Phytochemical screening and *in-vitro* evaluation of Free Radical Scavenging Activity of *Pistia stratiotes* extracts

Adeyemi D* and Shonekan O
Department of Pharmaceutical Chemistry, Faculty of Pharmacy. University of Lagos, Nigeria.

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ABSTRACT :

Medicinal plants have been identified and used throughout human history and many of the pharmaceuticals currently available to physicians have a long history of use as herbal remedies A large number of trado-medical uses were attributed to the *Pistia stratiotes* particularly the leaves. The root was applied as emollient and diuretic, while leaves infusions have been used for treatment of dropsy, kidney afflictions, dysentery, anaemia, eczema, leprosy, piles and syphilis and when boiled in coconut oil was applied externally in chronic skin infections. Current study was aimed at investigating the phytochemicals and antioxidant activities of methanol and n-hexane extracts of the leaves of *Pistia stratiotes*. Antioxidant activity was determined by spectrophotometric at 517 nm using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical mechanism with Vitamin C as reference standard. Phytochemical analysis confirmed the presence of flavonoids, tannins, alkaloids, steroids, reducing sugars, glycosides, deoxy sugars, resins and saponins. However, anthraquinones and volatile oils were not detected, while detection of phenolic compounds in extracts was confirmed by the positive test for flavonoids. From calibration curves, linearity of absorbance and % inhibition with concentration of Vitamin C was 0.9687 and 0.9685 respectively. Also, the linearity of absorbance with concentration of methanol and n-hexane extracts was 0.9528 and 0.9263 respectively. Also the linear correlation between the % inhibition and concentrations of methanol and n-hexane extracts were 0.9554 and 0.9520 respectively. In this study, hexane shows a better antioxidant activity (32.5-53.1%) than methanol extracts (31.5-43.4%) at 20-200µg/ml. Optimum inhibition of n-hexane extract (60.3%) was lower when compared to Vitamin C (93.4 %) at 300.0µg/ml. The IC_{50} for methanol and n-hexane extracts were 203.1 and 155.7µg/ml respectively, indicating a moderate antioxidant activity when compared to Vitamin C (13.9µg/ml). The results of this study confirms that the aquatic plant contain some natural compounds that could be used as antioxidants.

Keywords: *Pistia stratiotes,* extracts, antioxidant activities, phytochemical, inhibition

INTRODUCTION:

Medicinal plants find application in medicines, pharmaceuticals, agricultural and food industry and the use in for curing disease have been documented in history of all civilizations [1, 2]. Medicinal plants serve as important source of natural products or chemical substances which is produced by living organisms either by pathways of secondary metabolism called phytochemicals. The phytochemicals are biologically active and play important roles in preventive and therapeutic medicines and also protect plants cells from environmental hazards and pathogenic attacks as well as contribute to their aroma and flavour [3]. Phytochemicals are capable of accumulating in different parts of plants including leaves, stems, fruits, flowers, seeds, barks or roots and concentration varies across different tissues and species [4].

Free radicals are molecular fragment, which contains one or more unpaired electrons in its outermost atomic or molecular orbital and are highly reactive to initiate chain reaction, thereby causing damage to molecules in the tissues by extracting electrons from them in order to attain stability [5]. The main sources of free radicals include endogenous or exogenous; endogenous sources arise from auto-oxidation or inactivation of small molecules intracellularly, while the exogenous sources may arise from exposure to smoke, anaesthetics, pesticides as well as exposure to excessive radiation [6]. The sites of free radical generation may include lysosomes, peroxisomes, plasma membrane, cytosol and mitochondria and may be formed in human during phagocytosis as well as arachidonic acid and xenobiotic metabolism [7]. Different reactive oxygen species (ROS) such as oxygen radicals, superoxide anion and hydroxyl radical can be formed in vivo and cause oxidative stress. For instance, the superoxide oxygen ($O_2\bullet^-$ one electron) can be generated by direct auto-oxidation of O_2 during mitochondrial electron transport reaction [8]. Also, O_2 can be produced enzymatically by xanthine oxidase and cytochrome P450 in the mitochondria or cytosol and may be catabolized by superoxide dismutase to produce H_2O_2, which is the most commonly produced ROS in humans [9]. Hydroxyl radical (OH^-, three electrons) can be formed by radiolysis of water and by reaction of H_2O_2 with Fe^{2+} ions (Fenton reaction). OH^- have been reported to be the

***Corresponding author:**
Adeyemi D
Department of Pharmaceutical Chemistry, Faculty of Pharmacy. University of Lagos, Nigeria.
Telephone: +2348033871465
Email: dadeyemi@unilag.edu.ng

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most reactive of the ROS and is capable of damaging cell membranes and lipoproteins by lipid peroxidation [10]. Other adverse effects of free radicals oxidative stress are inflammation, aging acceleration as well as degenerative conditions including cardiovascular diseases, atherosclerosis, cataract and rheumatoid arthritis [11]. Antioxidants are phytochemicals which help man deals with oxidative stresses often caused by free radical damage and are capable of neutralizing free radicals by accepting or donating an electron to eliminate the unpaired condition and in the process becomes a free radical which is usually much less reactive than the free radical neutralized [12]. By nature, living organisms are moderately endowed with antioxidant defence systems [13]. The scavenging of DPPH free radical is the basis of a common antioxidant assay [14] which has been successfully utilized for investigating antioxidant properties of wheat grain, bran, vegetables, conjugated linoleic acids, herbs, edible seed oils, and flours in several different solvent systems including aqueous acetone, methanol and alcohol [15]. It is a convenient method for the antioxidant assay of cysteine, glutathione, ascorbic acid, tocopherol and polyhydroxy aromatic compounds for olive oil, fruits, juices and wines [16].

Pistia stratiotes is an aquatic plant which grows in large numbers in tropical countries and subtropical region of Asia, Africa and America. It is found floating on lakes, streams as well as on stagnant water which rich is in lime The leaves, arranged in whorls, are fan-like, containing numerous soft hairs; leaf blades are spongy, velvety-hairy, sessile and waterproof, while the tiny flowers are white to pale green and are followed by small, berrylike fruit. The roots are up to 20 inches long and dangle in water. The plants usually propagate by stolon and can completely take over ponds, marshes and may choke out some native aquatic plants to disrupting the aquatic ecosystem [17]. However, Ayurvedic medicine found uses for *P. stratiotes* and a large number of medicinal properties are attributed to the plant, particularly the leaves. The leaves were pounded to paste and placed on swell and haemorrhoids. When boiled in water, leaves were used to cure coughing of blood, diabetes and in bath water to lessen problem of flatulent stomach [18]. The leaves were burned and the ash brushed over skin with mange, water from the braise plant was drunk to ease menstrual flow and for cure of dysentery and urinary problems. The leaves were also applied to boils, wounds, syphilitic lesions and skin infections, while the boiled root water was drunk to ease constipation in Malay folklore medicine [19]. Also in Gambia, the plant was used as an anodyne for eyewash. The leaves were mixed with cooked hot rice and coconut milk and eaten for cure of dysentery, while alternately, oil extract of the leaves were given to rid the intestines of worms as well as in treatment of tuberculosis, dysentery and asthma [20]. Not surprisingly, the use of water lettuce by traditional doctors worked well, most especially with skin and hair infections caused by various dermatophytes as well as fungi [21]. Apart from tradomedical uses, the plant could bioaccumulate considerable quantities of heavy metals and can therefore be used as a bio adsorbent material to remove metals derived from industrial activities [22] and hence the living plant is often sought after to clear biological waste of water treatment plants or polluted ponds as well to reconstruct wetlands and monitor water quality in rivers [23]. The current study was aimed at investigating the phytochemicals and antioxidant activities of methanolic and n-hexane extract of the leaves of *P. Stratiotes*. Antioxidant activity was determined by spectrophotometric at 517 nm using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical mechanism and ascorbic acid as standard.

The taxonomical classification is as follows:
Kingdom: Plantae
Division: Magnoliophyta
Class: Liliopsida
Order: Arales
Family: Araceae
Genus: *Pistia*
Species: *stratiotes*

EXPERIMENTALS:

Reagents and Equipments

Reagents including sterile water, methanol, ferric chloride, alpha-naphthol, sulphuric acid, benzene, n-hexane, dilute hydrochloric acid, ammonia, ethanol, glacial acetic acid, acetic anhydride, chloroform, sodium nitroprusside, Fehling's solution, Dragendorffs reagent (potassium bismuth iodide) and 99.9% DPPH were obtained from Sigma-Aldrich; ascorbic acid standard 99-101% (BDH laboratories, Poole England), Equipments include Mettler Toledo weighing balance, rotary evaporator and ultra–violet/visible spectrophotometer (Supelco Belle-fonte, PA, USA), filter paper discs and aluminium foil paper (Whatman AA, Whatman International Limited, Maidstone, U.K.). Glasswares including pyrex tubes and glass funnels were thoroughly cleaned and dried prior to use.

Collection of plant material and solvent extraction

Fresh samples of *P. stratiotes* leaves was obtained from medicinal herb sellers at Mushin and thereafter authenticated by Mr. Oyebanji, the herbarium curator of the botany department, university of Lagos and deposited in the herbarium as a voucher specimen, and voucher number LUH6531 was given to the plant. The leaves (750, 904g) in duplicate were air dried for seven days and then pulverized into powder through manual hand grinding. The powdered yield of the plant material were 620 and 800g (82.6 and 88.5%) which was each macerated separately in 2.5 L of methanol and n-hexane respectively and allowed to stand for 72 hrs and then filtered. The residue was again macerated separately in 500ml of methanol and n-hexane and allowed to stand for 24 hrs and then filtered. Each extracts were concentrated to dryness at 40^0C under a reduced pressure using rotary evaporator to give a yield of 1.291g and 1.286g for methanol and n-hexane respectively. The dried extract was then transferred into universal bottles, labelled appropriately and kept in a refrigerator.

Phytochemical analysis

Phytochemical analysis for qualitative detection of alkaloids, flavonoids, reducing sugars glycosides, tannins, saponins, anthraquinones, volatile oils, resins, deoxysugars and steroids was performed on the extract as described [24, 25].

DPPH'S Free radical scavenging activity

The free radical scavenging activity (FRSA) of methanol and hexane extracts of *P. stratiotes* was determined against

DPPH by measuring UV absorbance at 517nm employing a slightly modified method [26]. The concentrations range of extract (20-300 µg/ml) was prepared separately in methanol and n-hexane respectively. Ascorbic acid was used as reference standard and same concentrations were prepared as the test solution. A 2.0ml of each concentration were placed into test tubes and 0.5ml of 1mM DPPH solution in methanol (or n-hexane) was added and samples incubated for 15 minutes at room temperature. The UV/Visible absorbance was read at 517nm and the experiment was in triplicates, using 1mM DPPH solution in methanol (or or n-hexane) as the blank solution. A lowered absorbance of the reaction mixture indicates a FRSA, which was calculated using the formula:

DPPH scavenging effect (%) = {[AB-AA]/ [AB]} x 100

Where AB is the absorbance of blank and AA is the absorbance of tested extract and all values were expressed as mean % of triplicate measurement ± SD.

RESULT :

Table 1: Phytochemical analysis of the extracts of *Pistia stratiotes*

S/N	Secondary metabolites	methanolic extracts of *Pistia stratiotes*	n-hexane extracts of *Pistia stratiotes*
1	Flavonoid	+	+++
2	Hydrolysable tannins	+	+++
3	Condensed tannins	+	+++
4	Alkaloid	++	+++
5.	Glycosides	++	
6	Reducing sugar	+	++
7	Anthraquinones	-	-
8	Volatile oil	-	-
9	Resins	+	-
10	Saponin	++	--
11	Keller-killiani test for deoxy sugar	+	+
12	Lieberman's & Salkowski test for steroidal nucleus	+	+++

Table 2: Free radical scavenging activity of *Pistia stratiotes* **extracts compared with Vitamin C standards**

Concentration (µg/ml)	Mean absorbance			Percent inhibition		
	P. stratiotes (methanol extracts)	*P. stratiotes (n-hexane extracts)*	Vitamin C standard	*P. stratiotes (methanol extracts)*	*P. stratiotes (n-hexane extracts)*	Vitamin C
20	0.425	0.418	0.292	31.5	32.5	52.9
40	0.406	0.394	0.281	34.5	36.5	54.7
60	0.404	0.374	0.257	34.8	39.7	58.6
80	0.403	0.344	0.238	35.0	44.5	61.6
100	0.393	0.321	0.176	36.6	48.2	71.6
120	0.391	0.315	0.155	37.0	49.2	75.0
140	0.384	0.312	0.131	38.1	49.7	78.9
160	0.369	0.307	0.125	40.5	50.5	79.8
180	0.357	0.305	0.117	42.4	50.8	81.1
200	0.351	0.291	0.087	43.4	53.1	86.0
220	0.258	0.273	0.068	58.4	60.5	89.0
240	0.246	0.245	0.053	60.3	60.5	91.5
260	0.246	0.246	0.046	60.3	60.3	92.6
280	0.247	0.247	0.043	60.2	60.2	93.1
300	0.247	0.246	0.041	60.2	60.3	93.4

Absorbance of DPPH blank (20 µg/ml) = 0.620.

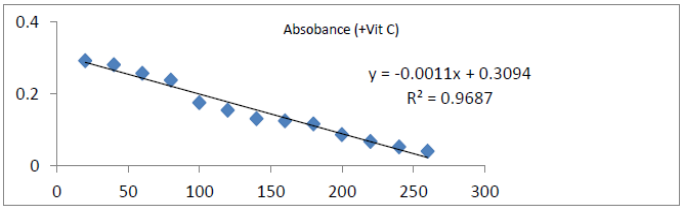

Figure 1: Calibration curve of absorbance versus concentration (µg/ml) for Vitamin C standards.

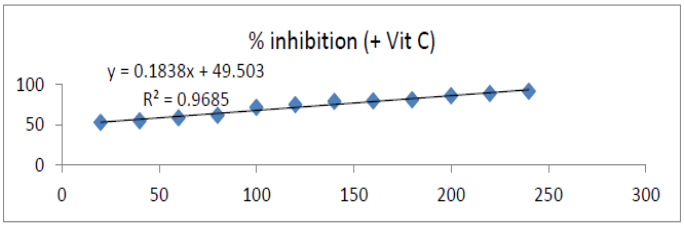

Figure 2: Calibration curve of % inhibition versus concentration (µg/ml) for Vitamin C standards

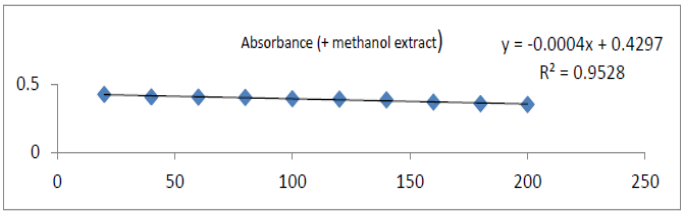

Figure 3: Calibration curve of absorbance versus concentration (µg/ml) for methanolic extract of *Pistia stratiotes*

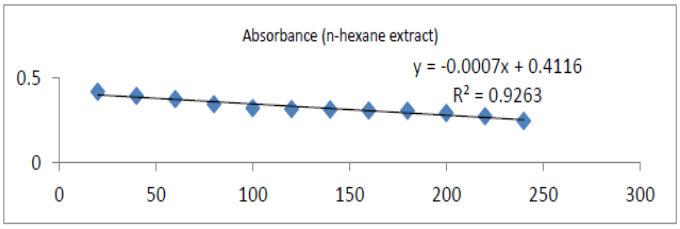

Figure 4: Calibration curve of absorbance versus concentration (µg/ml) for n-hexane extract of *Pistia stratiotes*

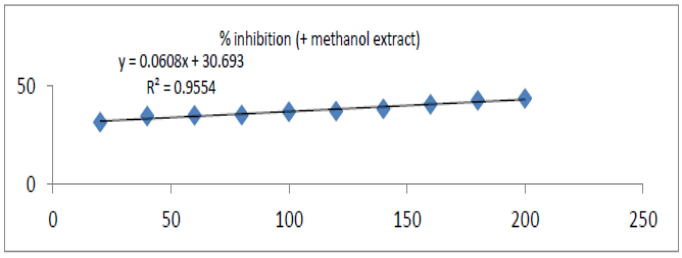

Figure 5: Calibration curve of % inhibition versus concentration (µg/ml) for methanol extract of *Pistia stratiotes*

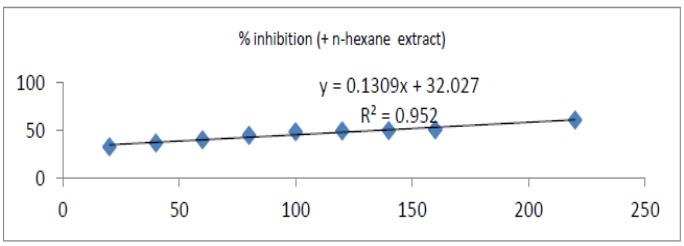

Figure 6: Calibration curve of % inhibition versus concentration (µg/ml) for n-hexane extract of *Pistia stratiotes*

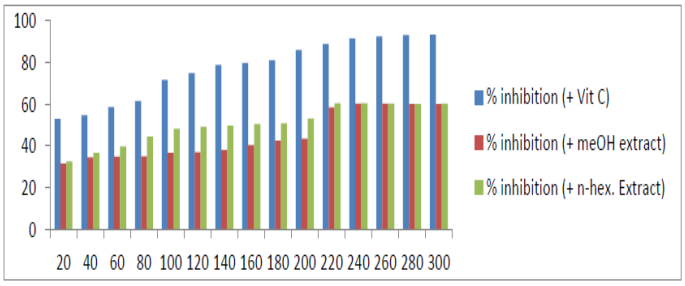

Figure 7: Graphical illustration of % inhibition of extracts of *P. stratiotes* in comparison with Vitamin C at various concentrations (µg/ml)

DISCUSSION:

As illustrated in Table 1, a preliminary phytochemical analysis of methanol and n-hexane extracts confirmed the presence of flavonoids, tannins, alkaloids, steroids, reducing sugars, deoxy sugars, glycosides, resins and saponins. However, anthraquinones and volatile oils were not detected in both extracts while the detection of phenolic compounds in both extracts was confirmed by the positive test for flavonoids. The DPPH free radical scavenging activity (FRSA) of both methanol and hexane extracts of *P. stratiotes* with corresponding absorbance is shown in Table 2 and values were compared with those of Vitamin C standard. The principle of antioxidant activity of the extracts towards DPPH radical employed in this study have been used extensively as a pre-screening method for new antioxidants from natural resources due to its stability, simplicity, rapidity and reproducibility [27]. The DPPH radical can be neutralized by either direct reduction via single electron transfer or by radical quenching via hydrogen atom transfer and upon reduction, the color of the solution fades from purple to yellow and the progress of the reaction is conveniently monitored by a UV-Visible spectrophotometer [28]. From the calibration curves (Figure 1 and 2), the linearity of absorbance and % inhibition with concentration of Vitamin C was 0.9687 and 0.9685 respectively. Also, the linearity of absorbance with concentration of methanol (Figure 3) and n-hexane (Figure 4) extracts was 0.9528 and 0.9263 respectively. Also the linear correlation between the % inhibition and concentrations of methanol (Figure 5) and n-hexane (Figure 6) extracts were 0.9554 and 0.9520 respectively. In this study, the hexane shows a better antioxidant activity (32.5-53.1%) than methanol extracts (31.5-43.4%) at relatively low concentrations (20-200µg/ml) and there was a linear correlation between % inhibition and concentrations of the extracts. The optimum inhibition of the n-hexane extract (60.3%) was however lower when compared to ascorbic acid (93.4 %) at 300.0µg/ml. The IC_{50} value as determined for vitamin C, methanol and n-hexane extracts were 203.1 and 155.7µg/ml respectively indicating moderate antioxidant activities when compared to vitamin C (13.9µg/ml). The increasing order of antioxidant activity include vitamin C > n-hexane > methanol (Figure 7). The antioxidant activities can be attributed to detection of flavonoids which is relatively higher in n-hexane compared to methanol extracts. Flavonoids are polyphenolic compounds and have been widely reported for vasoprotective, anti-inflammatory as well as antioxidants properties. The antioxidants plays significant role in

maintaining integrity of the cell membrane by prevention of lipid peroxidation and DNA damage caused by a cascade of free radical reaction [29]. It has been reported that more lipophillic flavonoids and phenolic derivatives may also disrupt the bacterial cell membrane [30] and inhibit bacterial growth depending on their concentrations [31]. This is probably due to their ability to form complex with soluble extracellular proteins and bacterial cell wall [32, 33]. Future studies should aim at investigating the pharmacological activities as well as fractionation and isolation of bioactive compounds in the plant extracts which may probably lead to discovery of new medicinal compounds.

CONCLUSION:

The tested plant extracts contains major secondary metabolites including flavonoids, tannins, alkaloids, steroids, reducing sugars, deoxy sugars, glycosides, resins and saponins. Both extracts shows a moderate antioxidant activity at investigated concentrations confirming that the aquatic plant extracts contain some natural compounds that could be used as antioxidants.

RECOMMENDATION:

The phytochemicals and antioxidant activity of *P. stratiotes* should be extensively studied to improve the understanding of the effects of tradomedical uses of the plant. The extracts should be purified, fractionated and isolates identified and tested for biological activities in search of new medicinal compounds.

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