

PRELIMINARY IDENTIFICATION OF THE CONSTITUENT SECONDARY METABOLITES OF A FRESHWATER WEED (*Nymphaea lotus*) AND TOXICITY TO SHRIMP LARVAE

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ABSTRACT

This study investigated the bio-active constituents and toxic threat of excessive population of *Nymphaea lotus* in aquaculture pond through extraction, phyto-screening and shrimp-lethality. The phyto-chemical analysis of the ethanolic leaf-extract revealed the presence of alkaloids, anthraquinone, cardiac glycoside, steroid, tannins, and triterpenes. Saponins and flavonoids were absent in the crude leaf-extract. Brine shrimp lethality bioassay involved a completely randomized design (CRD) which accommodated four (4) concentrations (0.0µg/mL, 10.0µg/mL, 100.0µg/mL and 1000.0µg/mL). A static non-renewal method was employed and each treatment accommodated 30 larvae and was replicated thrice. LC_{50} s of 1000.0µg/mL and 11.4µg/mL were obtained at 24 and 48-hour of exposures, respectively. Presence of metabolites in the leaf-extract of *N. lotus* indicated its medicinal and toxic potentials. A reduction in LC_{50} value, as the time of exposure of shrimp-larvae to leaf-extract increased, is a warning to aqua-culturists and other static water managers to disallow accumulation of die-offs for a long period of time.

Keywords: *Nymphaea lotus*, Secondary metabolites, Toxicity, Lethality, Aquatic weeds

INTRODUCTION

Aquatic weeds are found in aquatic ecosystems like lakes, reservoirs and ponds but may constitute a hindrance to effective or optimal utilization of these water bodies if in excessive density. They only grow in water or soil that is permanently saturated with water and include water hyacinth (*Eichornia crassipetals*), duck weed (*Spirodela polyrrhiza*), water lettuce (*Pistia stratiotes*), water meal (*Woffia globosa*), Egyptian water lily (*Nymphaea lotus*), and others (FAO, 2013). The excessive growth of these aquatic weeds or abundance constitutes hindrances to the primary focus of managing a concern water body, and sometimes toxic to other aquatic organisms because of their chemical compositions (Patel, 2012). Therefore, these invasive water plants become difficult to manage in larger ponds especially when there is nutrient rich run-off from the surrounding agricultural land (Royal Horticultural Society, 2012).

Nymphaea lotus is commonly known as Egyptian water lily. It is a warm water aquatic plant belonging to the family Nymphaeaceae and native to Egypt, Central and West-Africa and Madagascar. It is one of the commonest invasive species (aquatic macrophytes) in ponds, lakes and reservoirs (FAO, 2009). The conversion of seemingly under-use aquatic weeds to productive functions is now a global approach in maximizing utilization. The current study therefore focuses the presence of active compounds in the leaf part, and possibility of toxic threat to other aquatic lives in pond-water through brine shrimp lethality.

METHODOLOGY

Collection and preliminary processing of plant: Whole plants of *Nymphaea lotus* were harvested from earthen pond at Olugbon Fish Farm in Igboora (Within latitude 7° 15' N latitude and 7° 55' N longitude), Ibarapa, Oyo State, Nigeria. Plant was then sorted into leaf, rhizome and fibrous root/tuber. The weight of each plant part was then determined with the aid of an electronic weighing balance (Melter Teledo JA 2000 3N) after six (6) hours of shade drying. The shade dried leaf-parts were then oven-dried (Model OHG -9053A) at 50°C for 24 hours. Pestle and mortar were used to crush (homogenize) the leaves before soaking in ethanol in a ratio of 1:3 (W/V) for 24 hours

(Adebayo *et al.*, 2004). The solution was then filtered using Whatman filter paper, No. 42 (125mm). A rotary evaporator (RE-52-2) was used to concentrate the aqueous extract to a semi-solid residue. Phytochemical analysis of leaf extract of *N. lotus*: Phytochemical screening of the ethanolic extract was carried out to determine the presence of key secondary metabolites through different analytical tests and reagents. These include alkaloids by using Mayer and Wagner's reagent (Harbone, 1998), Saponins by using frothing and emulsion tests (Harbone, 1998), Tannins by adding a 10% jolt (Harbone, 1998), Flavonoids by adding 10% NaH (Harbone, 1998), Cardiac glycosides by using Lieberman's test and Kiliani test (Odebiyi and Sofowora, 1978; Trease and Evans, 1989), Steroids by using Salkowski test (Lynch *et al.*, 1970) and Anthraquinone (Odebiyi and Sofowora, 1978).

Brine shrimp test: Sea water from Kuramo beach (Atlantic ocean) in Lagos was sieved and later allotted into hatching chamber (plastic soap dish) which accommodated 250mL of sea water. A spoonful of eggs was added before placing the rectangular plastic container beside a window to receive adequate rays of light and proper ventilation. A complete hatching of eggs was observed after 72 hours such that the nauplii aggregated at the clean side of the hatching chamber by passing through an initial perforation of the divider, having been separated by the perforated divider of the plastic from the shell and other dirt. Assay procedure involved the dissolution of 20 mg of plant extract in 2mL of analar grade of Dimethyl sulfoxide ((CH₃)₂ SO) to give a concentration of 10,000ppm and was used as a stock solution. The lower concentrations were then prepared by 1:10 dilution as described by McLaughling *et al.* (1998). A completely randomized design (CRD) was used and involved four concentrations (0.0ppm, 10.0ppm, 100.0ppm and 1,000.0ppm) in triplicates inside test tubes (10 naupli per tube) before probit analyses (Finney, 1978).

RESULTS

Table 1: Qualitative analysis of the phytochemicals in the leaf extract of *Nymphaea lotus*.

S/NO	Phytochemicals	Availability in leaf
1.	Alkaloids	++
2.	Anthraquinone	++
3.	Cardiac glycoside	++
4.	Flavonoid	—
5.	Saponins	—
6.	Steroid	++
7.	Tannin	++

Table 2: Mortality pattern of shrimps exposed to varying concentrations of leaf extract of *Nymphaea lotus* at the end of 24 hours of exposure.

Sample size	Treatment (µg/mL)	Number of Survivors	Mortality	Percentage mortality
30	0.0	30.0	00.-	00.0
30	10.0	24.0	06.0	20.0
30	100.0	19.0	11.0	36.7
30	1000.0	15.0	15.0	50.0

Table 3: Mortality pattern of shrimps exposed to varying concentrations of leaf extract of *Nymphaea lotus* at the end of 48 hours of exposure.

Sample size	Treatment (µg/mL)	Number of Survivors	Mortality	Percentage mortality
30	0.0	30.0	00.0-	00.0
30	10.0	16.0	14.0	46.7
30	100.0	08.0	22.0	73.3
30	1000.0	05.0	25.0	83.30

DISCUSSION

The current study identified the presence of alkaloids, anthraquinone, cardiac glycosides, steroids, tannins and triterpenes but absence of flavonoids and saponins in the crude extract of an abundant leaf of *Nymphae lotus*. Consequently, revealing its suitability in drug making or combating ailments and toxic potential due to the presence of chemical constituents. The group of compounds such as alkaloids, tannins, anthraquinones and flavonoids are metabolites which are anti-pathogenic and explains the application of such plant-part in traditional medicine (Lata and Dubey, 2010). The pros and cons of the utilization of whole plant or other crude preparations have been reported (Colegate and Molyneux, 2008).). For example, the importance of these active compounds include the followings: Saponins (active as effective fish poisons, irritants of skin and mucous membranes and causes haemolysis by releasing haemoglobin from erythrocytes to alter permeability); alkaloids (marked physiological impacts on human and animal organisms); Cardiac glycosides (toxic impacts); Fouracoumarin (poisoning in animals, injury to skin and meadow dermatitis (blistering); tannins (binding and precipitation of proteins and organic compounds such as ammonia and alkaloids); plant acids (poisoning, mechanical irritation of mucous membranes, insolubility of calcium salts, disturbs blood coagulation); tannic acid (reduction of growth and feed utilization in fish) (Ajibade, 2016).

The evaluation of the bioactivity of the ethanol leaf-extract of *Nymphae lotus* through brine shrimp lethality, in the current study, resulted in a median lethal concentration of 1000µg/ml and 11.46µg/ml; at 24th and 48th hour of exposures, respectively. The classification of bioactivity in cytotoxicity studies into two (2) levels of performance; high if lethal concentration is less than 250µg/ml and low when LC₅₀ is equal or greater than 1000µg/ml has been reported by Mbwanbo *et al.* (2007), and Mwangi *et al.* (1999) when investigating 34 plant-extracts and the latex of 2 plants in medicinal uses in Kenya. Similarly, 3 levels of performance (very high when LC₅₀ is < 100 µg/ml; high when LC₅₀ equals 100µg/ml or < 1000 µg/ml; Low when LC₅₀ is ≥ 1000 µg/ml) were reported by Ajibade (2016). The current study therefore, indicated a general exertion of a low cytotoxic ability of the extract in the leaf of *N. lotus* on the surrounding water habitats (such as fish ponds) within twenty four hours of release; as evident through the LC₅₀ value of 1000µg/ml. However, as the period of exposure increased to forty eight (48) hours the median lethal concentration reduced to 11.46µg/ml which corresponds to a very sharp increase in toxicity. Consequently, an increase in the time of exposure of shrimp-larvae to leaf-extract of *N. lotus* increased its toxic-threat to the nauplii. Fish- pond managers should, therefore, disallow an excessive growth or survival of *N. lotus* in their water environments and accumulation of die-offs beyond twenty four (24) hours to avoid a toxic water environment due to a reactive chemical nature of the leaf with water as time progresses. Adequate and timely harvests of the whole plant in lotic and slow-moving waters are recommended. Additional research is needed to establish the specific quantity of each metabolite in the crude extract of the leaf.

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