PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF *Nymphaea lotus* FROM LAPAI –RESERVOIR, NIGERIA

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Abstract

Phytochemical analysis of three plant parts (leaves, flowers, and rhizomes) of Nymphaea lotus from a reservoir in Lapai Niger State were studied to ascertain there medicinal value. The phytochemical analysis revealed the presence of bioactive compounds such as tannins, tarpenes, flavonoids, alkaloids, anthraquinones, saponins, glycosides. The anthraquinonoes, tarpenes, and glycosides were the most prominent bioactive compounds in the leaves, while anthraquinones and glycosides were the prominent bioactive compounds in the flowers and the rhizome. The crude, aqueous and ethanolic extracts of the medicinal plants were tested against Stapylococcus aureus, Candida albicans, Salmonella typhi, Streptococcus sp Escherichia coli, and Proteus vulgaris. Enthanolic exracts of the leaves produced greater zones of inhibition on Staphylococcus aureus and Strepococcus sp. while the aqueous extracts produced no zones of inhibition on any of the organisms. The enthanolic extract of the leaves produced zone of inhibition on Candida albican while the enthanolic extract of the flowers produced zones of inhibition on Candida albicans. Escherichia coli, Salomonella typhi and Proteus vulgaris were the most resistant to the leaves enthanolic extracts, while Staphylococcus aureus, Salmonella typhi, Streptococcs sp, Escherichia coli and Proteus vulgaris were the most resistant to the leaves enthanolic extracts, while Stapylococcus aureus, Salmonella typhi, Streptococcus sp, Escherichia coli and Proteus vulgaris were the most resistant to the flowers enthanolic extracts. The organisms showed resistance to the enthanolic extracts of the rhizomes. The interest in the scientific investigation of these medicinal plants from Lapai reservoir, Nigeria is based on the claims of their effective use for the treatment of many diseases. Therefore, research into the effects of these local medicinal plants is expected to enhance the use of these plants against diseases caused by test pathogens.

Keywords: Phytochemical analysis, *Nymphaea lotus* and antimicrobial activities.

1.0. Introduction

Plants for long had formed the basis of complicated traditional medicine systems of natural products and higher plants have been used for centuries as remedies for human diseases (Saadabi, 2007). Approximately 80% of the world's inhabitants rely on traditional plant medicine for their primary health care solutions (Cragg *et al*., 1999) and over 60% of people in Nigeria rural areas depend on the traditional medicine for the treatment of their ailments (Ghani *et al*., 1989). Different plants have been used as a source of inspiration in the development of novel drugs (Robbers *et al*., 1996). The rediscovery of the connection between plants and health is responsible for the hybrid of botanical drugs, dietary supplements and plant-produced recombinant proteins (Raskin *et al*., 2002). Plant derived medicines are widely used because they are relatively safer than the synthetic alternatives, they are also easily available and cheaper (Iwu *et al*., 1999).

Macrophytes refer to all plants large enough to be visible to the naked eye, not only flowering plants but also ferns, bryophytes and algae. Aquatic macrophytes are important component of many watercourses, providing structures and habitat for fish and invertebrates, offering protection against currents and predators, and forming substrate for the depositioin of eggs. As primary producers, macrophytes represent important food resources, and they play a signifigant role in the oxygen balance and nutrient cycle of many watercourses. *Nymphaea lotus* (water lily) is perennial aquatic macrophyte that grows up to 45cm in height; it is an herbaceous aquatic plant whose leaves submerges or floats in water (Abu-Zaida *et al.*, 2008). It is one of the most beautiful water plants with submerged rhizomes. It possesses heart shaped leaf, which lies flat on water surfaces.

Nymphaea lotus provides relaxing euphoric sensation. It may help relieve muscle spasms. Ancient Egyptians used lotus flower as a strong aphrodisiac for both men and women and general remedy for all kinds of illness. Egyptian medicinal practitioners used flower to stimulate blood flow and anti-aging treatment. Also, the ancient Egypt used the plant as a key to good health, sex and re-birth. The ancient's worshipped lotus as a visionary plant and it was the symbol for the origin of life. When this flower is soaked in water or wine, and then ingested it acts as intoxicant. The Indian Buddhist highly respected it for visionary properties. The plant creates a feeling of wellbeing, euphoria and ecstasy and increase memory without the use of narcotics. In Korea, lotus seed is one of the most well known traditional herbal medicines used to treat cardiovascular symptoms (Kim *et al.*, 2006). The stamens assist consolidation of kidney function and are particularly useful in the treatment of male sex disorders and female leucorrhea (Nguyen, 1999).

Many plant species have been evaluated for their antimicrobial activity for the past 20years (Castello *et al.*, 2002). And since then efficacy of many medicinal plants in the treatment of many diseases have been put to test in many laboratories (Castello *et al.*, 2002). The emergency of the multi-drug resistant (MDR) bacteria is of great concern to both clinical and pharmaceuticals industries, since it is a major treatment failure in many infectious diseases (Davies, 1994; Akinjogunla *et al.*, 2009). The active principles of many drugs are found in plants secondary metabolites (Ghani, 1990; Dobelis, 1993). This has encouraged research into screeninig of plants for bioactive compounds and antibacterial as well as antifungal activities (Omer and Elnima, 2003; Saadabi, 2006). Many of the active compounds has found place in modern therapy, with compounds varying from flavonoids, terpenes, alkaloids and glycosides, to many minor classes of plant constituents (Vardamides *et al.*, 2001; Saadabi, 2006; Saadabi *et al.*, 2007). Phytochemicals are naturally occurring biologically active chemical compounds in plants and the prefix "Phyto" is from a Greek word meaning plants. Phytochemicals act as a natural defense system for host plants and provide colour, aroma and flavor. They are protective and disease-preventing particularly for some form of cancer and heart diseases.

Since fungi and bacteria are more and more resistant to common antibiotics, there is the need to use plants in traditional medicine practices to serve as remedy for these fungi and bacterial infections.

2.0. Materials and Methods

2.1. Study Area

This study was carried out in Lapai dam. Lapai is a local government headquarters (Latitude 9^0 27 North and Longitude 6^0 41 East) in Niger state Nigeria. Lapai local government is bordered in the north by Paiko LGA, Agaie LGA, to the west and FCT Abuja to the east and Kogi state area to the south. Lapai dam takes its source from river Ndakostu also found in Lapai LGA.

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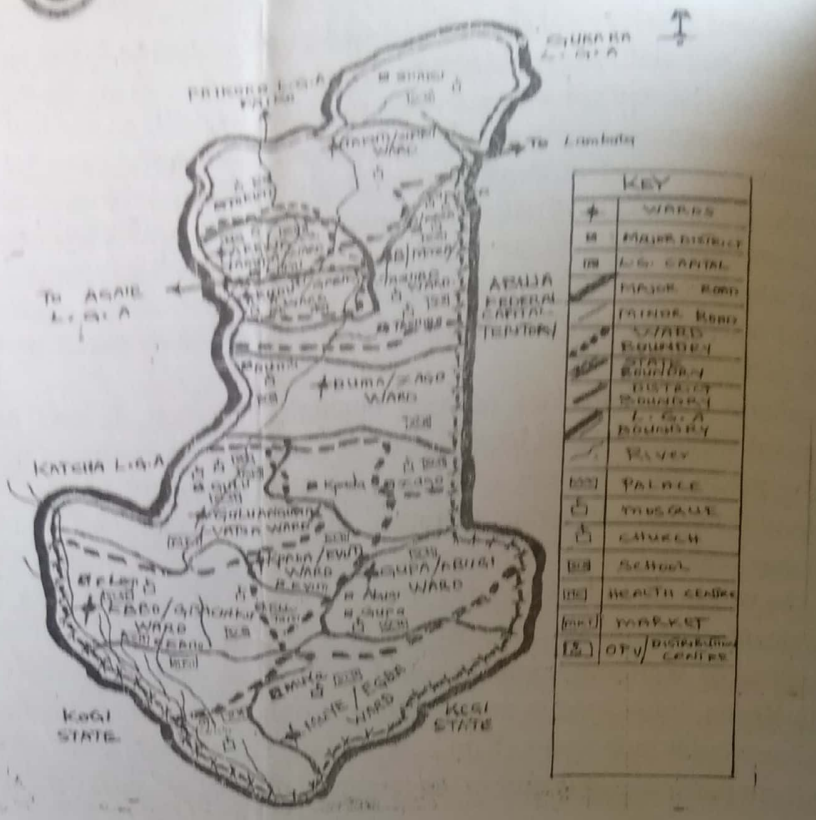

Fig 1: Political Map of Lapai Local Government Area Council in Niger State

2.2 Sample Collection

Nymphaea lotus was collected by hand into a polythene bag from Lapai dam and taken to the laboratory in Biological Sciences Department Ibrahim Badamasi University, Lapai, Niger State, Nigeria. The leaves, flowers and rhizome were removed separately and cleaned of all extraneous materials using sterile distilled water. Necrotic parts were removed and discarded and the remaining parts were rinsed thoroughly using distilled water and kept for further analysis.

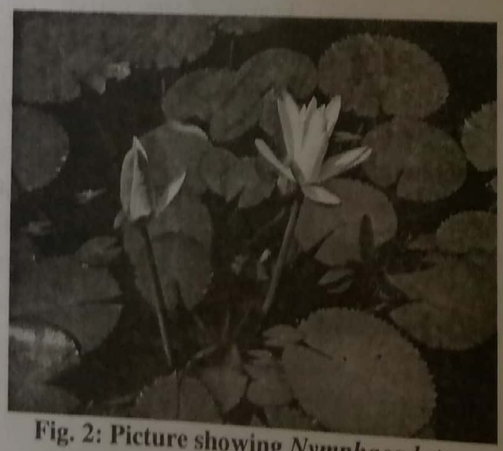

Fig. 2: Picture showing *Nymphaea lotus*

2.3. Test Organisms

Clinical strain of *Escherichia coli, Staphylococcus aureus, Salmonella typhi, Streptococcus* sp, *Proteus vulgaris and Candida albicans* were obtained from the Department of Microbiology, Federal University of Technology, Minna, Niger State.

2.4. Preparation of the Plant Extracts

The rinsed leaves, flowers and rhizomes were air dried in the laboratory at room temperature for a period of two months and then blended using a sterile electric blender, into powdered form.

Aqueous and ethanolic extraction of the plant materials was done by suspending a sample (50g) of the powdered form of each part and separately soaked each in 200ml of distilled water and 95% ethanol (200ml). The aqueous extraction was done at 70^0C for 30minutes and then it was allowed to stand for 72hours at 28^0C$\pm$ 2^0C laboratory temperature and then filtered using Whatman No.1 filter paper. The filtrate was concentrated in a vacuum at 30^0C. The extracts were transferred into sterile bottles and stored at 5^0C.

2.4.1. Media Preparation

The agars used in all the analysis were prepared according to manufacturer standard specification. For nutrient agar 28g weight was dissolved in 1litre of distilled water and sterilized at 121^0C for 15minutes, while Nutrient broth was also prepared; 12.5g weight in 500ml distilled water, the solution was caped with aluminum foil at 121^0C for 15minutes.

2.4.2. Phytochemical Analysis

The preliminary phytochemical analysis of the plant extracts was performed to analyze for the presence of bioactive components present in the leaf, flower and rhizome with modifications on the methods described by (Evans, 1989; Sofowora, 1993).

2.4.3. Analysis for Tannins

1ml of freshly prepared 10% KOH was added to 1ml of the extracts in different test tubes. A dirty white precipitate indicates the presence of tannins. The presence of tannins was reconfirmed by putting the powdered plant leaf, flower and rhizome (1.0g) into different beakers and 10ml of distilled water was added to each beaker. Two drops of 5% $FeCl_3$ was added and the production of a greenish precipitate was an indication of the presence of tannins.

2.4.4. Analysis for Alkaloids

The extract samples (0.5ml) each were stirred with 1% HCl on a steam bath. The solutions obtained were filtered and 1ml of the filtrate was treated with two drops of Mayer's reagent. The two solutions (of the plant parts) were mixed and made up to 100ml with distilled water. Turbidity of the extract filtrates on addition of Mayer's reagent was regarded as evidence for the presence of alkaloids in the extracts.

Also a few drops of freshly prepared Dangendorff's reagent was added to 0.5ml of the plant extracts in the test tubes and a brown colored solution was observed. A few drops of freshly prepared Picric reagent was added to 0.5ml of the plant extracts in the test tubes and a colored brown solution was observed, showing the presence of alkaloids.

2.4.5. Analysis for Saponins

0.5ml of plant extracts was introduced into different test tubes containing 5.0ml of distilled water, the mixture was then vigorously shaken for 2minutes, and formation of froth indicates the presence of saponins.

2.4.6. Analysis for Flavonoids

Four pieces of magnesium fillings were added to ethanolic extracts of the plant materials, this is followed by a drop wise addition of concentrated hydrochloric acid. Color varying from orange to red indicated flavones, red to crimson indicated flavonoid and crimson to magenta indicates flavonones.

2.4.7. Analysis for Terpenes

0.5ml of the plant extracts, 3.0ml chloroform was added and filtered, 10 drops of acetic anhydride and 2 drops of H_2SO_4 were then added to the filtrates and the color change from blue to green observed indicate the presence of terpenes.

2.4.8. Analysis for Glycosides

0.5ml of each plant extract was dissolved in 2ml of acetic anhydride and cooled in ice bath. Concentrated H_2SO_4 was carefully drop by drop. A color change from violet to blue to green indicated the presence of glycoside. Also 0.5g of each of the plant extract was dissolved in 2ml of chloroform each. Concentrated H_2SO_4 was carefully added drop by drop to form a lower layer. A reddish-brown color at the interface indicates the presence of glycoside.

2.4.9. Analysis for Anthraquinones

0.5ml of each of the plant extract was shaken with 10ml of benzene and filtered and 5ml of 10% ammonia was added to the filtrates. The mixture was shaken and presence of pink, red or violet color indicates the presence of anthraquinones.

2.5. Antimicrobial Susceptibility Testing

This was done using the agar diffusion method of Boakye-yiadon (1979) and Chessbrough (2004), where 15ml of the nutrient broth (oxoid) and yeast broth were turned into sterile petri-dishes and allowed to solidify. The test organisms were separately inoculated using sterile wire loop on solidified nutrient agar plates for bacterial and yeast extract agar plates for fungi and spread uniformly using spread plate method. Holes of 4mm and 8mm diameter were punctured on the agar medium using a sterile cork-borer and cut agar disc were aseptically and carefully removed with sterile forceps. A sterile Pasteur pipette was used to introduce 0.5ml and 0.2ml concentrations of the plant extract into 8mm and 4mm wells or holes bored on the surface of the agar medium containing the cultures. Control experiments were also set. The plates were allowed to stand for one hour at room temperature to allow for diffusion of the substrates to proceed before the growth of the organisms commenced. The plates were finally incubated at 37^0C for 24hours for bacterial and at room temperature for fungi. The presence of zone of inhibition around the hole containing the extracts indicates the antimicrobial activity against the test organisms. Antimicrobial activity was expressed in terms of diameter of zones of inhibition (mm).

3.0. Results

The result of the preliminary phytochemical analysis of the extract showed the concentrations of bioactive compounds in the ethanolic crude extracts of *Nymphaea lotus* leaves, flowers and rhizomes (Tables 1-3). The following bioactive compounds were identified from the ethanolic crude extracts of *Nymphaea lotus* leaves, tannins, flavonoids, alkaloids, anthraquinones, saponins, terpenes and glycosides (Table 1), from the crude extract of the flowers the following were present flavonoids, alkaloids, anthraquinones, saponins, terpenes and glycosides (Table 2) while table 3 show the crude extract of the rhizome tannins, flavonoids, alkaloids, anthraquinones, saponins, terpenes and glycosides compounds present.

In *Nymphaea lotus* leaves (Table 1), anthraquinones, terpenes and glycosides were the most prominent bioactive compounds in the extract; in the *lotus* flowers extract (Table 2), anthraquinones and glycoside were the most prominent bioactive compounds; in the rhizomes (Table 3), anthraquinones and glycoside were the most prominent bioactive compounds in the extract.

Also in *Nymphaea lotus* leaves extract alkaloids (using Dangredorff's reagent test), and flavonoids were present in moderately high concentrations. In the flowers extract, flavonoids, saponins and glycosides (using Salkowki's test) were present in moderately high concentrations; while in the rhizome's extract only flavonoids were present in moderately high concentrations (tables 1- 3).Alkaloids (using Mayer's reagent and picric acid test), saponins and tannins were present in small concentrations in the crude extract of the leaves (Table 1), in the crude extract of the flowers alkaloids and terpenes were present in small concentrations (Table

2) and in the crude extract of the rhizome alkaloids, saponins, tannins and terpenes were present in small concentrations (Table 3).

Furthermore in the *Nymphaea lotus* leaves, no compound was absent, tannins and alkaloids (using picric acid test) were absent in the flowers (Table 2), while in the rhizome alkaloids was absent (using picric acid test) (Table 3).The antimicrobial activity carried out on the tested medicinal plant parts are summarized in Table 4-6. It is interesting to note that the extract of *Nymphaea lotus* revealed an antimicrobial activity against *Staphylococcus aureus*, *Candida albicans* and *Streptococcus species*, the flower extract exhibited antimicrobial activity against *Candida albicans* while the rhizome exhibited no antimicrobial activity.

Table 1: Phytochemical analysis of the ethanol extracts of *Nymphaea lotus* leaves from Lapai Reservoir.

Plant Constituents	Tests Used	Occurrence
Alkaloids	Drangendorff's test	++
	Mayer's Reagent test	+
	Picric Acid test	+
Flavonoids	General test	++
Saponins	General test	+
Tannins	General test	+
Anthraquinones	General test	+++
Glycosides	Liberman's test	+++
	Salkowski's test	+++
Terpenes	Chloroform test	+++

Keys: +=Present in small concentrations ++=Present in moderately high conc +++=Present in high conc.

Table 2: Phytochemical analysis of the ethanol extracts *Nymphaea lotus* flowers from Lapai Reservoir

Plant Constituents	Tests Used	Occurrence
Alkaloids	Drangendorff's test	+
	Mayer's Reagent test	+
	Picric Acid test	–
Flavonoids	General test	++
Saponins	General test	++
Tannins	General test	–
Anthraquinones	General test	+++
Glycosides	Liberman's test	+++
	Salkowski's test	++
Terpenes	Chloroform test	+

Keys: +=Present in small concentrations ++=Present in moderately high conc +++=Present in high conc

Table 3: Phytochemical analysis of the ethanol extracts *Nymphaea lotus* rhizomes

Plant Constituents	Tests Used	Occurrence
Alkaloids	Drangendorff's test	+
	Mayer's Reagent test	+
	Picric Acid test	–
Flavonoids	General test	++
Saponins	General test	+
Tannins	General test	+
Anthraquinones	General test	+++
Glycosides	Liberman's test	+++
	Salkowski's test	++
Terpenes	Chloroform test	+

Keys: +=Present in small concentrations ++=Present in moderately high conc +++=Present in high conc

Table 4: Susceptibility Patterns of Extracts of *Nymphaea lotus* leaves on Test Organisms

Test organisms	Ethanolic extracts Average Zone of Inhibition(mm)	Aqueous extracts
Staphylococcus aureus	17.0	R
Candida albicans	16.5	R
Streptococcus species	18.5	R
Escherichia coli	R	R
Salmonella typhi	R	R
Proteus vulgaris	R	R

Key: R= resistance

Table 5: Susceptibility Patterns of Extracts of *Nymphaea lotus* flowers on Test Organisms

Test organisms	Ethanolic extracts Average Zone of Inhibition(mm)	Aqueous extracts
Staphylococcus aureus	R	R
Candida albicans	17	R
Streptococcus species	R	R
Escherichia coli	R	R
Salmonella typhi	R	R
Proteus vulgaris	R	R

Key: R= resistance

Table 6: Susceptibility Patterns of Extracts of *Nymphaea lotus* rhizome on Test Organisms

Test organisms	Ethanolic extracts Average Zone of Inhibition(mm)	Aqueous extracts
Staphylococcus aureus	R	R
Candida albicans	R	R
Streptococcus species	R	R
Escherichia coli	R	R
Salmonella typhi	R	R
Proteus vulgaris	R	R

Key: R= resistance

Plate 1: Zone of inhibition on *S. aurens*

Plate 2: Zone of inhibition on *Candida albicans*

4.0. Discussion

The phytochemical screening of *Nymphaea lotus* in this study revealed the presence of alkaloids, tannins, flavonoids, saponins, anthraquinones, terpenes and glycosides. The leaves extract contains all the analyzed bioactive compounds, this result therefore agrees with the findings of Akinjogunla *et al.* (2009). In the flower extract, tannins is absent and the concentrations of other compounds are moderately present compared to the leaves and the rhizome extracts which reveals a minimal concentrations of these bioactive compounds. (Sofowora, 1986, and Akinjogunla *et al.*, 2009) attributed the antimicrobial effect of plant extracts to the presence of these secondary plant herb metabolites. Hence, the presence of the secondary metabolites such as anthraquinones, glycosides, saponins, tannins, alkaloids, flavonoids and phenolics in *Nymphaea lotus* may be responsible for its potential use as antimicrobial activity. According to Ebana *et al.* (1991) and Cushnie and Lamb (2005) both alkaloids and flavonoids have antimicrobial activities. Tannins are important in herbal medicine in treating wounds and the stoppage of bleedings (Nguyi, 1988). Phytoconstituents such as saponins and phenolics compounds have been reported to inhibit bacterial growth.

The antimicrobial activity in this study showed that the ethanolic extracts of *Nymphaea lotus* leaves produced zones of inhibition on the organisms (*Staphylococcus aureus, Candida albicans and Streptococcus* sp which is in conformity with those earlier reported by Jonathan Yisa (2009), with the ethanolic extracts of the flowers produced zone of inhibition on (*Candida albicans)* greater than 14mm while the rhizome with the zone of inhibition. It has been found out that for enterobacteriacea to be regarded as sensitive to any antimicrobial agent, it must produce a zone of inhibition of greater or equal to 14mm (Barry and Thornsberry, 1985). The plant parts (leaves and flowers) under study are therefore an effective antimicrobial agent. Also the study revealed that the alcoholic extract of the plant posses antimicrobial activity against the test organisms than aqeous extract. This tends to explain greater solubility of the active ingredients in alcohol than water, where the zone of inhibition varied from one organism to another and from one plant part extract to another.

The zones of inhibition showed that the susceptibility of this organisms to leaves extract, with gram positive organisms being more susceptible to the extract than fungi and gram negative organisms, and also the zone of inhibition showed that the susceptibility of the organism to the flowers extract, with the fungi organism being susceptible to the extract than gram positive and gram negative organisms. According to Hugho (1998), the extent of the diameter of zones of inhibition is dependent on the initial population density of the organisms, the rate of diffusion of the antimicrobial agent and the growth rate of the organisms.

5.0. Conclusions

Plants contain thousands of constituents and are valuable sources of new and biologically active molecules possessing antimicrobial properties. The results obtained from the study have shown that ethanolic and aqeous extracts of *Nymphaea lotus* shows the presence of some major bioactive compounds (tannins, saponins, terpenes, anthraquinones, alkaloids, flavonoids and glycoside) which are used in pharmaceutical industries. It is also shown that the ethanolic extracts of *Nymphaea lotus* leaves and flowers produce antimicrobial activity of varying degrees on some of the test organisms used. This then means that the constituents of these plants extract could serve as a source of industrial drugs useful in the chemotherapy of some microbial infections.

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