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Phytochemical , Antioxidant and Antidiabetic Activities of *Pistia stratiotes* L. Assam, India

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Abstract

Diabetes Mellitus is a very common metabolic disorder. The digestive enzymes α-glucosidase and α-amylase are pivotal in breaking down starch into glucose. Consequently, inhibiting the activity of these enzymes is recognized as significant approach in the management of diabetes. *Pistia stratiotes* L. arecommonly used in traditional remedies due to their various health benefits. The main objective of this study was to analyze the total phenolic content, total flavonoids content, antioxidant properties, enzyme inhibitory activity against α-glucosidase and α-amylase. The Phenol and flavonoid content was found to be highest in *P. stratiotes* leaf methanolic extract 169.15 mg GAE/100 g extract weight and 224.74 mg QE/100 g extract weight respectively. The methanolic of *P. stratiotes* leaf showed higher antioxidant potential than the *P. stratiotes* root methanolic extract according to the DPPH and $ABTS^+$ assay**.** Furthermore, methanol extract *P. stratiotes* showed significant inhibitory activity against α-amylase and α-glucosidase with IC_{50} values of IC_{50} 41.72 µg/mL IC_{50} 47.72 respectively. Based on these results, it is understood that methanolic extract is the potential source of an antioxidant as well as antidiabetic activity.

Key words : *Pistia stratiotes*, phytoconstituents, antioxidant, antidiabetic.

Medicinal plants have a wide range of uses, including their application in medicine, pharmaceuticals, the agricultural sector, and in food industries. Traditional medicine has been made from plants for many thousands of years. Ayurveda, the ancient medication system of India, has a written documentation of more than 800 herbal remedies. Charaka Samhita and Sushruta Samhita are two valuable documentation of plant based drugs knowledge wealth that are even today given esteem importance world over[23].

Diabetes mellitus stands as one of the most serious and unmanageable metabolic conditions. The rising prevalence of diabetes

can be attributed to urbanization, demographic expansion, the aging population, increasing rates of obesity and a lack of physical activity[2]. Over time, complication of diabetes can impact vital organs including the kidneys, eyes, nerves, heart and blood vessels. In the absence of effective treatment, these complications can ultimately lead to death[18]. Recent data indicates that there are approximately 415 million individuals worldwide living with diabetes mellitus, and it is projected that this number will double by the year 2040.

Reactive oxygen species (ROS) and free radicals, which includes hydrogen peroxide (H_2O_2), superoxide anion (O_2^-) and hydroxyl radical (•OH), exhibit a persistent in the human body[20]. The continual increase in free radicals, coupled with a high ratio compared to antioxidants, results in oxidative stress, a condition linked to several some chronic and low-inflammation diseases. These ailments encompass insulin resistance in type-2 diabetes, cardiovascular diseases, rheumatoid arthritis and aging disease[13]. Consequently, the body requires exogenous antioxidants, which can be obtained through functional food products, vegetables, fruits and food supplements[4]. An antioxidant is a substance capable of diminishing the harmful effect of free radicals[17].

Pistia stratiotes L. belongs to Araceae Family[21]. It commonly known as 'Jal kumbhi', water lettuce, nile cabbage, water cabbage, and shellflower is a free floating aquatic plant of lake, ponds and streams[12]. *P. stratiotes* is a free floating aquatic plant with sessile leaves forming a rosette, leaves are pale green in color and lower surface is coverd with whitish hair[1,5,9]. Leaves of *P. stratiotes* possess antifungal properties which explain the use of plant in folk medicine for the treatment of various diseases[16]. Ash of *P. stratiotes* is applied to scalp for curing ringworm and leaf extract is used in eczema, leprosy, ulcers, piles and syphilis. Leaf extract of *P. stratiotes* boiled in coconut oil is applied to the skin in chronic dermatitis[15]. It is also useful in the treatment of stomach disorder, throat and mouth inflammation. *P. stratiotes* extract possessed significant antioxidant activity, which would help to prevent oxidative damage and promote wound healing process[28]. *P. stratiotes* phenolic compound is present which possess properties such as antiapoptosis, antiaging, anticarcinogen, antiinflammation as well as inhibition of angiogenesis and cell proliferation activities[24]. *P. stratiotes* is used traditionally for the treatment of diabetes mellitus in Nigeria. The aqueous extract of *P. stratiotes* possesses anti-hyperglycaemic and hypolipidemic effect and ameliorated hepatic and renal alteration in alloxan-induced diabetic rats. Extract of *P. stratiotes* was shown to reduce FBG, attenuate dyslipidemia, renal and kidney complications[3,30]. Therefore, this study was to explore the antioxidant activity of methanol extract against DPPH and $ABTS^+$ and evaluate antidiabetic activity based on α-amylase and α-glucosidase from *P. stratiotes*.

Collection and preparation of voucher specimens :

The collection of live specimens from Gauhati university was done January, 2021. Voucher specimen of the collected specimens was prepared following the standard herbarium techniques[11].

Figure 1. Habitat of *P. stratiotes*

Preparation of plant extract :

Collected sample was washed and cleaned with distilled water. It was allowed to shade dry under controlled environment to prevent the sample from dust. After shade drying, 50 g of each of the parts *i.e.* leaves and roots was sequentially extracted in Soxhlet Apparatus at 20 °C for 24 hours. Extraction was done following polarity index of the solvents viz., Hexane, Ethyl Acetate, Methanol and Distilled Water. After extraction, the solvents were separated using Buchi Rotary Evaporator at 40°C under 200 mbar pressure. Whereas, after the aqueous extraction, the water was removed by using Lyodel Freeze Dryer Lyophiliser at -75°C. The extracts thus obtained were stored in Borosil screw cap glass vials in 4°C refrigerator for further analysis.

Quantitative estimation of total phenol and total flavonoids contents :

The total phenol content was determined using a protocol described by Singleton and Rossi,1965. 1ml of the extract solution under the concentration of 1 mg/mL and 2ml sodium carbonate under the concentration of 20 mg/mL. Incubated at 30 °C for 5 min. Then, 1ml Folin Ciocalteu reagent was added and incubated at 30 °C for 30 min in dark. The absorbance was measured at 760 nm in Thermo Muliskan Go Multiplate Spectrophotometer. Gallic acid was used as a standard. It

was calculated using the formula:

$$TPC=(c \times V) / m$$

Where,

TPC = total phenol content in gallic acid equivalent (mg/g plant extract)
c = concentration of gallic acid established from the calibration curve (mg/ml)
V = volume of extract (ml)
m = weight of crude plant extract (g)

The total flavonoid content was determined using a protocol described by Singleton and Rossi, 1965. The flavonoid content was determined by adding 0.1ml of the extract solution under the concentration of 1 mg/mL, 0.1 sodium nitrite solution (5%, w/v) and 0.6ml distilled water and incubated at 30 °C for 5 min. Then, 0.2ml $AlCl_3$ solution (10%, w/v) was added and incubated at 30 °C for 15 min and its absorbance was obtained at 510 nm in Thermo Muliskan Go Multiplate Spectrophotometer. Quercetin was used as the standard.It was calculated using the formula:

$$TFC=(c \times V) / m$$

Where,

TFC = total flavonoid content in quercetin equivalent (mg/g plant extract)
c = concentration of quercetin established from the calibration curve (mg/ml)
V = volume of extract (ml)
m = weight of crude plant extract (g)

Antioxidant activity :

DPPH radical scavenging assay:

DPPH radical scavenging assay was determined by using a method described by Turkoglu *et al*.,[29]. The antioxidant activity of the various extracts was determined by measuring their ability to decolorize the purple-coloured methanol solution of DPPH. In brief, 1 mL of a 0.2 mM DPPH methanol solution was added to 1 mL of various concentrations (0.2– 1.0 mg/mL) of the extracts and incubated at 25 for 30 min. Ascorbic acid was used as the standard. The absorbance was measured against blank at 517 nm using Thermo Muliskan Go Multiplate Spectrophotometer. The percentage inhibition rate (IC_{50}) on the DPPH radical was calculated using the formula:

$$\text{Percentage inhibition (\%)} = [(A_{control} - A_{extract}) / A_{control}] \times 100$$

Where,

$A_{control}$ is the absorbance of the control,
$A_{extract}$ is the absorbance of the extract.

$ABTS^+$ radical scavenging ability:

$ABTS^+$ radical scavenging ability was determined by using a standard protocol by Re *et al*.,[22]. The $ABTS^+$ was generated by reacting 7 mM ABTS aqueous solution with $K_2S_2O_8$ (2.45 mM, final concentration) in the dark for 16 h and adjusting the pH to 0.7 with ethanol. Exactly 0.2 mL of the various dilutions of extracts (0.2–1.0 mg/mL) was added to 2.0 mL $ABTS^+$ solution. Gallic acid was used as the standard. The absorbance was measured at 734 nm after 15 min using Thermo Muliskan Go Multiplate Spectrophotometer. The percentage inhibition rate (IC_{50}) on the DPPH radical was calculated usin g the formula :

$$\text{Percentage inhibition (\%)} = [(A_{control} - A_{extract}) / A_{control}] \times 100$$

Where,

$A_{control}$ is the absorbance of the control,
$A_{extract}$ is the absorbance of the extract.

Antidiabetic activity :

In-vitro α-amylase inhibitory activity *:*

α-amylase inhibitory activity was determined by using a protocol described byYonemoto, 2014. 0.1ml of plant extract solution (0.01–0.1 mg/mL) and 0.1ml starch solution (1%, w/v; 20 mM phosphate buffered saline having pH 6.9 and containing 6 mM sodium chloride was mixed and incubated at 37 °C for 5 min. Then, 0.2ml α-amylase solution (0.5 mg/mL) was added to it and incubated at 30 °C for 15 min. The reaction was terminated by adding 0.2ml of dinitrosalicylic (DNS) acid colour reagent and immediately heated in boiling water for 10 min in water bath. After the reaction solution cools down upto room temperature, further 0.2ml distilled water was added to dilute each reaction. The absorbance was measured at 540 nm in Thermo Muliskan Go Multiplate Spectrophotometer. The inhibitory activity was calculated as:

$[\{1– (A_{sample}$ O.D. 540 ÷ $A_{control}$ O.D. 540)$\} \times$ 100%]

Acarbose was used as positive control.

In-vitro α-glucosidase inhibitory activity :

The *in-vitro* α-glucosidase inhibitory assay of the plant extracts was determined using a standard protocol with some modification kim, 2013. Briefly, 0.1ml of plant extract solution (0.01–0.1 mg/mL) and 0.2ml α-glucosidase solution (0.1 Unit/mL) was added to it and incubated at 37 °C for 15 min. Thereafter, 0.2ml p-nitrophenyl-α-D-glucopyranoside (PNPG) solution (5 mM) was mixed and incubated at 37 °C for 15 min. The reaction was terminated by adding 0.2ml of Na_2CO_3 and further diluted by adding 0.2ml distilled water to each reaction. The absorbance was measured at 405 nm in Thermo Muliskan Go Multiplate Spectrophotometer. The inhibitory activity was calculated as:

$[\{1 – (A_{sample}$ O.D. 405 ÷ $A_{control}$ O.D. 405)$\} \times$ 100%]

Acarbose was used as positive control.

The α-amylase and α-glucosidase inhibitory activities were expressed as percentage of inhibition (IC_{50}).

Statistical analysis :

The statistical analysis was analyzed taking triplicate data as mean ± standard deviation. The ANOVA was performed and the significance difference between means ($P \leq 0.05$) was evaluated by Tukey test.

Identification of the specimen :

Voucher specimen was prepared and herbarium was deposited to the Herbarium of Gauhati University (GUBH), located in the Department of Botany, Gauhati University, Assam, India. The specimen was identified as *Pistia stratiotes* (Accession number: 019665).

Quantitative determination of total phenolic content (TPC) and total flavonoid content (TFC): In the present findings revealed that among all extracts, methanolic extracts of *P. stratiotes* root and leaves had shown the highest phenolic and flavonoid contents respectively tabulated in table-1. This corroborated with the previous findings where the phenolic content of methanol extract was the highest with the value of 109.05 ppm [26]. Earlier research finding indicate that 70% ethanol extracts exhibited significantly higher levels of total polyphenolic and flavonoid contents incomparison to water extract in *P. stratiotes*[8].

Table-1. Quantitative determination of total phenolic content and total flavonoid content

SI No	Sample	Total Phenolic Content(mg GAE/gm)	Total Flavonoid Content(mg QE/gm)
1	PSLHx	53.45 ± 1.34	116.87 ± 2.13
2	PSRHx	47.67 ± 1.28	93.21 ± 3.58
3	PSLEa	67.79 ± 1.45	82.17 ± 2.69
4	PSREa	74.04 ± 1.61	102.43 ± 1.83
5	PSLMe	169.15 ± 4.36	224.74 ± 2.56
6	PSRMe	138.08 ± 3.28	191.77 ± 4.13
7	PSLAq	116.29 ± 2.67	167.28 ± 2.47
8	PSRAq	102.54 ± 3.92	147.79 ± 1.36

PSLHx = hexane extract of *P. stratiotes* leaf, PSRHx = hexane extract of *P. stratiotes* root, PSLEa = ethylacetate extract of *P. stratiotes* leaf, PSREa= ethylacetate extract of *P. stratiotes* root, PSLMe= methanolic extract of *P. stratiotes* leaf, PSRMe= methanolic extract of *P. stratiotes* root, PSLAq= aqueous extract of *P. stratiotes* leaf, PSRAq= aqueous extract of *P. stratiotes* root.

Anti-oxidant activity :

The DPPH and $ABTS^+$ free radical scavenging ability of *P. stratiotes* leaves and root methanolic extracts was determined and the result is presented in (Table-2, Figure3, 4). Ascorbic acid and Gallic acid served as the standard, and the IC_{50} value was determined to be 55.49±2.81 and 37.81 ± 0.84 µg/mL respectively. The antioxidant activity of methanol extract *P. stratiotes* leaf was also significantly higher than the antioxidant potential of *P. stratiotes* root methanolic extract according to the DPPH and $ABTS^+$ assay. Earlier research has also documented that the methanol extract exhibited the most pronounced antioxidant activity when compared to the n-hexane and ethyl acetate extract[19]. Previous study also concluded that methanolic extract of *P. stratiotes* was good source of natural antioxidants[10]. Previous study reported that according to FRAP and DPPH assay, the antioxidant activity of 70% ethanol extract was significantly higher[6-8,27].

Table-2. IC_{50} (µg/mL) of the samples against DPPH and $ABTS^+$

IC_{50} (µg/mL) of the samples against DPPH		
SI No	Sample	IC_{50} (µg/mL) DPPH
1	PSLMe	72.38 ± 2.73
2	PSRMe	115.25 ± 4.26
3	Ascorbic acid	55.49 ± 2.81
IC_{50} (µg/mL) of the samples against $ABTS^+$		
SI No	Sample	IC_{50} (µg/mL) $ABTS^+$
1	PSLMe	51.23 ± 0.93
2	PSRMe	66.49 ± 2.07
3	Gallicacid	37.81 ± 0.84

PSLMe= methanolic extract of *P. stratiotes* leaf, PSRMe= methanolic extract of *P. stratiotes* root

The values obtained are Mean ± SD; where n = 3 independent replicates; p value ≤ 0.05 were considered significant. Gallic acid and Ascorbic acid acts as the standard.

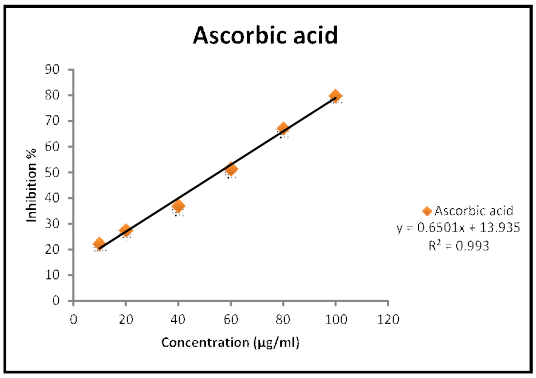

Figure 2.Graph of Concentration vs. Inhibition % of standard Ascorbic acid (DPPH)

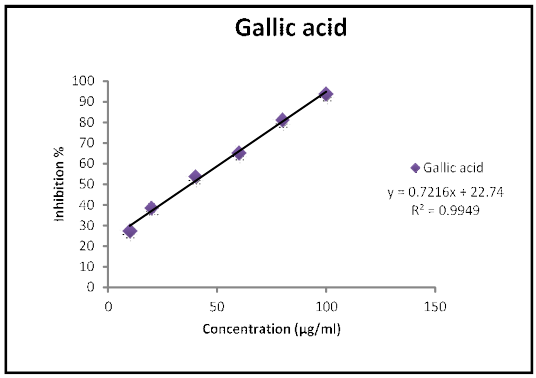

Figure 3. Graph of Concentration vs. Inhibition % of standard Gallic acid ($ABTS^{\cdot}$)

Table-3. IC_{50} (µg/mL) values showing inhibitory potentials of *P. stratiotes* extracts on their activities on α-amylase and α-glucosidase

SI No	Sample	IC_{50} (µg/mL)	
		α-amylase	α-glucosidase
1	PSLHx	88.82 ± 1.41	94.19 ± 2.32
2	PSRHx	95.08 ± 1.73	98.39 ± 1.24
3	PSLEa	48.54 ± 2.43	64.89 ± 3.32
4	PSREa	74.64 ± 3.02	87.87 ± 1.91
5	PSLMe	41.72 ± 1.12	47.72 ± 2.72
6	PSRMe	46.53 ± 2.08	52.79 ± 1.51
7	PSLAq	83.24 ±3.18	91.34 ± 1.82
8	PSRAq	79.28 ±1.45	88.23 ± 2.61
9	Acarbose	32.74 ± 1.69	43.15 ± 1.27

The values obtained are Mean±SD; where n=3 independent replicates; p value ≤ 0.05 were considered significant. Acarbose acts as the standard for α-amylase and α-glucosidase inhibitor.
PSLHx= hexane extract of *P. stratiotes* leaf, PSRHx= hexane extract of *P. stratiotes* root, PSLEa= ethylacetate extract of *P. stratiotes* leaf, PSREa= ethylacetate extract of *P. stratiotes* root, PSLMe= methanolic extract of *P. stratiotes* leaf, PSRMe= methanolic extract of *P. stratiotes* root, PSLAq= aqueous extract of *P. stratiotes* leaf, PSRAq= aqueous extract of *P. stratiotes* root.

Antidiabetic activity :

In vitro α–amylase and α–glucocidase activity of Pistia stratiotes extracts:

The half minimal inhibition concentration of the *P. stratiotes* plant parts extracts are shown below in (Table-3). Acarbose served as the positive control for both enzymes, with IC_{50} values of 32.74 ± 1.69 µg/mL and 43.15 ± 1.27 µg/mL for α-amylase and α-glucosidase respectively. *P. stratiotes* leaf methanol extract and *P. stratiotes* root methanol extract both

demonstrated strongest inhibition against the enzyme activity of α-glucosidase (IC_{50} 47.72 and 52.79 µg/mL respectively) when compared with the standard acarbose (IC_{50} 43.15 µg/mL). The methanol extracts tends to be strong dose dependant inhibitor of α-amylase and α-glucosidase. A previous study reported that the aqueous extract of *P. stratiotes* possesses anti-hyperglycaemic and hypolipidemic effect and ameliorated hepatic and renal alteration in alloxan-induced diabetic rats. Extract of *P. stratiotes* was shown to reduce FBG, attenuate dyslipidemia, renal and kidney complications[3,30].

The study found that *P. stratiotes* is a rich source of total phenol and flavonoid compounds. Methanol extract of *P. stratiotes* tends to be strong dose dependant inhibitors of α-amylase and α-glucosidase when compared to the standard Acarbose. *P. stratiotes* could easily be replace with synthetic anti-oxidant drugs. It will serve the purpose of herbal anti-oxidant rich food and easily adaptable by the human body with no toxic or side-effects. As a whole our finding support that *P. stratiotes* is a potent plant rich in active phytoconstituents, which could boon the pharmacological industry as well as human healthcare system, in management of Diabetes mellitus by using it as an alternative herbal drug in place of synthetic drugs.

Conflict of Interest

The authors declare no conflict of interest.

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