

Antimicrobial and Phytochemical Analysis of Water Lily

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ABSTRACT

Bacteria are organisms that causes diseases that affect brain, lungs, skin and other parts of the body. Phytochemical analysis was carried out using standard methods and antibacterial activity of water lily (Leaves, Roots and Seeds) ethanolic extract was carried out using agar diffusion method. The phytochemical analysis revealed the presence of anthraquinone, alkaloids, flavonoids, glycosides, tannins and saponin in which alkaloids, flavonoids and tannins were highest in leaves, glycoside was highest in seeds while saponin was highest in roots. The highest antibacterial activity was found in seeds at 50 mg/ml, 70 mg/ml and 90 mg/ml against *Staphylococcus aureus*, *Salmonella taphi* and *Pseudomonas aeruginosa*. This study suggested the use of water lily for the treatment of various infection cause *E. coli*, *Staphylococcus aureus*, *Salmonella taphi* and *Pseudomonas aeruginosa*.

Key Words: Bactria, Phytochemical analysis, Water lily, Antibacterial activity

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I. INTRODUCTION

Water lily (Bado in Hausa) is aquatic plants growing and distributed in streams, rivers and ponds, whose leaves submerged in water (fayed and Abdel-shafey, 1985 and Fulckar, 2005). The medicinal benefits of water lily are ascribed to a large number of nutritional constituents it contains. A tea made from the roots makes a good gargle for irritation or inflammation in the mouth and throat. It is used as a lotion, helps to heal sores, soften skin and sometimes both leaves and roots are sometimes made into poultice for wounds, cut and brushes (Steven and James, 1974 and Edith, 1990). Based on findings of Alma (1974), Native American use the roots tea for cough. Hausa people use whole plant as traditional medicine, to treat guinea worm infection; and also used as an antitumor agent, and rheumatic pains by Yoroba people (Ogbadoyi et al., 2007).

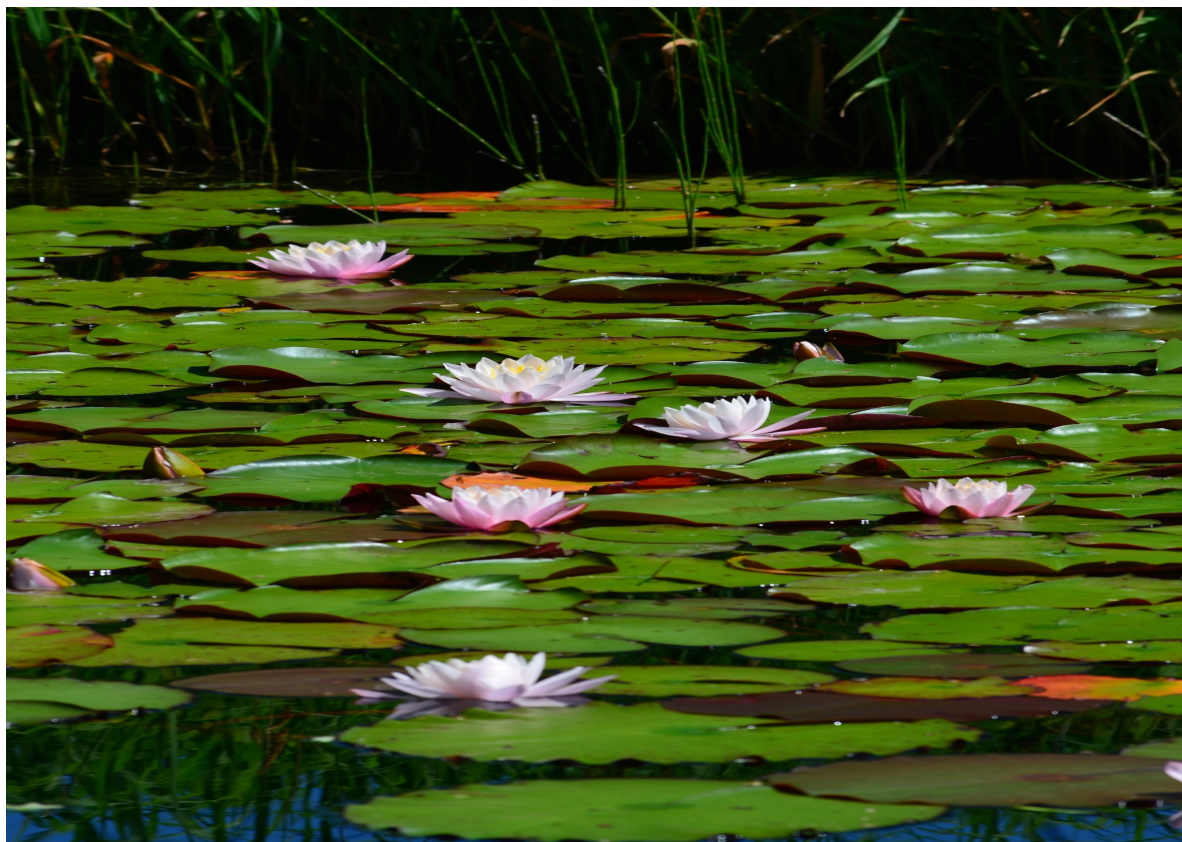
Plant materials remain an important resource to combat serious diseases in the world. The traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries. The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannin, flavonoid and phenolic compounds (Edeoga *et al.*, 2008).

Phytochemicals are chemical compounds produce naturally by plant for protection and controlling other essential functions of growth and reproduction (Molyneux et al., 2007). Phytochemicals in general senses as 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 in maintaining colour, aroma and flavour (Gibson et al., 1998 and Mathai, 2000). This is aimed to determine phytochemical constituents and antibacterial activity of water lily against *E. coli*, *Staphylococcus aureus*, *Salmonella taphi* and *Pseudomonas aeruginosa*.

II. MATERIALS AND METHODS

Sample collection

Water lily was obtained from five villages of Sabon birni Local Government, Sokoto State. The villages include Kurawa, Tsamaye, Makuwana, Yar bulutu and Alkalawa.



Preparation of the sample

The water lily parts were sorted into leaves, seeds and roots and then washed under running tap water to remove dust. The selected plant part sample were then air dried until constant weight was obtained, grounded into powder using pestle and mortar, and stored in polythene bag prior to use.

Extraction

Thirty (30) grams of the grounded powder of the plant material would be placed into 300mls of distilled water in 500ml conical flask. The mixture would be shaken at 30-minute interval, allow to stand for 72 hours. It would then be filter and evaporated to dryness in a steam bath. It would then be stored into universal sample bottles in a refrigerator for further analysis and percentage yield would be recorded (Sofowora, 1986).

Phytochemical screening

The preliminary phytochemical analysis of the plant extracts was 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).

Determination of Alkaloid

Alkaloids was determined using method as reported by (Trease and Evans, 1978).

Principle

As with most alkaloids, salts are soluble in water, while the alkaloids free bases are soluble in organic solvent. This fact is made use of in extraction of the free alkaloids with organic solvent.

Procedure

Five grams of powdered plant sample was extracted with 100 ml of methanol water (1:1 v:v) mixture and solvent evaporated. The resultant residue would mix with 20 ml of 0.0025M H₂SO₄ and partitioned with ether to removed unwanted materials. The aqueous fraction was basified with strong NH₃ solution and then extracted with excess chloroform to obtain the alkaloids fraction or separated by filtration. The chloroform extraction was repeated several times and the extract would be concentrated to dryness. The alkaloid would be weighed and the percentage was calculated with reference to initial weight of powder.

Weight of alkaloid residue x 100

Weight of sample

Where A = Absorbance of the colour at 495 nm

Determination of Flavonoids

The precipitation method was used (Bohm and Kocipai, 1994).

Excess iodine was determined by titration, rendering acidic with sodium thiosulphate standard solution

Procedure

a weighed sample five (5) gram of water lily was hydrolyzed by boiling in 100 mls of hydrochloric acid solution for about 35 minutes. The hydrolysate was filtered to recover the extract (filtrate), the filtrate was treated with ethyl acetate drop wise twice until in c0The precipitated flavonoid would be recovered by filtration using a weighed filter paper after drying in the oven 100°c for 30 minutes, it was cool in a desiccator and weighed. The difference in weighed gave the weighed of flavonoid which was expressed as a percentage of the weighed of sample analyzed.

% flavonoid

Where

eight sample

weight of filter paper

weight of filter paper sample

Determination of Tannins

Tannins was determined by the method of (Trease and Evans, 1978).

Principle

The method is based on quantitative consumption of tannins and pseudo tannins to iodine in alkaline medium, a character which is attributed to their phenolic nature. True tannins, in contrast to pseudo tannins can be removed from the extract by precipitation with gelatin. This can permit the determination of each group of constituents alone.

Procedure

Powdered sample (0.1) was put into a 100 cm³ conical flask and 50 cm³ volumetric flask. The residue would be washed several times and the combined solution made up with distilled water to 0, 1, 2, 3, 4 and 5 cm³ of the standard tannic acid and 10 cm³ of the sample solution in a 50 cm³ volumetric flask, 2.5 cm³ Folin-Denis reagent and 10 cm³ of Na₂CO₃ solution was added and made to volume with distilled water. The flask would be allowed to stand for 20 minutes after which optical density would be measure at 760 nm. The calibration curve was plotted from which the concentration of tannic acid in the sample was extrapolated.

Determination of Saponins

Saponins was determine using method of (El-Olemyl et al., 1994)

Principle

Saponins are soluble in water or boiling dilute alcohol and are precipitated on the addition of acetone.

Procedure

From powdered plant extract, five (5) grams was place in a 250 ml flask containing 30ml of 50% alcohol. The mixture would be boiled under reflux for 30minute and would immediately filter while hot through a coarse filter paper.

Two grams (2g) of charcoal was added; the content was boiled and filtered while hot. The extract was cool (some saponins may be separated) and an equal volume of acetone was added to complete the precipitation of saponins. The separated saponins was collected by decantation and dissolved in the least amount of boiling 95% alcohol and filtered while hot to remove any insoluble matter.

The filtrate was allowed to cool at room temperature thereby resulting in the precipitation of saponins. The separated saponins would be collected by decantation and suspended in about 2 ml of alcohol and filtered. The filter paper was immediately transferred to a desiccator containing anhydrous calcium chloride and the saponins would be left to dry. They was weighed with reference of extract used.

Where

eight sample

weight of filter paper

weight of filter paper sample

Determination of Glycosides

Glycosides were determine using spectrometric method (El-Olemyl et al,1994)

Procedure

One gram (1g) of the extract was extracted in 10ml of 70% alcohol and mixture was filtered. From the filtrate, eight (8 ml) of the mixture would be added to 8ml of 12.5% lead acetate (to precipitate resins, tannin and pigments). The mixture was shake well, completed to volume (100 ml) with distilled water and filtered. The filtrate (50 ml) was pipetted into another 100ml volumetric flask and 8ml of 4.7% disodium hydrogen phosphate (Na_2HPO_4) solution (to precipitate excess lead) would be added. The mixture was made up to the volume with distilled water and mixed. The mixture was filtered twice through a Whatman No. filter paper. Baljet reagent (10 ml) would be added to 10 ml of purified filtrate. A blank sample of 10 ml of distilled water was also added to 10 ml Baljet reagent. The two would be allowed to stand for one hour (time maximum for colour development). The intensity of the colour was read at 495 nm using spectrophotometer against a blank (20 ml distilled water). The colour was stable for several hours.

The percentage of total glycosides was calculated digitoxins by simply using the $E^{1\text{cm}}$ 1% of given digitoxins

The percentage

Where A = Absorbance of the colour at 495 nm

Determination of Antibacterial Activity

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 was impregnated with extract solution of graded concentrations (50, 70, and 90 mg/ml) and then placed on agar plates which had previously been inoculated with the tested microorganisms (*Staphylococcus aureus*, *Salmonella taphi* and *Pseudomonas aeruginosa*). Control experiments comprising Ciprofloxacin was set up. Following the 24-hours incubation at 37°C, clear zone was observed as zone of inhibition which was measured in millimeters (mm).

III. RESULT AND DISCUSSION

Table 1: Phytochemical Screening of Water lily Parts

Parameters	Seeds	Roots	Leaves
Anthraquinone	+	-	-
Alkaloids	++	++	+++
Flavanoids	+	++	+++
Tannins	+	+	++
Glycosides	++	+	+++
Saponins	+	++	++

Key: +: trace amount, ++: moderate amount, +++: large amount and -: not detected

Table 2: Phytochemical Composition of Water lily Parts

Parameters mg/%	Seeds	Roots	Leaves
Alkaloids	0.043±0.002 ^a	0.067±0.002 ^b	0.084±0.004 ^c
Flavanoids	0.093±0.003 ^a	0.123±0.0135 ^a	0.130±0.014 ^a
Tannins	4.28±0.024 ^a	1.58±0.0590 ^b	5.30±0.002 ^c
Glycosides	2.66±0.240 ^a	0.002±0.002 ^b	2.10±0.020 ^c
Saponin	0.23±0.004 ^b	0.038±0.003 ^a	0.032±0.002 ^a

All values are expressed as mean± standard deviation, any column with same superscript is statistically not significant (P greater than 0.5) and those with the same superscript are statically significant (P less than 0.5).

Table 3: Effect of Ethanolic Extract of Water lily Against Bacteria

Bacteria	Conc. (mg/ml)	Seeds	Roots	Leaves	Ciprofloxacin (Control)
<i>E. coli</i>	50	8.0 32±0.45	-	6.10±0.61	15.10±0.21
	70	10.86±1.20	-	7.90±0.56	20.90±0.34
	90	11.49±0.36	-	8.93±0.32	26.93±0.48
<i>Staphylococcus aureus</i>	50	10±0.0.51	-	9.30±1.20	19.0±0.61
	70	10.9±0.45	-	9.91±1.0	18.90±0.67
	90	12.5±0.71	-	12.0±2.0	19.30±0.20
<i>Salmonella taphi</i>	50	7.12±0.82	-	9.30±1.20	16.10±0.11
	70	8.90±0.53	-	9.91±1.0	20.90±0.16
	90	11.0±0.36	-	12.0±2.0	30.93±0.27
<i>Pseudomonas aeruginosa.</i>	50	12.41±1.63	-	12.00±1.63	36.10±0.61
	70	15.2±0.72	-	13.2±0.72	38.90±0.56
	90	16.91±0.31	-	16.10±0.31	45.93±0.32

Values were expressed as Mean \pm standard deviation

Phytochemical compounds such as alkaloids, flavonoids, tannins, saponins, anthraquinone and glycosides and other aromatic compounds are secondary metabolites of plants that serve a defense mechanism against predation by many microorganisms, insects and other herbivores (Bonjar *et al.*, 2004). The present study carried out on the water lily parts revealed the presence of medicinally active constituents. The phytochemical compounds of the water lily part in Table-2. Analysis of plant extracts revealed the presence of alkaloids, flavonoids, glycosides, anthraquinone, saponins, steroids and tannins which might be responsible for its antimicrobial activity. The compounds are known to act by different mechanism to exert antimicrobial action. Tannins bind to proline rich proteins and interfere with the protein synthesis (Shimada, 2006).

The antibacterial result (Table 3) shows that seeds and leaves ethanolic extract possess activity even at 50 mg/ml which might be due the presence fact that it contents phytochemical compound such as flavonoids that are hydroxylated phenolic substance known to be synthesized by plants in response to microbial infection and it should not be surprising that they have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls (Marjorie. 1999).

IV. CONCLUSION

Water lily Seeds extract shows highest antibacterial activity and reasonable concentration of phytochemical compounds. Thus, it can be use to treat various infection caused by studied *Staphylococcus aureus*, *Salmonella taphi* and *Pseudomonas aeruginosa*.

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