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# *Efficacy of Aqueous Extract of Pistia Stratiotes (Water Lettuce) Against some Tropical Fish Bacterial*

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***ABSTRACT***

**Background:** The study was undertaken to determine the phytochemical screening and the antibacterial activities of *Pistia stratiotes* (water lettuce) against some clinically isolated fish pathogens. **Methods:** The healthy and disease free plants samples were collected from Jebba Upper Basin of River Niger and tested for their antibacterial activities and phytochemical properties. *Pistia stratiotes* aqueous extract was extracted using sterile distilled water. The Agar diffused methods were used to determine the antimicrobial actions of the plant against fish pathogens of *Pseudomonas fluorescens*, *Escherichia coli*, *Salmonella typhi*, *Aeromonas hydrophila*, and *Vibrio anguillarum*. **Results:** The antibacterial investigation of aqueous extracts revealed the significant activity against these bacterial. Moreover, phytochemical examination of the crude extract showed the occurrence of saponins, alkaloids, tannins, anthraquinones, flavonoids, and catechins, which completely inhibited the growth of *V. anguillarum* and *E. coli*. However, *Salmonella typhi*, *Pseudomonas fluorescens*, and *Aeromonas hydrophila* exhibited moderate varying levels of resistance. **Conclusion**: The finding of this study showed that bioactive resistance from *Pistia stratiotes* can help disrupt the activities of various fish bacterial in aquaculture practices

**Keywords:** *Antibacterial; Aqueous extract; Fish; Pathogens; Pistia stratiotes*

## Introduction

Fish is an important source of animal protein globally that provides many essential nutrients for human health benefits. In Nigeria, fish is a reputed important staple food considering its high quality proteins and important nutrients. Hence, aquaculture (fish farming) industry is regarded as one the country's fastest growing profitable agricultural subsector (Olaoye *et al.*, 2013) and will continue to be lucrative if its sustainability planning and management are well implemented. However, sustainable fish production can only succeed when fish are healthy and free from disease. Diseases infection in fish farming is among the major complications faced by fish farmers globally because fish are susceptible to several pathogenic bacterial especially when stocked at high density (Antunes *et al.*, 2006).

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Given that pathogenic bacterial could be antagonistic to successful aquaculture operation, its management is invaluable in aquaculture industry because its mismanagement, which is more severe in developing countries, could lead to disease invasion and eventual loss of revenue, food insecurity, and ecological damage. In the face of managing fish health, commercial fish farming has probably resulted to indiscriminate use of synthetic antibiotics, which has consequently led to the development of resistant bacterial in the aquatic bodies (Abutbul *et al.*, 2005) and eventual mortality of fish (Figueiredo *et al.*, 2006, Hatha *et al.*, 2005). Thus, using conventional antibiotic drugs should be discouraged (Cabello, 2006) and replaced with biodegradable plant sourced remedies against fish bacterial.

In this regard, aquatic plants could contribute to the enhancement and discovery of plant-based phytomedicines of immense economic and ecological importance in the treatment of fish pathogen for prosperous aquaculture practices. Therefore, this study investigated the efficacy of aqueous extract of *Pistia stratiotes* (water lettuce) to establish a potential alternative processes to a degradable, effective and less expensive resources in treating fish bacterial.

*Pistia stratiotes, as* an aquatic plant and weed of stagnant water, can be more problematic in the future (Marwat *et al.*, 2010). It grows in a very wide variety of aquatic habitats, requires a moist habitat, and is found in lakes and rivers, but prefers relatively stagnant water. It can survive terrestrial condition by anchoring to the hydro-soil for a few weeks when the water level recedes. It is very sensitive to frost; the best range of temperature is 15-35°C (Khan *et al.*, 2014). It has a low salinity tolerance; salt concentrations of 1.66% are toxic to the plant (Haller *et al.*, 1974).

It is also well known that *Pistia stratiotes* contain anti-oxidants and several natural-occurring chemicals of pharmacological importance (Khan *et al.*, 2014, Tulika and Mala, 2015). Some of these chemicals are known as “secondary metabolites” and have been shown to be high in biological actives against certain microbes (Hossain *et al.*, 2018, Khan *et al.*, 2014, Mukhtar and Tukur, 2000). The phytochemical in the aquatic plants include saponins, flavonoids, alkaloids, sterols, phenolic compounds, *etc.* (Adelakun *et al.*, 2015, Hossain *et al.*, 2018). This study then investigated antibacterial potential of aqueous extract of *Pistia stratioties* (water lettuce) to help in the treatment of fish bacterial infections.

## Materials and Methods

*Plant collection:* Fresh whole of *Pistia stratiotes* (water lettuce) was collected along the bank of River Niger at Kainji Dam in Borgu Local Government Area of Niger State, Nigeria. The collection was immediately taken for identification and quality authentication at the Biological Laboratory of National Institute for Freshwater Fisheries Research, New Bussa, Nigeria. In the laboratory, the plant was identified using the instructions provided by Obot *et al.* and confirmed healthy for experimental research (Obot and Ayeni, 1987).

*Preparation of plant aqueous extract:* Fresh healthy samples were initially cleaned of dirt using distilled water, chopped, and dried at room temperature. The dried sample was ground into a fine powder using electric grinder machine (Rico MG 1803 Mixer Grinder, India). For extraction, ten grams of the ground sample was weighed into conical flask containing 50 ml distilled water, corked undisturbed for 24 hours, and then filtered with Whatman filter paper (No. 1). The resulting filtrate was placed in rotary evaporator and lyophilised to remove water and the standard extract was immediately transferred to Eppendorf tube until use.

*Standard solution preparation:* Two stock solutions of final extract dissolved in distilled water were obtained at concentrations of 100 mg/ml in preparation for phytochemical and antibacterial assays.

*Phytochemicals assay:* Standard quantitative analyses of phytochemical constituents (such as



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tannin, saponins, alkaloids, flavonoids, catechins, quinine and anthroquinones) were carried out on the aqueous extract of *Pistia stratiotes* according to the method of Raaman (2006).

*Bioassay for antibacterial activity:* Antibacterial effect of the aqueous extract was tested against *Escherichia coli* (E. coli), *Vibrio angullarum*, *Aeromonas hydrophila*, *Salmonella typhi*, and *Pseudomonas fluorescens* through agar well diffusion. Later, 50 ml of the pure selective medium was decanted into different sterilised petri-dish and solidified at room temperature. Pure cultures of each bacterial (100 ml containing 108 colony forming units per gram (cfu/g) in 10 ml of soft agar were inoculated evenly on agar plates to obtain uniform inoculums and the solid agar was pricked at 8mm diameter across using sterile borer. Each prick was filled from 100ml of stock solution prepared earlier from aqueous extract of plant sample. The set-ups were placed in refrigerator for 30minutes to allow for diffusion before incubation at $37^0$C for 24 hours. All bioassay tests were performed in triplicates and the average results were taken.

## Results

This study elucidated phytochemical ingredients and the efficacy of aqueous extract of *Pistia stratiotes* against some pathogenic bacterial of fish. The phytochemical examination verified the presence of seven bioactive compounds. Alkaloids (3.86 ± 0.01) and saponins (1.35 ± 0.01) exhibited high presence in the aqueous extracts of *Pistia stratioties* while catechins, quinine, anthroquinones, and tannins showed moderate quantities (0.44 ± 0.01, 0.42 ± 0.12, 0.26 ± 0.02, and 0.17 ± 0.01 respectively), though only traces of flavonoids (0.03 ± 0.00) were present (**Table 1**). Antimicrobial efficacy of the understudied plant showed broad spectrum inhibitory activity against investigative susceptible bacterial species (**Table 2**). The growth of both *E. coli* and *V. angullarum* was completely inhibited to 0 cfu/g, while the moderate level of inhibition was recorded for *S. typhi*, *A. hydrophila* and *P. fluorescens* with inhibitions of $2.3x10^3$, $3.5x10^3$, and $4.2x10^3$ (cfu/g), respectively.

**Table 1.** Phytochemical properties of *Pistia stratiotes*

| Phytochemical | Quantity (µg/mg Dry weight sample) [a] |
|---|---|
| Flavonoids | 0.03 ± 0.00 |
| Saponins | 1.35 ± 0.01 |
| Alkaloids | 3.86 ± 0.01 |
| Anthroquinones | 0.26 ± 0.02 |
| Catechins | 0.44 ± 0.01 |
| Quinine | 0.42 ± 0.12 |
| Tannin | 0.17 ± 0.01 |

[a]: *Mean ± standard error*

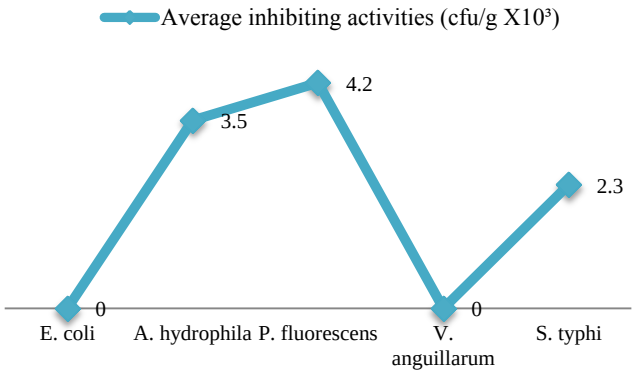



**Figure 1.** Antibacterial activities of *Pistia stratiotes*

## Discussion

Fish farming remains undoubtedly an important source of animal protein globally. The intensification in fish production has led to an increasing interest in fish disease management through different effective and low cost innovation. From the study, *Pistia stratiotes* contains phytochemical ingredients mainly consisting of alkaloids and saponin, with moderate levels of catechin, quinine, anthroquinine, and tannins as well as traces of flavonoids. Presence of alkaloids and saponins had been reported earlier in this plant from Bangladesh (Hossain *et al.*, 2018).

Based on the findings, complete inhibitions were reported for *E. coli* and *V. angullarum*, while the extract significantly hindered the growth of *P.*



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*fluorescens, S. typhi,* and *A. hydrophila*. This result is in consonance with the study by Daboor *et al.* showing that the understudied plant extract exerted antimicrobial action on the pathogenic bacterial (Daboor and Haroon, 2012). This finding was probably as a result of different metabolites present that had high relevance in the treatments against potentially fish pathogenic bacterial. Similarly, Sridevi and Fareed had earlier shown that *Pistia stratiotes* inhibited the growth of *E. coli, Pseudomonas spp.* and *S. typhi (Fareed et al., 2008, Sridevi et al., 2010)*.

The occurrence of high concentration of alkaloids can be attributed directly to its anti-oxidative exploit (Edu *et al.,* 2015). Action of tannins and flavonoid present can affect the function of bacterial cell membrane and consequently inhibit their growth (Hendrich, 2006, Trombetta *et al.,* 2005). Newman and Killeen reported that saponins played a major role in the efficacy of plants against bacterial through membranolytic quality rather mere modification of extracellular part, which could lead to eventual microbial increasing density (Killeen *et al.,* 1998, Newman *et al.,* 2000). Varying degrees of resistance to the plant aqueous extract by pathogenic bacterial confirmed that bacteria varied widely in their degrees of susceptibility to certain specific bioactive compounds (Arekemase *et al.,* 2011) in the plant. The effectiveness of *Pistia stratiotes* against some fish bacterial may reveal the existence of wide range antibiotic ingredients in the plant (Srinivasan *et al.,* 2001). This study corroborated the study by Arekemase Arekemase *et al.* (2011), who reported that aqueous extract of plants inhibited the growth of bacterial. High concentration of saponins in the plant possibly inhibits pathogenic bacterial activity as it can hydrolyse to release compounds that can inhibit microbial growth (Aboaba and Efuwape, 2001). The efficacy of *Pistia stratiotes* against some fish pathogens affirmed the report by another study, who described its antimicrobial action using various solvents for extraction (Sridevi *et al.,* 2010).

## Conclusion

The antimicrobial efficacy of *Pistia stratiotes* can be due to high level of alkaloids and saponin as well as the presence of other bioactive ingredients. The presences of these phytochemicals are responsible for the complete elimination of *E. coli, V. angullarum* and moderate inhibition of *P. fluorescens, S. typhi,* and *A. hydrophila*. Since synthetic drugs resistance against pathogens is currently established in aquaculture practices, this study confirmed that aqueous extract of *Pistia* stratiotes could be one of the many new herbs based on antibiotics to combat pathogenic bacterial especially gram-negative *V. anguillarum,* and *E. coli,* therefore, justifying the scientific basis for using this aquatic plant for disease management in aquaculture practices.

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## Authors' contribution

Adelakun, KM and Halidu S.K. designed the research, searched for the literature, revised, and corrected the manuscript. Fajobi, EA and Joshua DA carried out the experiment and drafted the manuscript. Final manuscript was approved by all authors for publication.

## Conflict of interest

There is not any conflict of interest.

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