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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL PROPERTIES OF ETHANOLIC EXTRACT OF *Nymphaea lotus* L. STEM

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ABSTRACT

Medicinal properties of Nymphaea lotus L. may be attributed to the phytochemical constituents. As such the study was aimed to determine the phytochemical constituents and the antimicrobial properties of Nymphaea lotus stem extract. In this study, the powdered stem of Nymphaea lotus was subjected to maceration (extraction) using ethanol (90%, v/v). After extraction 2.2 grams of the crude extract was obtained, which is oily and sticky in texture and brownish green in color. The phytochemical screening of the extract was carried out using standard methods with some minor modifications. The result for phytochemical screening revealed the presence of tannins, terpenoids, phenolic, saponins, steroids, flavonoids, alkaloids and anthraquinone which all may accounted to its medicinal properties. The antimicrobial activity test was evaluated qualitative through agar disc diffusion method using the following microbial isolate: Salmonella typhi, Pseudomonas aureginosa, Staphylococcus aureus, Enterococcus faecalis and Candida albicans. The results showed that Salmonella typhi and Enterococcus faecalis were highly susceptible to Nymphaea lotus stem extract with highest zone of inhibition 17 and 14 mm respectively. While Pseudomonas aureginosa were moderately susceptible to Nymphaea lotus stem extract with the highest zone of inhibition 13 mm. However, the extract showed no activity against Staphylococcus aureus and Candida albicans. Nevertheless, the result showed that the stem of Nymphaea lotus can be a promising source of naturally occurring antibiotics and hence justify the claim made by traditional medicine practitioners.

Keywords: Phytochemicals, Nymphaea lotus, maceration, antimicrobial, antibiotics, agar,

INTRODUCTION

Phytochemicals are those chemical compounds produce naturally by plant mainly for protection, but also control other essential functions of growth and reproduction (Molyneux *et al., 2007).* Phytochemicals in general senses or terms are those plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure, and pathogenic attack; and also contribute to the plant's color, aroma and flavor (Gibson *et al.,* 1998 and Mathai, 2000). These compounds are known as secondary constituents (or metabolites) and possess many biological functions or properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property (Narasinga, 2003).

Literature survey as reported by Saxena *et al.,* (2013) indicated that phenolic compounds are the most numerous and structurally diverse plant phytochemicals with 45% plant abundance. Terpenoids and steroids are the second diverse group with 27% then followed by alkaloid with 18% and lastly others with 10% plant natural abundance (Saxena *et al.,* 2013). It is well-known that plants produce these chemical compounds to protect themselves, but recent researches have shown that many phytochemicals can also protect human against diseases (Narasinga, 2003).

Nymphaea lotus L. (bado in Hausa) one of the members of Nymphaeceae family, commonly known as white water lily and it is herbaceous aquatic plant which usually grows up to about fifty centimetre in height whose leaves float or submerge in water (Abu-zaida *et al.,* 2008).

This plant is localized to both temperate and tropical regions (such as Asia, Central and Southern Europe, the Middle East, North Africa, tropical mountains in Africa and West Africa more especially in Nigeria). The plant has medicinal, social (Irvine and Trickett, 1953; Encyclopedia of Life, 2015) and also used in bioremediation of heavy metal from polluted water (Mohamed and Serag, 2003).

In traditional medicine, the whole plant aqueous decoction is used by Hausa people to treat guinea worm infection; and also used as an antitumor agent, and rheumatic pains by Yoroba people (Ogbadoyi *et al.,* 2007). Lim, (2014) also reported that the plant plays a vital role in the treatment of fever, skin diseases, cancer, abnormal heart beat, and urinary difficulties.

A study was reported in 2009 on the antimicrobial activity of Ethanolic extracts of *Nymphaea lotus* leaves against gram-positive and gram negative bacteria (Akinjogunla *et al.,* 2009). Adelakun, *et al.,* (2015) also reported the antibacterial activity of aqueous extract of *Nympheae lotus* against some gram-negative fish pathogenic bacteria. With increasing prevalence of multi-drug resistant strains of microbes (bacteria and fungi) and the recent appearance of strains with reduced susceptibility to antibiotics, currently aquatic plant sources are explored as new sources of plant based antibiotics. This study was aimed to determine the phytochemical constituents of ethanol (90%) extract of *Nymphaea lotus* stem and also its in-vitro antimicrobial properties against *Salmonella typhi*, *Pseudomonas aureginosa*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Candida albicans*.

MATERIALS AND METHODS

Sample collection and identification

Fresh plant sample of *Nymphaea lotus* (water lily) was collected from Daberan dam, Daura Local Government, Katsina State. The plant was identified and authenticated as *Nymphaea lotus* (voucher no. 353) by a taxonomist from the department of Biology, Faculty of Natural and Applied Sciences, Umaru Musa Yar'adua University Katsina (UMYUK), Katsina state.

Preparation of the sample

The stems of water lily (*N. lotus*) were sorted out from the plant and then washed under running tap water to remove dust. The selected plant part sample were then air dried until constant weight was obtained, crushed into powder using motor and pestle, and stored in polythene bag prior to use.

Extraction

Fifty grams (50g) of the *Nymphaea lotus* powdered was dissolved in 500ml of ethanol (90% v/v) in a stoppered container with regular shaking. After 72hours, the mixture was filtered using filter paper (what man No.1) into a conical flask. The filtrate was then concentrated using rotary evaporator. The concentrate (which contains remains of residual water) was cooled (dried) under fan to obtain the crude extract. After this texture and color were recorded; also the weight of the extract was measured. The percentage yield of extraction was calculated using the following equation:

$$\text{Percentage yield (\%)} = \frac{weight\ of\ the\ dry\ concentrated\ crude\ extract}{weight\ of\ the\ powdered\ sample\ used} X\ 100\%$$

Bioassay for antimicrobial properties

The antimicrobial activity was evaluated using disc diffusion method (NCCLS, 2004). Nutrient agar (NA) and Saboroud dextrose agar (SDA) were sterilized in flasks cooled to 45-50°C and then poured into sterilized Petri dishes. Sterile filter paper (What man no.1) discs of 6 mm diameter were impregnated with extract solution of graded concentrations (62.5, 125, 250 and 500mg/ml) and then placed on agar plates which had previously been inoculated with the tested microorganisms (*E. faecalis, S. aureus, S. typhi, P. aeruginosa and C. albicans*). Control experiments comprising Ciprofloxacin and Fluconazole were set up. Following the 24-hours incubation at 37°C, clear zone was observed as zone of inhibition which was measured in millimeters (mm).

Phytochemical screening

The preliminary phytochemical analysis of the plant extracts were carried out for the presence of alkaloids, tannins, saponins, terpenoids, steroids, anthraquinones, Phenolics and flavonoids using standard methods with some minor modifications as described by Shaikh and Patil, (2020).

RESULTS AND DISCUSSION

In Table1, the colour of the crude extract was brownish green, texture was oily and sticky and the weight was 2.2g. Percentage yield was calculated to be 4.4%. Basri *et al.,* (2011) reported that percentage yield determines solvent ability to extract the bioactive constituent. Even though, the amount recovered may be depending on the effective mass transfer rate from the solid.

Table 1: Physical properties of the crude extract of *N. lotus*

Weight of the extract	2.2g
Percentage yield	4.4%
Texture	Oily and sticky
Color	Brownish green

The qualitative phytochemical screening of *N. lotus* stem extract showed the presence of tannins, terpenoids, alkaloids, flavonoids, phenolics, saponins, steroids and anthraquinone (Table 2). According to a report by Lata and dubey (2010), the medicinal benefits of a plant might be attributed to the quality of the bioactive constituents.As such, presence of these bioactive constituents such as terpenoids, anthraquinones, steroids, saponins, tannins, alkaloids, flavonoids and phenolics in *N. lotus* stem extract might be responsible for its potential use as a drug against pathogenic microbes. According to Omulokoli *et al.,* (1997), Newman *et al.,* (2000) and Zakaryan *et al.,* (2017) alkaloid, tannins, saponins and flavonoids possesses antimicrobial activities. Also Saxena *et al.,* (2013) reported that terpenoids and phenolics had both antibacterial and antifungal activity.

Table 2: Result for qualitative analysis of phytochemical constituents of *N. lotus* stem extract

Phytochemical constituents	Observations
Alkaloids	+
Flavonoids	+
Terpenoids	+
Phenolic	+
Saponins	+
Tannins	+
Steroids	+
Anthraquinone	+

Key: + = present, - = absent

The results in Table 3 revealed the *in-vitro* antimicrobial activity of the extract against the microorganisms employed by disc diffusion assays (to determine the antimicrobial potentials of the extract). Standard drugs, Ciprofloxacin and Fluconazole were used as positive controls and compared with that of the extracts. The result showed that ethanol (90% v/v) extract of *Nymphaea lotus* stem was active against both *Pseudomonas aeruginosa* and *Salmonella typhi* (gram-negative bacteria); and also active against *Entrecoccus faecalis* (gram-positive bacteria). From the result the extract shows moderate activity toward *Enterococcus faecalis* and *Pseudomonas aeruginosa* with highest zone of inhibition 14 and 13 mm respectively. And this support the research work of Akinjogunla *et al.,* (2009) and Akaka *et al.,* (2020) which shows the antimicrobial activity of the *N. lotus* extract. The extract also shows a better result towards *Salmonella typhi* with highest zone of inhibition 17 mm. And this substantiates the findings of Adelakum *et al.,* (2015) who reported the antimicrobial activity of the *N. lotus* crude extracts. The extract showed no activity towards both *Staphylococcus aureus* (gram-positive bacteria) and *Candida albicans* (fungi). The antimicrobial activity of *Nymphaea lotus* ethanol (90% v/v) extract may be related to the presence of phytochemical constituents. However, specific compound(s) that attribute to the activity have not been determined in the present study.

Table 3: Antimicrobial properties of *N. lotus* extract

Microbial Isolates	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml	Control	
	Mean Zone of inhibition in mm ± Sd				**+ ve**	**- ve**
S. typhi	17±1.41	15±2.82	11±1.41	9±0.00	21±0.00[1]	06
E. faecalis	14±0.00	10±1.13	7±0.00	06	33±0.00[1]	06
S. aureus	06	06	06	06	44±0.00[1]	06
P. aeruginosa	13±1.13	10±1.41	9±0.00	06	21±0.00[1]	06
C. albicans	06	06	06	06	26±0.00[2]	06

Key: Positive control (+ve) = [1]Ciprofloxacin, [2]Fluconazole, (-ve) = negative control, mm = millimeter, M±Sd = Mean±Standard deviation

CONCLUSION

In conclusion, this study revealed eight bioactive compounds presence in the ethanol (90% v/v) extract of *N. lotus* stem and its in-vitro antimicrobial activity against *Pseudomonas aeruginosa, Salmonella typhi and Entrecoccus faecalis;* as such, it can be used to treat diseases caused by or related to these organisms. And also this study justifies some of the claims made by the traditional medicine practitioners.

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