such losses, various medications are used for prevention and treatment. These are often used as additives in fish feeds to promote growth, and sometimes, in the form of baths or injections (Zhang *et al*., 2022). The use of antibacterial drugs could lead to emergence of resistance in microbial pathogens and accumulation of residues in fish tissues (Hassan *et al*., 2021). Alternatives have been sought over the last few years, which could replace antibiotics, and medicinal plants can be one such alternative. Even though medicinal plants have been used to treat illnesses for millennia, studies on their effects against fish pathogens are relatively few. Plants are rich in a wide variety of phytochemical compounds which have the beneficial capabilities to improve the health status of fish (Ahmadifar *et al*., 2021). *Allium sativum* bulb is a well-known medicinal plant with many health benefits (Alam *et al*., 2016; Nasir *et al*., 2020; Tesfaye, 2021 and Choudhary *et al*., 2022). In the current study extract of *Allium sativum* exhibited varying degree of antibacterial activity against suspected *Clarias gariepinus* bacterial pathogens. The antibacterial activity was largely concentration dependent as inhibition zones increases with

increasing concentration of the extract. With significantly highest antibacterial activity observed at 1000mg/ml for all test organisms. Various investigations and reports have highlighted the antimicrobial activity of *Allium sativum* in the management of various diseases in aquaculture (Hoseinifar *et al*., 2020; Valenzuela-Gutiérrez *et al*., 2021). This result is consistent with the findings of Manpreet *et al*. (2021), who reported that *Allium sativum* extracts had anti-microbial activity against various microorganisms. The antimicrobial activity of ginger may be due to the considerable amounts of phytochemical compounds present in garlic (Panpatil *et al*., 2013; Phan *et al*., 2019; Bhatwalkar*et al.,* 2021; Akullo *et al*., 2022). Allicin has been found to be the active ingredient in garlic and it works as an antimicrobial agent by inhibiting DNA and protein synthesis moderately and inhibiting RNA synthesis completely as a primary target (Salehi *et al*., 2019). Garlic is also rich in anionic components such as nitrates, chlorides and sulfates and other water soluble components found in plants and these components may have antimicrobial properties (Hanane *et al*., 2022).

Yield of crude extract of *Allium sativum* bulb

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.

Conclusion

Eight bacteria which include; *Staphylococous epidemis*. *Bacillus subtilis*, *Streptococcus sp.*, *Entrobacterae rogenes*, *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 to lower concentration of 80Omg/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

i. 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.
ii. The extract mechanism of all ingredients and their long terms effect are not fully understood. Therefore, studies should be carried out this area.
iii. Further studies on the isolation and purification of active compounds present in *Allium sativum* bulbs should be carried out.

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