



Original Article
JBS. 2015 Dec; 2(4):38-42

Journal of Biomedical Sciences

Official Publication of NHRWS

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Cite this Article

Adelakun KM, Mustapha MK, Muazu MM, Omotayo OL, Olaoye O. Phytochemical screening and antibacterial activities of crude extract of *Nymphaea lotus* (water lily) against fish pathogens. *Journal of Biomedical Sciences*. 2015;2(4):38-42.

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Phytochemical screening and antibacterial activities of crude extract of *Nymphaea lotus* (water lily) against fish pathogens



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Information about the article

Received: May. 16, 2016

Revised: June. 25, 2016

Accepted: July 8, 2016

Published online: Aug. 4, 2016

ABSTRACT

Background

This study was designed to determine the role of aqueous extract of water lily (*Nymphaea lotus*) for potential antibacterial activity against some gram-negative fish pathogenic bacteria.

Material and methods

Aqueous extracts of the dried whole plant *Nymphaea lotus* was tested by the agar disc diffusion method against four bacteria strains, namely, *Escherichia coli*, *Vibrio anguillarum*, *Pseudomonas fluorescens*, *Aeromonas hydrophila* and *Salmonella typhi*. Inhibitory growth values were determined in colony forming unit per gram (cfu/g) for each pathogen while examination of phytochemical constituents of the crude extricate of the plant was also carried out qualitatively.

Results

Phytochemical screening of the crude aqueous extract revealed the high presence of saponins, alkaloids and carbohydrate, moderate presence of cardiac glycosides, tannins, phenolics, anthroquinones, terpenoid, quinones, catechins and trace of flavonoids.

Conclusion

The antibacterial analysis of crude aqueous extracts showed moderate varying degrees of resistance against the microbes especially *Escherichia coli* and *Vibrio anguillarum* where complete growth inhibition was observed. Hence, this plant aqueous extract can help in the treatment of fish bacterial infections..

Keywords

Antibacterial activity, aqueous extract, fish pathogens, *Nymphaea lotus*, phytochemicals

Introduction:

Ecological factors including aquatic environment, season, food and feeding habits, susceptibility of the subjected individual as well as contact with affected organisms play major role fish in infection instigated by pathogens transmitted by pathogens from fish. Transmission of fish pathogens from fish or its habitat which instigated fish infection are relatively associated with some ecological factors such as relationship or direct contact with infected species, habitat degradation, season, food and feeding habit as well as resistance mechanism of the exposed individual to a particular disease. These pathogens are usually facultative bacterial species for fish and may be quarantined from fish with no evident signs of disease. The source of this infection may be from fish may be fish reared for consumption or ornamental purposes [1].

Bacteria pathogens related to fisheries resources are include some species of *Vibrio*, *Salmonella*, *Streptococcus* as well as aeromonads, mycobacteria and others and may be zoonotic [1-4]. Antimicrobial sources are basically relevant in reducing the global onus of infectious diseases. However, as specific pathogens develop resistance mechanism, the efficacy of a particular antibiotics is reduced and therefore could be an intense risk to general public health and even for the wide range of antibiotics, the incidence of resistance are growing worldwide [5, 6]. Botanical antimicrobials remained major untapped resources globally, though its utilization for medicinal purposes has been well acknowledged when people understood that the efficacy of antibiotics is restricted and its abuse is basis for microbial resistance [7]. According to Ali and Qaiser, [8] 35,000 to 70,000 plant species has been used in folk medicine worldwide. Use of medicinal plants as a drug is alternative method for the management of pathogenic microbes like bacteria, fungi and viruses is eco-friendly. Different researches conducted worldwide to proof the antimicrobial efficacy of traditional used medicinal plants [7, 9, 10]. Traditional medicines are well and widely documented in developing countries. The natural products of medicinal plants may give a new source of antibacterial, antifungal and antimicrobial agents in fish production [11]. However, application of aquatic plant biocides as antimicrobial has not been widely explored until very recent [12-14].

Nymphaea lotus (Water lily) is an aquatic plant usually with white blossom and perpetual rhizomes or rootstocks affixed down with mud, floating or submerged solitary leaves and flashy flowers [15]. In traditional medicine, the plant species is adjudging to be a sedative with reputation of arousing sexual desire and detoxifying human body system. It is also considered effective in the treatment of fever, diarrhoea, urinary difficulties, enteritis, dyspepsia, and abnormal heart beat [16]. El-Ghazali *et al.* [17] reported the significant of the leaves in the treatment of bowel problems and tumours by the traditional Sudanese. With the increasing resistance and the

acknowledgement of limiting factors of antibiotics, currently aquatic plant sources are vigorously explored as new sources of plant based antibiotics. The present study was designed to determine the role of aqueous extract of water lily (*Nymphaea lotus*) for potential antibacterial activity against some gram-negative fish pathogenic bacteria i.e. *Escherichia coli*, *Vibrio anguillarum*, *Pseudomonas fluorescens*, *Aeromonas hydrophila* and *Salmonella typhi*. The observed inhibition zones were measured in Colony forming unit per gram (Cfu/g). The study also included the analysis of phytochemical properties of the plant extracts to determine the bioactive constituents responsible for its antimicrobial status.

Material and methods

Plant material

The plant materials used in this study consisted of mature whole plant species of water lily which was collected from Upper Jebba Basin during the dry season (February to early April, 2015). The healthy and disease free plant identified with the aid of keys compiled by Obot and Ayeni [18] were initially rinsed with distilled water and dried on paper towels in the laboratory at (37±1)°C for 24 h [9] for antibacterial activities test and phytochemical analysis.

Preparation of aqueous extract

10g of the sample was weighed out and soaked in 50ml of distilled water contained in an undisturbed conical flask stoppered with cork for 24hrs. It was then filtered off with sterile filter papers (Whatman No. 1) into clear conical flask in readiness for water bath, where the aqueous solvent was allowed to evaporate at 100°C. The standard extracts thus obtained transferred to eppendorf tubes.

Bioassay for antimicrobial activity

In the presence study to test antibacterial activity: *Nymphaea lotus* crude extract of the whole plant was used. The antibacterial activity was studied by agar well diffusion. Approximately 50ml of sterilized selective medium was poured into each petri-plate and solidified at room temperature. A fresh bacterial culture of 100ml having 108cfu/g was taken in 10ml of soft agar were poured on agar plates and allowed to solidify. It was then punched off 8mm diameter across earlier marked petri-dish into agar medium using sterile cup borer and later filled with 100ml of sample plant aqueous extract. Plates were placed for 30 minutes in refrigerator for diffusion of extract and then incubated at 37°C for 24 hours. Bioassay was performed in duplicate for better result.

Bacterial strains

The gram negative *Escherichia coli*, *Vibrio anguillarum*, *Pseudomonas fluorescens*, *Aeromonas hydrophila* and *Salmonella typhi* were selected for screening.

Preparation of inoculum

Newly culture microbes were inoculated by smearing dropful of bacterial suspension of each bacterium uniformly into optimal temperature hence sustaining homogeneous growth rate of each bacterium.

Phytochemical analysis

Qualitative phytochemical analysis of plant extracts were carried out for phenolics, Cardiac glycosides, Anthraquinones, tannins, saponins, alkaloids and Catechins as described by Raaman *et al.* [19].

Results

In *Nymphaea lotus*, the phytochemical analysis proved the presence of 9 bioactive compounds which persist to inhabit the activity of the aforementioned susceptible bacteria species.

Antimicrobial activities of the plant extract in table 1 shows maximum antibacterial activity against *Escherichia coli* and *Vibrio anguillarum* with a cfu/g (colony forming unit per gram) 0 while other bacteria species such as *Salmonella typhi*, *Aeromonas hydrophila* and *Pseudomonas fluorescens* produced average growth resistance of (1.1×10^3 , 2.2×10^3 and 2.5×10^3), cfu/g respectively.

Table - 1 Antibacterial activities of *Nymphaea lotus*

Pathogens	Inhabiting activities (cfu/g X 10^3)		Average (cfu/g X 10^3)
<i>E. Coli</i>	0	0	0
<i>P. fluorescens</i>	2.6	2.4	2.5
<i>S. typhi</i>	1.1	1.1	1.1
<i>A. hydrophila</i>	2.3	2.1	2.2
<i>V. anguillarum</i>	0	0	0

Alkaloids, carbohydrate and saponin showed high presence in the extracts while cardiac glycosides, terpenoid, anthraquinones, catechin, phenolics, tannins and quinone indicated moderate scores whereas flavonoids showed trace presence while phlobatanin is absent (Table 2).

Table - 2 Phytochemical properties of *Nymphaea lotus*

Phytochemical	Status
Saponins	++
Phenolics	+
Phlobatanin	--
Quinones	+
Tannin	+
Terpenoid	--
Carbohydrate	++
Cardiac glycosides	+
Catechins	+
Alkaloids	++
Anthraquinones	+
Flavonoid	Trace

Keys: ++ Highly present, + Present

Discussion

Antibacterial activities

The results indicate that the aqueous extract of *Nymphaea lotus* showed antibacterial activity toward the gram-negative bacteria: *Escherichia coli*, *Vibrio anguillarum*, *Pseudomonas fluorescens*, *Aeromonas hydrophila* and *Salmonella typhi*. It was seen that the aqueous solvent extracts of the plant showed a maximum activity against *E. coli* and *V. anguillarum* and better result against *Salmonella typhi*, *Aeromonas hydrophila* and *Pseudomonas fluorescens* bacteria in significant order.

Phytochemical constituents

The phytochemical evaluations of the sample extract shows evidence of many bioactive metabolites that independently or in combinations could be accountable for its antimicrobial biocide. The demonstration of antimicrobial activity against the bacteria may suggest presence of expansive range antibiotics complexes [20]. Lata and Dubey [21] reported that medicinal benefit of plant depends on the quality of bioactive constituents including tannins, alkaloids, flavonoids and phenolic compounds and all these are present in the understudied aquatic plant. According to the study, alkaloid which is highly present in *Nymphaea lotus* plays some metabolic functions and manages growth in living cell most importantly steroidal alkaloids which play protective in animal cells.

Newman *et al.* reported antimicrobial activities of saponin and tannin [22]. According to Killen *et al.*, the form of activity of antibacterial efficacy of saponins includes membranolytic quality as opposed to just modifying the surface tension of extracellular part, consequently affected by microbial populace increase density [23]. Effectiveness of flavonoids and phenolic antimicrobial substances against some microorganisms may be due to their one carbonyl group structures synthesized by plant while responding to microbial infection [24]. Plant source alkaloids are acknowledged of antibacterial quality [25].

Anthraquinones are considered to be one of the most active agents in metastatic breast cancer. The presence of tannins confirmed the antimicrobial action of the extract which is in consonance with the report by Rates (2001) that the activities of tannins is related to their capacity to inhibit cell protein synthesis by binding proteins. Triterpenoids are a large class of natural isoprenoids *i.e.* terpenoid present in the plant, exhibit a wide range of biological activities [26, 27].

Conclusion

The antibacterial activity of *Nymphaea lotus* may be attributed to various chemicals such as saponins, alkaloid and carbohydrate detectable in its extracts. In addition, this medicinal plant could be a promising source of drugs against fish pathogen in traditional aquaculture practices.

Competing interests

None declared.

Authors' contribution

Omotayo OL and Olaoye O searched the literature, Adelakun, KM, Mustapha MK and Muazu MM carried out the experiment and drafted the manuscript, revised and corrected. Final manuscript was approved by all authors for publication.

Acknowledgments

This research work was supported by Forestry Research Institute of Nigeria (FRIN) and Federal College of Wildlife Management, New Bussa, Nigeria under the management of Dr. A.O. Adepoju and Dr. A.S. Kehinde respectively.

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