Phytochemical Screening and Antioxidant Activity of the Leaves Extract of *Nymphaea Lotus*

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***Abstract*:- *Nymphaea lotus*, Commonly known as white water lily (English) or Bado (Hausa), belongs to the family Nymphaeaceace. It is used in traditional medicine as an aphrodisiac, anodyne, astringent, cardiotonic, sedative, demulcent, analgesic and as anti-inflammatory agents. The present study, therefore is aimed at evaluating the chemical components of the plant as well as the antioxidant properties of its ethanolic, ethylacetate, chloroformic, petroleum ether and water extracts. The fresh leaves of *Nymphaea lotus* was collected from Bauchi LGA, Bauchi State and authenticated in the Department of Biological Sciences, Abubakar Tafawa Balewa University, Bauchi. The fine powdered sample (200g) was macerized until crude extracts was obtained. This was then concentrated in a rotary evaporator at 40°C to give a crude ethanol fraction (CF). Extraction yield was also determined (13.2 % recovery). Partition of the crude ethanol fractor was performed further with slight modifications. The preliminary phytochemical analysis of the plants extracts was performed using standard procedures. The results showed the presence of alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenes and steroids. The presence of one secondary metabolite in one solvent extract and the absence in another solvent extract might be due to difference in solvent polarity which agrees with the rule of thumb 'like dissolves like'. The antioxidants activity of the samples was determined using Reducing Power Assay. The reduction power of ethanol , petroleum ether , chloroform , ethyl acetate , and aqueous extracts of *Nymphaea lotus* leaf were expressed based on IC_{50} values and the result range from 86.60 ± 0.05 µg/cm^3 (IP = 11.57 ± 0.05) to 248.92 ± 0.43 µg/cm^3 (IP = 4.77 ± 0.55). Ethyl acetate extract has the lowest value of IC_{50} (86.60 ± 0.05 at 20 µg/cm^3) while petroleum ether has the highest value of $IC_{50.}$ (248.92 ± 0.43µg/cm^3. The lower the value of $IC_{50\ (higher}$ IP), the better the antioxidant activity. The result showed that ethyl acetate has the highest antioxidant activity (lower value of IC_{50}) followed by aqueous extracts and ethanol extract as the lowest. Although, ethyl acetate has the lowest polarity index, it has higher molecular weight compared to ethanol and water. It has been noted that the higher the molecular weight of the solvent the lower the polarity which allows other substances of about the same molecular weight to be easily extracted. However the activity of the extracts was found to be less when compared to the standard (ascorbic acid). IC_{50} value for standard was found to be 34.62 ± 9.39 µg/cm^3 at 20 µg/cm^3.**

***Keywords: Nymphaea Lotus,* Antioxidants, IC_{50}, Polarity index, Reduction potential, Ascorbic acid**

I. INTRODUCTION

Medical plants have been the chief support of traditional herbal medicine amongst rural dwellers worldwide since ancient times to date. Hippocrates (Ca.460-377 B.C), one of the ancient authors who described medicinal natural products of plant and animal origin, listed approximately 400 different plant species for medicinal purposes [1]. Natural products have been an integral part of the ancient traditional medicine system, example Chinese, Ayurvedic and Egyptian [2]. Over the years they have assumed a very central stage in modern civilization as natural sources of chemotherapy as well as amongst scientists in search for alternative sources of drugs. Various plants in different localities are known to be effective in alleviating disease condition of their users. In Africa, diseases such as fever, dysentery, cholera, diarrhea, cancer, ulcer and others which are major diseases of any tropical African country [3]. Although modern medicine may exist side-by-side with such traditional practice, herbal medicines have often maintained their popularity for historical and cultural reasons. Such products have become more widely available commercially, especially in the developed countries.

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation and reduction of molecules are essential to life, they represent normal phenomena that occur in cell metabolism. Among substances involved in oxidation-reduction reactions of molecules are free radicals, which are organic or inorganic compounds having one or more unpaired electrons on their valence shell, they are chemically unstable and very reactive [4]. In organisms, reactive oxygen species (ROS) and reactive nitrogen species are involved in metabolic processes such as energy production, regulation of cell growth, intercellular signaling, phagocytosis and synthesis of important biological molecules [4]. For many years, chemists have known that free radicals instigate oxidation, which can be controlled by a range of antioxidants substances. Antioxidants exert their activity by scavenging the free radicals, thereby giving rise to a fairly stable radical. The free radicals are metastable chemical species, which tend to trap electrons from the molecules in the immediate sorroundings. These radicals if not scavenged effectively in time, they may damage crucial bio molecules like lipids, proteins including those present in all membranes, mitochondria and the DNA resulting in abnormalities leading to diseases conditions

[1].Thus, free radicals are involved in a number of diseases including: tumor inflammation, hemorrhagic shock, diabetes, infertility, asthma, cardiovascular disorder, e.t.c, among others [1].

Description of the plant under study

Nymphaea lotus, Commonly known as white water lily (English) or Bado (Hausa), belongs to the family Nymphaea-ceace. The flowers are white, sometimes with a pink tinge. The leaves vary from green to red-brown, with a number of purple spots. The plant is native to the Nile and is grown in various parts of East Africa and Southeast Asia [5]. It is used in traditional medicine system as an aphrodisiac, anodyne, astringent, cardiotonic, sedative, demulcent, analgesic and as anti-inflammatory agent [5]. The plant produces calming and sedative effects on the nervous system, therefore, used for the treatment of insomnia, anxiety and other related disorders [5]. Many biological activities, including anticancer and antiviral, have been attributed to gallic acid and ellagic acid which are widely present in *Nymphaea lotus*[5]. Despite these medicinal uses of *Nymphaea lotus*, there is little information on its quantitative phytochemical composition and antioxidant potential.

The present study, therefore is aimed at evaluating the chemical components of the plant as well as the antioxidant properties of its ethanolic, ethylacetate, chloroform, petroleum ether and water extracts.

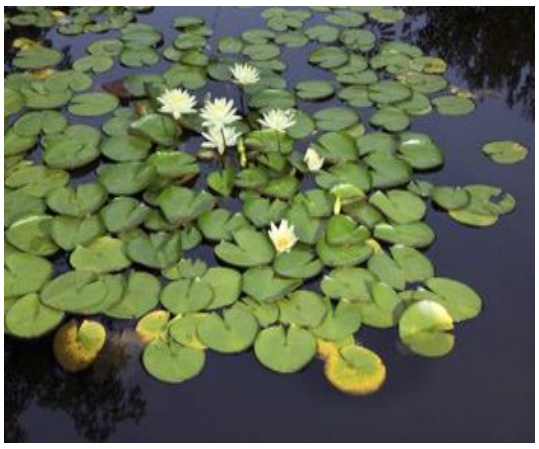

Photo show of *Nymphaea lotus* plant

II. MATERIALS AND METHOD

2.1 Materials

Rotary evaporator, Rotary shaker, UV visible Spectrophotometer, and other necessary laboratory apparatus and equipments.

2.2 Reagents and Solvents

All reagents are of analar and BDH grades

2.3 Sample Collection and Preparation

The fresh leaves of *Nymphaea lotus* was collected from Bauchi Local Government Area of BauchiState and the identification of the plant was authenticated in the Department of Biological Sciences, Abubakar Tafawa Balewa University, Bauchi. The leaves were cut into small pieces and ground to powder using a mortar and pestle. The powder was sieved with a sieve. It was kept for further analysis.

2.4 Preparation of Plant Extracts

Cold maceration method based on solvent popularity was used. The fine powdered sample (200.00 g) was macerized with 1000.00 cm^3 of 50 % ethanol for 48 hours on a ratory shaker at room temperature. The extract was then filtered using Watman N0.1 filter paper. This was then concentrated in a rotary evaporator at 40^oC to give a crude ethanol fraction (CF) the extract. Extraction yield was also determined. Partition of the crude ethanol fractor was performed further by the method of [6] with slight modifications as described by [9,7]. The dried crude ethanol fraction was dissolved in 50.00 cm^3 of distilled water and then partitioned sequentially with three portions(100.00 cm^3) each of petroleum ether, chloroform, ethyl acetate and water using separating funnel. The petroleum ether extract, chloroform extract, ethyl acetate extract and water extract were collected separately and then concentrated in a rotary evaporator at 40^oC. The various extracts were stored in a refrigerator until further use.

2.5 Phytochemical Screening

The preliminary phytochemical analysis of the plants extracts was performed using standard procedures [8,7,10,11] to detect the presences of bioactive components in the leaves of *Nymphaea lotus*.

2.5.1 Test for Phenolic Compounds

Ferric chloride test :

The extracts were dissolved in about 10.00 cm^3 of distilled water. To 2.00 cm^3 of each extract few drops of 2 % ferric chloride solution was added. Formation of a dark green color was an indication of the presence of phenolic compounds.

2.5.2 Test for Tannins

To 2.00 cm^3 of each extract 1 cm3of distilled water and 3 drops of 10 % ferric chloride solution were added. Formation of blue or green black color was an indication of the presences of tannins.

2.5.3 Test for Flavonoids

Lead acetate test :

To 1.00 cm^3 of each of the extracts few drops of lead acetate solution were added. Formation of yellow precipitate was an indication of the presences of flavonoids.

2.5.4 Test for Terpenoids

Salkowski' stest :

To 1.00 cm^3 of each of the extract, a 3.00 cm^3 of chloroform was added. The resultant solution was carefully mixed with 2.00 cm^3cconcentrated sulphuric acid. Formation of a reddish brown colour at the interface was an indication of the presence of terpenoids.

2.5.5 Test for Steroids

To 1.00 cm^3 of each of the extracts 2.00 cm^3 of each chloroform and a few drop of concentrated sulphuric acid were added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids.

2.5.6 Test for Alkaloids

To 1.00 cm^3 of the extracts few drops of concentrated Hydrochloric acid and Dragendorf's reagent were added. Formation of white precipitate indicated the presence of alkaloids.

To 2.00 cm^3 of the extracts 2.00 cm^3 of dilute hydrochloric acid was added. The resultant solutions were treated with few drops of Mayer' s reagent. Formation of a yellow colour precipitate was an indication of presence of alkaloids.

2.5.7 Test for saponins

Foam test :

A 2.00 cm^3 of extracts were shaken in the test tube for 30 seconds. Formation of foam which persisted for 10 minutes was an indication of the presence of saponins.

2.6 Preparation of Extracts

The stock solution of the extracts were prepared by weighing 87.00 mg, 40.00 mg, 50.00 mg, 52.00 mg and 37.00 mg of ethanol extract, water extract, petroleum ether extract, chloroform extract and ethyl acetate extract respectively. The various masses were dissolved in small amount of distilled water and transferred quantitatively into 100 cm^3 volumetric flask. The flasks were filled up to the mark with distilled water. This is equal to 0.87 mg/cm^3, 0.40 mg/cm^3, 0.50 mg/cm^3 and 0.37 mg/cm^3 of ethanol , water. petroleum ether , chloroform and ethyl acetate extract respectively. The stock solutions were diluted to the same concentration of 20 $\mu g/cm^3$ by measuring 1.20 cm^3 , 2.50 cm^3, 2.00 cm^3, 1.90 cm^3 and 2.70cm^3 from the stock solution of ethanol extract, water extract, petroleum ether extract, chloroform extract and ethylacetate extract respectively. The different aliquot volumes were transferred into a 50 cm^3 volumetric flasks and filled up to the mark with distilled water.

2.7 Antioxidant Activity Assay

The antioxidant property of *Nymphaea lotus* leaf extract was determined using Reducing power assay method. Substances, which have reduction potential, react with potassium ferricynide[Fe^{3+}] to form potassium ferricynide (Fe^{2+}) , which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm. This experiment was carried out as described by [12,13]. A 1 cm3 of each of the plant extracts solutions (20.00 $\mu g/cm^3$) was mixed with 2.50 cm^3 phosphate buffer (0.20 $Moldm^{-3}$, pH 6.6) and 2.50 cm^3 potassium ferricyanide $[K_3Fe(CN)_6]$ (1 g/100 cm^3) , then the mixture was incubated at 50 ^{0}C for 20 minutes. To this 2.50 cm^3 of trichloroacetic acid (10 g/100 cm^3) was added and centrifuged at 3000 rpm for 10 minutes. Finally, 2.50 cm^3 of distilled water and 0.50 cm^3 ferric chloride, $FeCl_3$ (0.10 g/100 cm^3) and the absorbance was measured at 700 nm in UV-visible spectrophotometer. Ascorbic acid was used as standard and phosphate buffer as blank solution. The absorbance of the final reaction mixture was expressed as mean ± Relative standard deviation. Increased absorbance of the reaction mixture indicated stronger reducing powder. The antioxidant activity of the leaf extract was expressed as IC_{50} and compared with the standard.

2.7.1 Assessment of % inhibition and IC_{50}

Radical Scavenging activity of the extracts and standard were expressed in terms of % inhibition. It is calculated by using the formula as follows :

$$\% I = \frac{(Acontrol - Asam)}{Acontrol} X100$$

A control = absorbance of the control and A sample = absorbance in the presence of the sample of ethanol, aqueous, petroleum ether, chloroform and ethyl acetate extracts. The IC_{50} value is defined as the concentration (in $\mu g/cm^3$) of extracts that produced 50 % antioxidant effect.

$$IC_{50} = \frac{(Concentrationofextracts)}{\%\ Inhibition} X50$$

2.8 Statistical Analysis

All data were expressed as mean ± Relative standard deviation, RSD. Statistical analysis was perform by one way ANOVA using manual method and confirmed by SPSS software version 2.0 and $P \leq 0.05$ was considered as statistically significant.

III. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Extraction

The leaves of *Nymphaea lotus* were extracted using various solvents and the nature of crude extracts recovered is presented in Table 1 below:

Table1: Nature and Recovery of the Crude Extracts of *Nymphaea lotus* leaves.

Extracts	Texture	Colour	Weight of Sample(g)	Weight of Extracts(g)	% Recovery
Ethanolic	Oily and sticky liquid	Dark green	200.00	26.40	13.20
Pet. Ether	Oily and sticky liquid	Dark brown	24.40	1.90	7.79
Chloroform	Sticky liquid	Dark green	22.50	2.40	10.60
Ethyl acetate	Sticky Liquid	Dark green	20.10	4.80	23.88
Aqueous	Sticky Liquid	Dark green	17.70	12.60	71.19

3.1.2 Phytochemical Screening

Phytochemical screening was performed on the crude extracts of *Nymphaea lotus* leaves for qualitative detection of various secondary metabolites and the results obtained are tabulated in table 2 below:

Table 2: Phytochemical screening of the solvent extracts of *Nymphaea lotus* leaves

Secondary metabolite	EE	PE	CE	EAE	AE
Alkaloids					
Dragendorff's test	–	–	–	–	–
Mayers Reagent test	+	+	+	+	+
Phenolics	+	–	–	+	+
Tannins	+	–	+	+	+
Flavonoids	+	–	–	+	+
Saponin	+	+	+	+	+
Terpenes	–	+	+	–	–
Steroids	–	+	+	–	–

KEY:+ = Detected, - = Not detected, EE = Ethanol extract, PE = Petroleum ether, CE = Chloroform extract, EAE = Ethylacetate extract, AE = Aqueous extract

3.1.3 In vitro Antioxidant assay:

The antioxidant activity of the crude extracts of *Nymphaea lotus* leaves was carried out by reducing power assay method and the results are presented in table 3 below:

Table 3: In vitro free radical scavenging effect of extracts of *Nymphaea lotus* leaves by reducing power assay.

Extracts (20 µg/cm^3)	% Inhibition (IP)	IC_{50} (µg/cm^3)
Ethanolic	9.40 ± 0.31^{bcde}	115.43 ± 0.38^{bcde}
Petroleum ether	4.77 ± 0.55^{a}	248.92 ± 0.43^{a}
Chloroform	8.09 ± 0.23^{abcde}	128.80 ± 0.26^{bcde}
Ethyl acetate	11.57 ± 0.05^{bcde}	86.60 ± 0.05^{bcde}
Aqueous	10.41 ± 0.13^{bcde}	97.27 ± 0.14^{bcde}

Key: IC_{50} = Concentration (µg/ml) of extract that produced 50% antioxidant effect.

NOTE: The results are mean ± relative standard deviation (n=3). Values on the same column with the same superscript letters are significantly the same at $p \leqslant 0.05$, while values on the same column with different superscript letters are significantly different at $p \leqslant 0.05$.

3.2 Discussion Of Results

Table 1 shows the nature and recovery of each solvent fraction. Exhaustive extraction of 200 g sample (*Nymphaea lotus* leaves) with 50 % ethanol recovered 26.40 g of extract (13.2 % recovery) which was partitioned with petroleum ether (7.79 % recovery), chloroform (10.67 % recovery), ethyl acetate (23.88 % recovery) and water (71.19 % recovery).

The result of the phytochemical screening of the crude extracts (table 2) showed the presence of active entities that elicit a major pharmacological response. The result proved the presence of alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenes and steroids. The presence of one secondary metabolite in one solvent extract and the absence in another solvent extract might be due to difference in solvent polarity which agrees with the rule of thumb 'like dissolves like".

The reducing capacity of a compound may serve as significant indicator of its potential antioxidant activity. Reducing power is the measure of the reductive ability of antioxidant and it is evaluated by the transformation of Fe^{3+} to Fe^{2+} in the presence of extract [12]. The reduction power of ethanol , petroleum ether , chloroform , ethyl acetate , and aqueous extracts of *Nymphaea lotus* leaf were summarized in table 3. Antioxidant activity was expressed based on IC_{50} values and the result range from 86.60 ± 0.05 µg/cm^3 (IP = 11.57 ± 0.05) to 248.92 ± 0.43 µg/cm^3 (IP = 4.77 ± 0.55). Ethyl acetate extract has the lowest value of IC_{50} (86.60 ± 0.05 at 20 µg/cm^3) while petroleum ether has the highest value of IC_{50}. (248.92 ± 0.43µg/cm^3. The lower the IC_{50} (Higher IP) value, the better the antioxidant activity. On the other hand, the higher the value of IC_{50} (lower IP) the lesser the antioxidant activity. Hence among the solvent extracts, ethyl acetate extract has exhibited the best antioxidant activity. Phenols and flavanoids were found to be present in ethyl acetate , ethanol and aqueous extracts which are the molecules rich in antioxidant activity [14]. The result showed that ethyl acetate has the highest antioxidant activity (lower value of IC_{50}) followed by aqueous extracts and ethanol extract as the lowest. Although, ethyl acetate has the lowest polarity index, it has higher molecular weight compared to ethanol and water. It has been noted [15] that the higher the molecular weight of the solvent the lower the polarity which allows other substances of about the same molecular weight to be easily extracted. However the activity of the extracts was found to be less when compared to the standard (ascorbic acid). IC_{50} value for standard was found to be 34.62 ± 9.39 µg/cm^3 at 20 µg/cm^3[12].

IV. CONCLUSION

The result of this study revealed that *Nymphaea lotus* leaves are very rich in phytochemicals that are of high medicinal importance. It was also discovered that the leaves of the plant possess high antioxidant power. It has been established [15] that the presence of phenolic compounds and flavonoids are responsible for the high antioxidant activity, and since these compounds have been found to be present in the leaves of this plant, it is not surprising that the plant possesses high antioxidant properties. Phenolic compounds and flavanoids can readily donate electrons to the free radical therefore can be used as a source of natural antioxidants since the synthetic antioxidants have been linked to different adverse effects.

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