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# In vitro Evaluation of Free Radical Scavenging Activity of Pistia stratiotes

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**ABSTRACT:** Free radical stress leads to tissue injury and progression of disease conditions such as arthritis, hemorrhagic shock, atherosclerosis, diabetes, hepatic injury, aging and ischemia, reperfusion injury of many tissues, gastritis, tumor promotion, neurodegenerative diseases and carcinogenesis. The present study was aimed to investigate the antioxidant activity of methanolic extracts of *Pistia stratiotes* leaves by different methods like reductive ability, nitric oxide radical assay and superoxide scavenging activity. PSLE showed strong antioxidant activities in different systems like reducing power, superoxide anion scavenging and nitric oxide radical scavenging activities when compared with different standards such as ascorbic acid and BHT. Plant extract showed concentration- dependent scavenging activity on all reactive species used. Presumably, *Pistia stratiotes* leaves extract (PSLE) functions as an antioxidant to scavenge free radicals and reduces free radical induced cell injury.
**Key words**: *Pistia stratiotes*, Atherosclerosis, Reducing power, Superoxide anion, BHT.

## INTRODUCTION

Consumers all over the world are becoming more conscious of the nutrition value, health benefits and safety of their food and its ingredients. In addition, there is a preference for natural functional food ingredients that are believed to be safer, healthier and less subject to hazards than their artificial counterparts. Evaluation of the functional properties of naturally occurring substances, especially those that are present naturally in human diets, has been of interest in recent years[1]. Antioxidants are found in varying amounts in foods such as vegetables, fruits, grain cereals, eggs, meat, legumes and nuts. Some antioxidants such as lycopene and ascorbic acid can be destroyed by long-term storage or prolonged cooking[2,3].Other antioxidant compounds are more stable, such as the polyphenolic antioxidants in foods such as whole-wheat cereals and tea [4, 5]. Antioxidants are the substances used by the body to protect itself from damage caused by oxidation. Antioxidants that can neutralize free radicals may therefore be used to protect the human body from diseases and retard rancidity in foods consumed by humans [6].

Oxidation is essential in many living organisms for the production of energy to fuel biological processes. However, the uncontrolled production of oxygen derived free radicals is involved in the onset of many diseases such as atherosclerosis, rheumatoid arthritis, and cancer as well as in degenerative processes associated with aging[7].

Cancer chemoprevention by using antioxidants approaches has been suggested to offer a good potential in providing important fundamental benefits to public health, and is now considered by many clinicians and researchers as a key strategy for inhibiting, delaying or even reversal of the process of carcinogenesis [8].

It is believed that higher intake of antioxidant rich food is associated with decrease risk of degenerative disieases particularly cardiovascular diseases and cancer. Several research studies have demonstrated that herbal plants contain diverse classes of compounds such as steroids, polyphenols, alkaloids, tannins and carotenoids [9].With this background and abundant source of unique active components harbored in plants, the present study was taken up on the medicinal plant namely *Pistia stratiotes* belongs to the family Araceae.

The plant *Pistia stratiotes* (Araceae) is an aquatic, floating stoloniferous herb commonly found in ponds and streams. The leaves are obovate, light green in color with many prominent longitudinal veins surrounded at its base by a membranous sheath which is free-floating and spreads in the water [10]. *Pistia stratiotes* leaves are used in traditional medicine for the treatment of ringworm infection of the scalp, syphilitic eruptions, skin infections, boils, and wounds [11].

## MATERIALS AND METHODS

### Preparation of plant material

The *Pistia stratiotes* leaves were collected from Lower lake, Bhopal (M.P), India during the month of October. The specimen was identified by Botanist, Department of Botany, Faculty of Science, M.V.M College, Bhopal through comparison with the voucher specimen deposited at herbarium unit of the department. The collected plant material was dried under shade and then powdered with mechanical grinder.100 g of freshly powdered leaves were evenly packed in soxhlet apparatus and the extraction was done with 50% alcohol. Then solvent was evaporated at low temperature under reduced pressure.

### Drugs and chemicals

2,2diphenyl-1picrylhydrazylhydrate (DPPH) and nitroblue tetrazolium (NBT) were obtained from Himedia, Mumbai. 2-Deoxy 2-ribose, xanthine oxidase and hypoxanthine oxidase were obtained from Sisco research laboratory Mumbai. Ascorbic acid and potassium ferricynide were obtained from SD Fine Ltd, Baisar. All other chemicals used were obtained commercially and were of analytical grade.

### *In vitro* Antioxidant activity

### Measurement of the reductive ability [12]

The reducing power of PSLE was determined according to the method previously described by Oyaizu [12] .For the measurement of the reductive ability, the Fe3+ - Fe2+ transformation in the presence of the extract was investigated.10mg of plant extract (PSLE) in 1ml of distilled water was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid TCA (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml. 0.1%) and the absorbance was measured at 700nm. BHT was used as a reference compound. Increased absorbance of reaction mixture indicates increased reducing power.

### Scavenging of Superoxide anion radical [13,14]

The superoxide anion radical was generated *in vitro* with hypoxanthine and xanthine oxidase. A reaction mixture with a final volume of 1 ml per tube was prepared with 50 mM KH2PO4 – KOH pH 7.4 containing 1 mM EDTA. 100 µM hypoxanthine, 100 µM nitro blue tetrazolium NBT. 0.066 U per tube of xanthine oxidase diluted in 100 µl of phosphate buffer and the PSLE in 10 µl of saline. The xanthine oxidase added last. The reaction mixture incubated at 25ºC for 5 minutes and absorbance was measured at 560 nm. Decrease in the absorbance of reaction mixture indicates an increase in superoxide anion scavenging activity. The results are expressed as the percentage inhibition of NBT reduction rate with respect to the reaction mixture without PSLE (saline only). Inhibition of reaction mixture was calculated in percentage inhibition (I %) using the formula.

$I\% = 100\times \{A_o - A_t / A_o\}$

Where Ao is the absorbance of the control and At is the absorbance of the test compound.

### Nitric oxide radical scavenging effect [15,16]

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions which were measured by Griess reaction [15,16].The reaction mixture (3ml) containing sodium nitroprusside (10mM) in phosphate buffer saline and the PSLE in different concentrations (10, 25, 50, 75,100 and 125µg) were incubated at 25ºC for 150 min. Each 30 min., 0.5ml the incubated sample was removed and 0.5 ml of griess reagent (1% sulphanilamide. 0.1% napthylethylene diamine dihydrochloride in 2% $H_3PO_4$) was added. The absorbance of the chromophore formed was measured at 546nm. The percentage inhibition of nitric oxide generated was measured. Ascorbic acid was used as reference compound.

## OBSERVATIONS AND RESULTS

### Measurement of reductive ability

The reducing power of PSLE and BHT is shown in Table.1.The reducing power increased as the extract concentration increased, indicating some compounds in *Pistia stratiotes* both electron donors and could react with free radicals to convert them into more stable products and to terminate radical chain

reactions. Increased absorbance of reaction mixture indicates increased reducing power.

**Superoxide scavenging activity**

Superoxides are produce from molecular oxygen due to oxidative enzymes of body as well as via non-enzymatic reactions such as auto oxidation by catecholamines. The alcoholic extract of *Pistia stratiotes* leaves and ascorbic acid at 50 µg/ml, inhibited NBT reduction by 63.24% and 74.90% respectively. $IC_{50}$ values obtained were 26 µg/ml in PSLE extract and 28µg/ml for the standard as shown in Table 2. This shows that the extract inhibited xanthine oxidase activity.

**Nitric oxide radical scavenging effect**

Nitric oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons etc. and involved in regulation of various physiological processes. Excess concentration of (NO) is associated with several diseases. In the present study the extract competes with oxygen to react with (NO) and thus inhibits generation of anions. The % inhibition of (NO) generation by PSLE is shown in Table-3. The concentration of PSLE at100 µg/ml exhibited 48.2% inhibition whereas BHT exhibited 68.6% inhibition.

**DISCUSSION AND CONCLUSION**

Herbs have played a significant role in maintaining human health and improving the quality of life for thousands of years. They have served as valuable ingredients for seasoning, beverages, cosmetics, dyes and medicines. From the present study, it can be concluded that the methanolic extract of the leaves of *Pistia stratiotes* possesses potent antioxidant and free radical scavenging properties. Further investigation on the isolation and identification of antioxidant components in the plant may lead to chemical entities with potential for clinical use.

**Table –1. Measurement of reductive ability of PSLE.**

| Concentration (µg/ml) | Absorbance 700 nm | | |
|---|---|---|---|
| | PSLE | BHT | Blank |
| 10 | 0.450± 0.24 | 0.517±0.25 | 0.437±0.19 |
| 20 | 0.493± 0.43 | 0.592 ±0.41 | |
| 30 | 0.567 ±0.12 | 0.626 ±0.28 | |
| 40 | 0.818±0.78 | 0.763±0.35 | |
| 50 | 0.819 ±0.66 | 0.866±0.19 | |

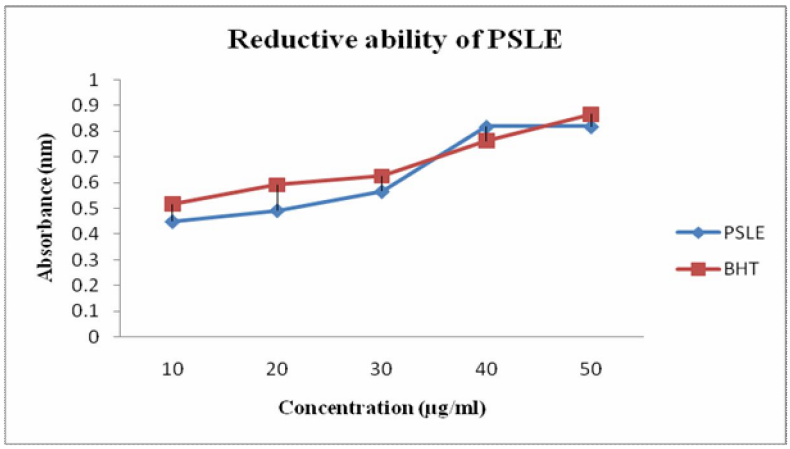
**Table -2. Scavenging of Superoxide anion radical (NBT)**

| Concentration (µg/ml) | % Antioxidant activity | |
|---|---|---|
| | PSLE | Ascorbic acid |
| 10 | 25.01± 2.44 | 31.60 ±7.43 |
| 20 | 40.03± 7.70 | 45.39 ±4.02 |
| 30 | 50.02 ±5.20 | 55.12 ±7.07 |
| 40 | 57.50 ±4.80 | 65.21 ±5.00 |
| 50 | 63.24 ±3.56 | 74.90 ±9.80 |
| $IC_{50}$ | 26 µg/ml | 28 µg/ml |

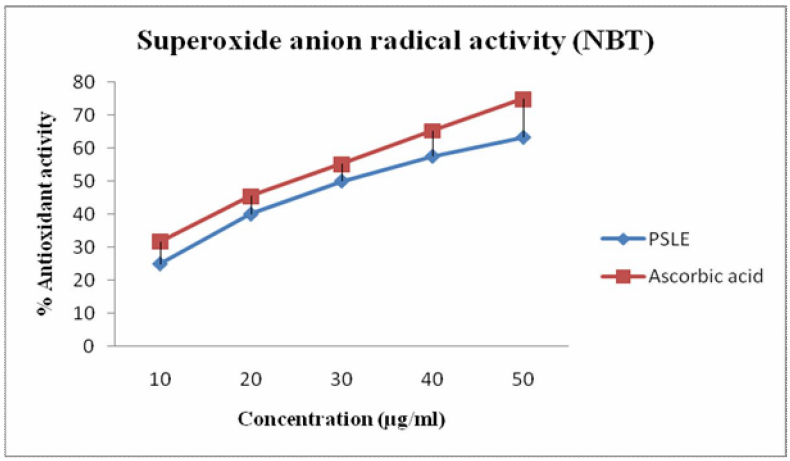
**Table -3. Nitric oxide radical scavenging effect**

| Concentration (µg/ml) | % Inhibition | |
|---|---|---|
| | PSLE | BHT |
| 10 | 26.0± 0.22 | 39.0±0.20 |
| 25 | 29.3± 0.45 | 42.0 ±0.31 |
| 50 | 35.6 ±0.11 | 54.6 ±0.29 |
| 75 | 42.1±0.80 | 60.6 ±0.25 |
| 100 | 48.2 ±0.70 | 68.6±0.20 |

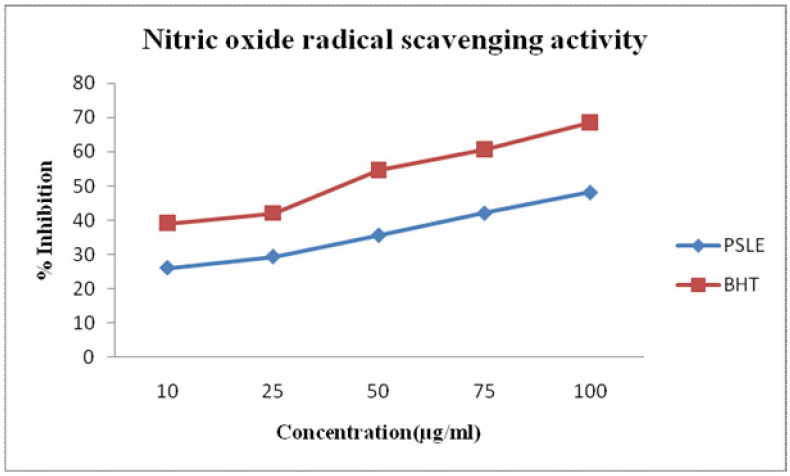
**Graph 1: Reductive ability of PSLE**




**Graph 2: Reductive ability of PSLE**




**Graph 3: Reductive ability of PSLE**

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