The Antioxidant Potential of *Salvinia molesta* D.S. Mitchell.

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ABSTRACT:
The present study was carried out to determine the antioxidant potential of *S. molesta*. The antioxidant activity was evaluated by DPPH, Hydroxyl Radical Scavenging and Total Antioxidant assays and its IC_{50} values were also determined. Two solvent extracts such as ethanol and acetic acid were chosen for the study. The results showed that the extracts had significant antioxidant activity in all the three tested assays. The ethanol extract revealed the highest antioxidant activity. DPPH assay showed better activity than other assays.

Keywords: Antioxidant, extract, free radicals, inhibition, and oxidative stress

INTRODUCTION:

Oxidative stress is the imbalance between oxidation and antioxidant reaction caused by the accumulation of free radicals in the body (Pizzino *et al.,* 2017). Cells produce free radicals through multiple metabolic pathways and free radicals are crucial factors that give rise to oxidative damage of proteins, showing high reactivity (Pieczenik and Neustadt, 2007). Reactive oxygen species (ROS) are the uppermost free radicals in cells and mainly produced by mitochondria (Al-Gubory *et al.,* 2012). Excess ROS break down cells and tissues, affect the metabolic function and cause different health problems (Liu *et al.,* 2014).

Conventionally, oxidative stress is managed using various synthetic antioxidant compounds such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG). The usage of these synthetic antioxidant compounds have been associated with undesirable effects (Ndhlala *et al.,* 2010).

Plants have high content of compounds with antioxidant properties that able to capture free radicals (Munteanu and Apetrei, 2021). The role of antioxidant is to neutralize the free radicals in biological cells. The free radicals having a negative impact on living organisms (Munteanu and Apetrei, 2021). Therefore, due to the profound consequences of oxidative stress and the drawbacks of synthetic antioxidants, the need for alternative antioxidants, which are safer, easily accessible, and potent are warranted (Mwihia, 2017).

Pteridophytes are enriched in antioxidants and have been used significantly and successfully in folk medicine for several years (Shebis *et al.,* 2013). *S. molesta* which is a free-floating fresh water plant that prefers to grow in slow-moving waters which is available in abundant can be utilized to generate novel medicinal compounds to cure emerging diseases (Nithya *et al.,* 2016). Thus, keeping in view of the health benefits of these plants, the current study was designed to evaluate the antioxidant potential of *S. molesta*.

MATERIALS AND METHODS

Collection, Identification and Preparation of extracts

The fresh samples of *S. molesta* were manually collected in bulk quantity from the pond of Poovangaparambu, Kanniyakumari district, Tamil Nadu, India and were identified by the experts. It is initially washed with pond water, followed by running tap water and finally with sterile distilled water to remove the adhering macroscopic epiphytes, animal's castings, attached debris, sand and salt particles. After rinsing, it was shade dried under the room temperature for 15 days and then powdered into fine particles using an electric mixer. The fine powdered samples were stored in an airtight container at 4°C for further use.

The dried powdered samples (10gm) were immersed in 100 ml of various solvents viz., acetic acid and ethanol separately. The extraction was carried out using agitator for 48 hrs. The plant extracts were collected and filtered through Whatmann filter paper No. 1 to separate the filtrate. Then the extracts were concentrated using a vaccum rotary evaporator at low temperature (40°C) and stored in a refrigerator for further use (Rebecca *et al.,* 2012).

DPPH Radical Scavenging Assay

The various concentrations of sample such as 12.5 µg/mL- 200 µg/mL from stock solution were made up to a final volume of 20 µl with DMSO and 1.48 ml DPPH (0.1 mM) solution was added. A control without the test compound, but an equivalent amount of distilled water was taken. The reaction mixture incubated in dark condition at room temperature for 20 minutes. After 20 minutes, the absorbance of the mixture was read at 517 nm. 3 ml of DPPH was taken as control (Chang *et al.*, 2001).

Hydroxyl Radical Scavenging Activity

Different concentration of sample such as 125-2000 µg from a stock concentration of 10 mg/mL were mixed with 500 µl reaction mixture ((2 deoxy 2 ribose (2.8 mM), FeCl3 (100 µm), EDTA (100 µm), H_2O_2 (1.0 mM), ascorbic acid (100 µm) in KH_2PO_4 - KOH buffer (20 mM pH 7.4)) was made up to a final volume of 1 ml. A control without the test compound, but an equivalent amount of distilled water was taken. After incubation for 1 hour at 37°C, added 1ml of 2.8 % TCA, then 1 ml 1 % aqueous TBA was added and the mixture was incubated at 90°C for 15 minutes to develop the colour. After cooling the absorbance was measured at 532 nm against an appropriate blank solution (Elizabeth and Rao, 1990).

Total Antioxidant Activity

The concentrations of sample such as 125 µg/mL -2000 µg/mL from a stock concentration of 10 mg/mL was obtained with 3 ml of reagent solution (0.6 ml H_2SO_4, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tube containing the reaction solutions were incubated at 95^0C for 90 minutes. The absorbance of the solution was measured at 695 nm against blank after cooling to room temperature (Methanol 0.3 ml). Ascorbic acid (10 mg/mL DMSO) was used as reference. The antioxidant activity is expressed as number of gram equivalent of ascorbic acid (Prieto *et al.*, 1999).

RESULTS AND DISCUSSION

The free radical scavenging potential of different solvent extracts (ethanol and acetic acid) were studied by DPPH, hydroxyl radical scavenging and Total antioxidant assays. A different concentration was used for the study such as 12.5, 25, 50, 100 and 200 µg/mL. All the assays showed significant antioxidant potential and the significant activity was noted in all the assay studied. The antioxidant activity of *S. molesta* was also previously reported by various scientist (Nithya *et al.*, 2016; Ace *et al.*, 2020).

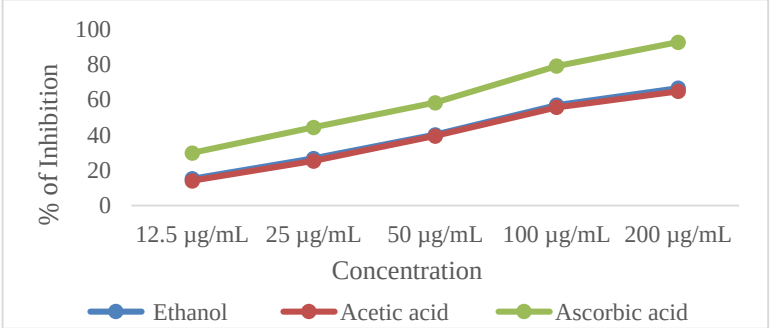
In all the assays studied dose dependency in its inhibition is recorded where, higher the concentration more was the percentage of inhibition. The dose-response curve for the free radical scavenging activity at different concentrations are presented in Fig. 1.

The DPPH method in testing antioxidant activity was an easy and fast procedure for evaluating the activity of capturing free radicals from non-enzymatic antioxidants (Ace *et al.*, 2020). The ability of each concentration of extract to scavenge DPPH radical or represented as percentage inhibition. Maximum inhibition of 66.46 ± 1.35 % was observed in ethanol extract at 200 µg/mL. The acetic acid showed maximum inhibition of 64.61 ± 0.66 % at 200 µg/mL. Ascorbic acid was used as a reference standard and it showed a value of 92.46 ± 0.30 % at 200 µg/mL. The (200 µg/mL) concentration of extracts showed the highest scavenging effects. Whereas the lowest concentration of the extract showed least scavenging activity. Zhou *et al.*, 2020 also noticed the scavenging activity that was found to be strongly dependent on concentration. The IC_{50} value for DPPH assay in ethanol extract was found to be 80.62 µg/mL. The IC_{50} value of acetic acid was 83.1713 µg/mL. The IC_{50} value of ethanol showed significant result. Ace *et al.*, (2020) also proved that ethanol fraction has a very high antioxidant activity in *Eleocharis dulcis* and *S. molesta* (Table: 1; Fig: 1).

Table:1 DPPH assay showing percentage of inhibition

Concentration µg/mL	Percentage of inhibition		
	Ethanol	Acetic acid	Standard Ascorbic acid
12.5	15.12 ± 0.05	13.98 ± 0.65	29.71 ± 0.79
25	26.63 ± 0.19	25.21 ± 0.28	44.29 ± 0.38
50	40.08 ± 0.98	39.32 ± 0.71	58.24 ± 0.85
100	56.82 ± 0.45	55.53 ± 0.82	78.95 ± 0.09
200	66.46 ± 1.35	64.61 ± 0.66	92.46 ±0.30

Figure:1 DPPH Scavenging activity in two different solvent extracts



Hydroxyl radical scavenging assay was tested at concentrations 125, 250, 500, 1000 and 2000 µg/mL. The percentage inhibition by ethanol extract was 57.73 ± 0.26 in 2000 µg/mL. In acetic acid extract the percentage inhibition was found to be 56.98 ± 1.65 in 2000 µg/mL. Gallic acid is used as a control. The control showed 92.38 ± 0.10 percentage inhibition in 2000 µg/mL. Hydroxyl is a very reactive free radical which can react with almost all biological macromolecules and is also supposed to be a highly potent contributor of oxidative stress -mediated tissue injuries (Gulcin *et al.,* 2003; Valko *et al.,* 2007; Ye and Huang, 2012). These radicals may undergo decomposition reactions to generate single oxygen and hydroxyl radicals because of which oxidative stress associated cellular damage, lipid peroxidation and diseases such as arthritis and Alzheimer's disease may get triggered (Liu *et al.,* 2010). The IC_{50} value of the ethanol extract was 1382.26 µg/mL. The acetic acid extract showed IC_{50} value of 1426.13 µg/mL. The hydroxyl radical scavenging activity was better in ethanol extract (Table:2; Fig: 2).

The Total antioxidant activity was also detected in different concentration. The maximum percentage inhibition recorded in ethanol extract with a value of 57.93 ± 0.21 % at 2000 µg/mL concentration. The acetic acid showed maximum percentage inhibition of 55.46 ± 0.28 % at 2000 µg/mL. Ascorbic acid a standard antioxidant drug that showed percentage inhibition of 93.94 ± 0.48 % at 2000 µg/mL concentration. Total antioxidant capacity is widely used as a parameter of food, pharmaceutical and medicinal extracts (Gulcin *et al.,* 2010). The IC_{50} value was also determined for the solvent extracts and it showed a significant value. The IC_{50} value of ethanol extract was found to be 1389.5 µg/mL. The IC_{50} value of acetic acid extract was 1525.76 µg/mL (Table: 2; Fig: 3). Ethanol was proved to significant activity than acetic acid extract. In the previous studies the ethanol extract at a concentration of 1.000 ppm from the *S.molesta* extract proved to have a highest absorbance value compared to n-hexane and ethyl acetate solvents, indicating that the greater the absorbance value, the higher the reducing power by antioxidant compounds found in the plant (Ace *et al.,* 2020) (Table: 2; Fig:3).

The IC50 value showed better results in DPPH assay. Tyagi and Agarwal (2017) also showed a better antioxidant activity in DPPH assay (Table: 3; Fig: 4).
The result of this study revealed that *S. molesta* possess high antioxidant activity in the ethanol extract and it can be used as a source of natural antioxidants.

Table: 2 Hydroxyl radical scavenging assay and Total antioxidant activity showing percentage of inhibition

Concentration µg/mL	Percentage of inhibition					
	Hydroxyl Radical scavenging Assay			Total Antioxidant Activity		
	Ethanol	Acetic acid	Standard Gallic acid	Ethanol	Acetic acid	Standard Ascorbic acid
125	12.91 ± 0.49	12.69 ± 0.27	29.46 ± 0.01	14.72 ± 0.43	12.21 ± 0.53	23.80 ± 0.96
250	25.74 ± 0.39	25.02 ± 0.38	51.43 ± 0.45	29.35 ± 0.39	23.01 ± 0.36	48.85 ± 0.78
500	40.40 ± 0.12	38.51 ± 0.95	64.18 ± 0.86	36.69 ± 1.62	35.41 ± 0.27	63.44 ± 0.99
1000	49.45 ± 0.50	48.54 ± 0.22	78.99 ± 0.33	47.76 ± 0.49	45.67 ± 0.42	75.13 ± 0.39
2000	57.73 ± 0.26	56.98 ± 1.65	92.38 ± 0.10	57.93 ± 0.21	55.46 ± 0.28	93.94 ± 0.48

Figure: 2 Hydroxyl Radical Scavenging activity in two different solvent extracts

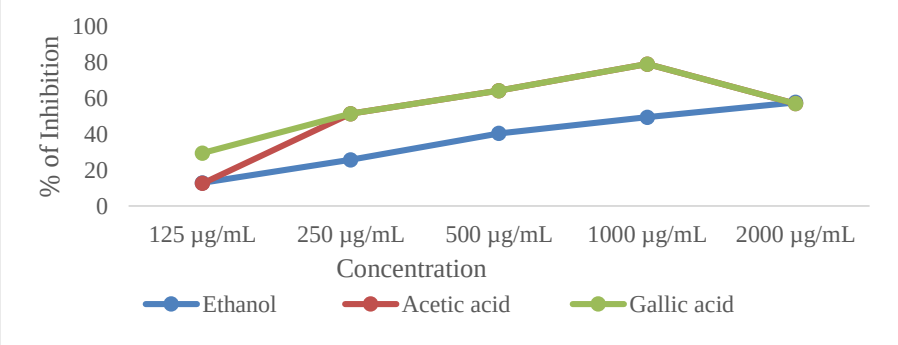


Fig: 3 Total antioxidant activity in two different solvent extracts

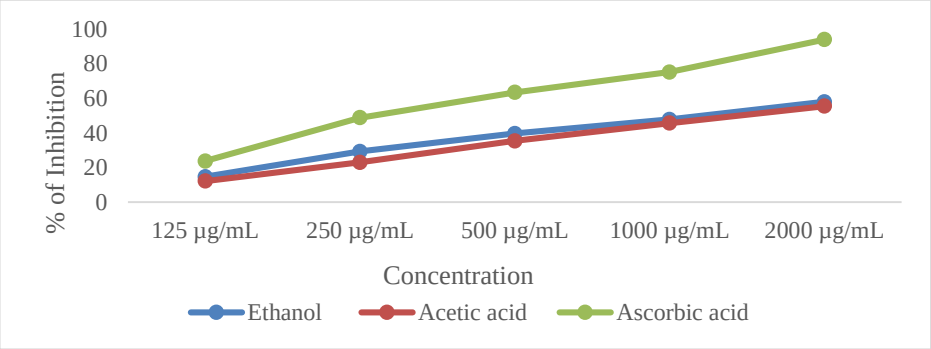
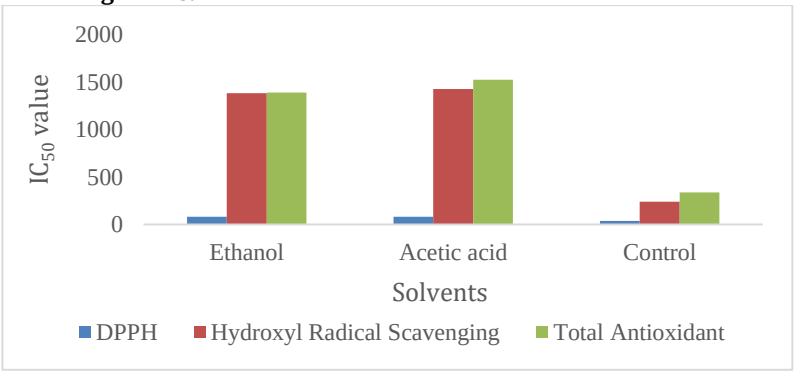


Table: 3 IC_{50} value of different solvent extracts

Antioxidant Activity	IC_{50} Value		
	Ethanol	Acetic acid	Control
DPPH Assay	80.62 µg/mL	83.1713 µg/mL	37.25 µg/mL
Hydroxyl Radical Scavenging Assay	1382.26 µg/mL	1426.13 µg/mL	241.86 µg/mL
Total Antioxidant Activity	1389.5 µg/mL	1525.76 µg/mL	338.52 µg/mL

Fig: 4 IC_{50} value of different solvent extract of *S. molesta*



CONCLUSIONS

The ethanol extract obtained from leaves of *S. molesta* have shown a good antioxidant activity. Further from this study it can be concluded that ethanol extracts of *S. molesta* can further may be used for pharmacological studies.

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REFERENCE

1. Ace, B, Herwati, TN & Dwita, LS 2020', Phytochemical compounds and antioxidant activity of water chesnut *(Eleocharis dulcis)* and giant molesta *(Salvinia molesta)* extract', Systematic Reviews in Pharmacy', vol. 11, no.12, pp. 916-924.
2. Al-Gubory, KH, Garrel, C, Faure, P & Sugino, N 2012, 'Roles of antioxidant enzymes in corpus luteum rescue from reactive oxygen species-induced oxidative stress', Reproductive BioMedicine Online, vol. 25, pp. 551-60.
3. Chang, ST, Wu, JH, Wang, SY, Kang, PL, Yang, NS & Shyur, LF 2001, 'Antioxidant activity of extracts from *Acasia confuse* bark and heartwood', Journal of Agricultural and Food Chemistry, vol. 49, no. 7, pp. 3420-3424.
4. Elizabeth, K & Rao, MNA 1990, 'Oxygen radical scavenging activity of Curcumin', International Journal of Pharmaceutics, vol. 58, no. 3, pp. 237-240.
5. Gulcin, I, Buyukokuroglu, ME, Oktay, M & Kufrevioglu, OI 2003, 'Antioxidant and analgesic activities of turpentine of *Pinus nigra* Arn. Subsp. Pallsiana (Lamb.) Holmboe', Journal of Ethnopharmacology, vol. 86, no. 1, pp. 51-8.
6. Gulcin, I, Kirecci, E, Akkemik, E, Topal, F & Hisar, O 2010, 'Antioxidant, antibacterial, and anticandidal activities of an aquatic plant: duck weed (*Lemna minor* L. Lemnaceae)', Turk J Biology,
7. Liu, HN, Liu, Y, Hu, LL, Suo, YL, Zhang, L, Jin, F, Feng, XA, Teng, N & Li, Y 2014, 'Effects of dietary supplementation of quercetin on performance, egg, quality, cecal microflora populations, and antioxidant status in laying hens', Poultry Science, vol. 93, no. 2, pp. 347-53.
8. Liu, W, Wang, H, Pang, X, Yao, W & Gao, X 2010, 'Characterization and antioxidant activity of two low-molecular-weight polysaccharides purified from the fruiting bodies of *Ganoderma lucidum*', International Journal of Biological Macromolecules, vol. 46, no. 4, pp. 451-7.

9. Munteanu, IG & Apetrei, C 2021, 'Analytical methods used in determinating antioxidant activity: A review', International Journal of Molecular Sciences, vol. 2, no. 7.
10. Mwihia, SK 2017, '*In vitro* antibacterial and antioxidant activities of methanolic and dichloromethanolic seed extracts of Kenyan *Annona squamosa* Linn', Doctoral dissertation, Kenyatta University, Nairobi, Kenya.
11. Ndhlala, M, Moyo, M & Staden, VJ 2010, 'Natural antioxidants: fascinating or mythical biomolecules?', Molecules, vol. 15, no. 10, pp. 6905-6930.
12. Nithya, TG, Jayanthi, J & Ragunathan, MG 2016, 'Antioxidant activity, total phenol, flavonoid, alkaloid, tannin and saponin contents of leaf extracts of *Salvinia molesta* D.S. Mitchell (1972)', Asian Journal of Pharmaceutical and Clinical Research, vol. 9, no. 1, pp. 200-203.
13. Pieczenik, SR & Neustadt, J 2007, 'Mitochondrial dysfunction and molecular pathways of disease', Experimental and Molecular Pathology, vol. 83, no. 1, pp. 84-92.
14. Pizzino, G, Irrea, N, Cucinotta, M, Pallio, G, Mannino, F, Arcoraci, V, Squadrito, F, Altavilla, D & Bitto, A 2017, 'Oxidative stress: Harms and benefits for Human Health', Oxidative Medicine and Cellular Longevity, no. 3, pp. 1-3.
15. Prieto, P, Pineda, M & Aguilar, M 1999, 'Spectrophotometric quantification of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E', Analytical Biochemistry, vol. 269, no. 2, pp. 337-341.
16. Rebecca, LJ, Dhanalakshmi, V & Shekhar, C 2012, 'Antibacterial activity of *Sargassum ilicifolium* and *Kappaphycus alvarezii*', Journal of Chemical and Pharmaceutical Research, vol. 4, no. 1, pp. 700-705.
17. Shebis, Y, Iluz, D, Tahan, KY, Dubinsky, Z & Yehoshua, Y 2013, 'Natural antioxidants: Functions and Sources', Food and Nutrition Sciences, vol. 4, pp. 643-649.
18. Tyagi, T & Agarwal, M 2017, 'Antioxidant properties and phenolic compounds in methanolic extracts of *Eichhornia crassipes*', Research Journal of Phytochemistry, vol. 11, no. 2, pp. 85-89.
19. Valko, M, Leibfritz, D, Moncol, J, Cronin, MTD, Mazur, M & Telser, J 2007, 'Free radicals and antioxidants in normal physiological functions and human disease', International Journal of Biochemistry & Cell Biology, vol. 39, no. 1, pp. 44-84.
20. Ye, CL & Huang, Q 2012, 'Extraction of polysaccharides from herbal *Scutellaria barbata* D. Don (Ban-zhi-lan) and their antioxidant activity', Carbohydrate Polymers, vol. 89, no. 4, pp. 1131-7.
21. Zhou J, Yang, Q, Zhu, X, Lin, T, Hao, D, Xu, J 2020, 'Antioxidant activities of *Clerodendrum cyrtophyllum* Turcz leaf extracts and their major components', PLoS ONE, vol. 15, no. 6.