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IN-VITRO ANTIOXIDANT POTENTIAL OF OF VARIOUS EXTRACTS FROM WHOLE PLANT OF *Alangium salvifolium*

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ABSTRACT

The present investigation was to examine the in-vitro antioxidant potential of various extracts of whole plant of Alangium salvifolium by different in-vitro methods. The antioxidant activity was determined by DPPH assay, super oxide radical scavenging, total antioxidant activity (Phosphomolybdic acid method), total phenol and flavonoid content with reference standard. An IC50 value was found that methanolic extract of Alangium salvifolium is more effective in free radical scavenging activity than that of other two extracts. So, the in-vitro studies clearly showed that the methanolic extract of Alangium salvifolium has a significant antioxidant activity. These in-vitro assays indicate that this plant extracts is a better source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

Keywords: DPPH assay, Super oxide radical activity, Total antioxidant activity, Total Phenaol & Flavonoid.

INTRODUCTION:

Oxygen is essential for the survival of all on this earth. During the process of oxygen utilization in normal physiological and metabolic processes approximately 5% of oxygen gets univalently reduced to oxygen derived free radicals1,2 like superoxide, hydrogen peroxide, hydroxyl and nitric oxide radicals. All these radicals known as reactive oxygen species (ROS) exert oxidative stress towards the cells of human body rendering each cell to face about 10000 oxidative hits per second3. Various reactive oxygen species (ROSs) are formed in the living organism in different ways i.e. normal aerobic respiration, stimulated polymorphonuclear leukocytes and macrophages and peroxisomes. These appear to be the endogenous source of oxidants. Exogenous sources of free radical include tobacco smoking, ionizing radiation, certain pollutants, organic solvents and pesticides4.

All these free radicals are capable of reacting with membrane lipids, nucleic acids, proteins, enzymes and other micro molecules resulting in cellular damage5. Free radicals are involved in the development of degenerative

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diseases6. They have also been implicated in the pathogenesis of diabetes, liver damage, nephrotoxicity, inflammation, cancer, cardiovascular disorders, neurological disorders, and in the process of aging7. To protect these free radical induced damage, antioxidants are the most popular agents that interactively and synergistically neutralize free radicals. Hence, there has been an increased interest in the food industry as well as in preventive medicine in the development of ''Natural antioxidants'' from plant materials. Therefore, the plants with antioxidant properties are becoming more and more popular all over the world3.

Alangium salvifolium (L.f) Wang belongs to family (Alangeaceae) also called as Ankola8 [1] and extensively cultivated in India. Its dried leaves, has traditionally been used to treat various ailments in Asia. Root is used in diarrhea, paralysis, piles and vomiting9. It is a popular folk medicine and has been studied for its anti-inflammatory, antimicrobial, antifertility and cardiotonic activities10-13. Traditionally Alangium salvifolium bark have been reported to exhibit a variety of biological activities, including antidiabetic, anticancer, diuretic, anti-inflammatory, antimicrobial, laxative, and antiepileptic activity14-15.

To the best of our knowledge, no reports are available on the antioxidant potential of various extracts of whole plant of A. salvifolium. Therefore, the current communication focused on the investigation and evaluation of the antioxidant activities of various extracts that were derived from Alangium salvifolium. Furthermore, the total phenolic and total flavonoid content of all extract were also determined.

MATERIALS AND METHODS

Chemicals

Chemical reagents nitroblue tetrazolium (NBT), 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Germany, Catechol (Loba Chemie, Mumbai), sodium carbonate, sodium phosphate, EDTA and Ammonium molybdate (S.D-fine chemicals, Mum- bai).

Plant Material

Whole plant of Alangium salvifolium were collected from Tirunelveli District, Tamil Nadu, India and plant authentication were done by the Botanical Survey of Medicinal Plants Unit Siddha, Government of India, Palayamkottai. The whole plant of Alangium salvifolium were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

Preparation of the extracts

The dried powder was extracted sequentially by hot continuous percolation method using Soxhlet apparatus16, using different polarities of solvents like petroleum ether, ethyl acetate and methanol. The dried powder was packed in Soxhlet apparatus and successively extracted with petroleum ether by for 24 hrs. Then the marc was subjected to ethyl acetate for 24 hrs, and the marc was subjected to methanol for 24 hrs. The extracts were concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

DPPH radical scavenging activity17

A methanolic solution of 0.5ml of DPPH (0.4mM) was added to 1 ml of the different concentrations of plant extract and allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol without the extracts served as the positive control. After 30 min, the absorbance was measured at 518 nm and converted into percentage radical scavenging activity as follows.

Where A518 control is the absorbance of DPPH radical+ methanol; A518 sample is the absorbance of DPPH radical+ sample extract/ standard.

Superoxide anion radical scavenging activity18

Superoxide radical (O2-) was generated from the photoreduction of riboflavin and was deducted by nitro blue tetrazolium dye (NBT) reduction method. The assay mixture contained sample with 0.1ml of Nitro blue tetrazolium (1.5 mM NBT) solution, 0.2 ml of EDTA (0.1M EDTA), 0.05 ml riboflavin (0.12 mM) and 2.55 ml of phosphate buffer (0.067 M phosphate buffer). The control tubes were also set up where in DMSO was added instead of sample. The reaction mixture was illuminated for 30 min and the absorbance at 560 nm was measured against the control samples. Ascorbate was used as the reference compound. All the tests were performed in triplicate and the results averaged. The percentage inhibition was calculated by comparing the results of control and test samples.

Table 1DPPH radical scavenging activity of petroleum ether extract of Alangium salvifolium

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Petroleum ether extract)	Standard (Rutin)
1	125	12.64 ± 0.03	28.39 ± 0.25
2	250	14.43 ± 0.09	48.98 ± 0.46
3	500	25.45 ± 0.30	65.18 ± 0.13
4	1000	47.53 ± 0.02	70.65 ± 0.20
		IC_{50} = 1250µg/ml	**IC_{50} = 270µg/ml**

* Values are expressed as mean ± SEM of 3 observations

Total antioxidant activity (Phosphomolybdic acid method) 19

The antioxidant activity of the sample was evaluated by the transformation of Mo (VI) to Mo (V) to form phosphomolybdenum complex19. An aliquot of 0.4 ml of sample solution was combined in a vial with 4 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The vials were capped and incubated in a water bath at 950C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The antioxidant activity was expresses relative to that of ascorbic acid.

Determination of total phenolic content20

A 1.0ml aliquot of sample was added to 1.5ml of deionized water and 0.5 ml of Folins phenol reagent, and the contents were mixed thoroughly. After 1min, 1.0ml of 20% sodium carbonate was added, and the mixture was again mixed thoroughly. After 30min of incubation at 37ºC, the absorbance was measured at 650nm in a spectrophotometer.

Determination of total flavonoids content21

A 0.5 ml of aliquot of sample was added with 4 ml of the vanillin reagent (1% vanillin in 70% conc. H¬¬2¬SO4¬) was added and kept in a boiling water bath for 15 mins. The absorbance was read at 360 nm. A standard was run by using catechol (110 µg/ml).

RESULTS AND DISCUSSION

Inhibition of DPPH radical

The DPPH assay constitutes a quick and low cost method has frequently been used for the evaluation of the antioxidative potential of various natural producats22.

The percentage of DPPH radical scavenging activity of petroleum ether extract of Alangium salvifolium was presented in Table 1. The DPPH radical scavenging activity of the petroleum ether extract was increases with increasing concentration, 47.53% DPPH radical scavenging. Nevertheless, it was 70.65% in the presence of 1000µg/ml Rutin. The IC50 values of the petroleum ether extract of Alangium salvifolium and Rutin were

Table 2DPPH radical scavenging activity of ethyl acetate extract of Alangium salvifolium

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetae extract)	Standard (Rutin)
1	125	14.61 ± 0.04	28.39 ± 0.25
2	250	19.58 ± 0.06	48.98 ± 0.46
3	500	25.97 ± 0.27	65.18 ± 0.13
4	1000	53.45 ± 0.14	70.65 ± 0.20
		IC_{50} = 875µg/ml	**IC_{50} = 270µg/ml**

* Values are expressed as mean ± SEM of 3 observations

Table 3DPPH radical scavenging activity of Methanolic extract of Alangium salvifolium

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	Standard (Rutin)
1	125	38.65 ± 0.16	28.39 ± 0.25
2	250	56.51 ± 0.03	48.98 ± 0.46
3	500	59.76 ± 0.18	65.18 ± 0.13
4	1000	72.02 ± 0.43	70.65 ± 0.20
		IC_{50} = 225µg/ml	**IC_{50} = 270µg/ml**

* Values are expressed as mean ± SEM of 3 observations

Table 4. Superoxide anion radical scavenging activity of Petroleum ether extract of Alangium salvifolium

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Pet. ether extract)	Standard (Quercetin)
1	125	13.26 ± 0.48	49.44 ± 0.48
2	250	25.17 ± 0.42	61.88 ± 0.49
3	500	32.06 ± 0.52	78.39 ± 0.18
4	1000	39.14 ± 0.43	89.28 ± 0.09
		IC_{50} = 1365 µg/ml	**IC_{50} = 145 µg/ml**

* Values are expressed as mean ± SEM of 3 observations

recorded at 1250µg/ml and 270µg/ml respectively.

The percentage of DPPH radical scavenging activity of ethyl acetate extract of Alangium salvifolium was presented in Table 2. The DPPH radical scavenging activity of the ethyl acetate extract was increases with increasing concentration, only 53.45% DPPH radical scavenging. Nevertheless, it was 70.65% in the presence of 1000µg/ml Rutin. The IC50 values of the ethyl acetate extract of Alangium salvifolium and Rutin were recorded at 875µg/ml and 270µg/ml respectively.

The percentage of DPPH radical scavenging activity of methanolic extract of Alangium salvifolium was presented in Table 3. The DPPH radical scavenging activity of the ethyl acetate extract was increases with increasing concentration, only 72.02% DPPH radical scavenging. Nevertheless, it was 70.65% in the presence of 1000µg/ml Rutin. The IC50 values of the methanolic extract of Alangium salvifolium Rutin were recorded at 225µg/ml and 270µg/ml respectively.

On the DPPH radical, methanolic extract of Alangium salvifolium had significant radical scavenging effect with increasing concentration in the range of 125-1000µg/ml when compared with that of Rutin (standard), the scavenging activity of other two extracts were little lower. An IC50 value of methanolic extract of Alangium salvifolium and Rutin was recorded as 225µg/ml and 270µg/ml respectively.

Table 5. Superoxide anion radical scavenging activity of Ethyl acetate extract of Alangium salvifolium

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetate extract)	Standard (Quercetin)
1	125	21.87 ± 0.33	49.44 ± 0.48
2	250	33.84 ± 0.58	61.88 ± 0.49
3	500	40.67 ± 0.22	78.39 ± 0.18
4	1000	49.42 ± 0.15	89.28 ± 0.09
		IC_{50} = 1005 µg/ml	**IC_{50} = 145 µg/ml**

* Values are expressed as mean ± SEM of 3 observations

Inhibition of Superoxide anion radical scavenging activity

Superoxide radical is known to be very harmful to cellular components as a precursor of the more reactive oxygen species, contributing to tissue damage and various diseases23.

The percentage of superoxide anion scavenging activity of petroleum ether extract of Alangium salvifolium was presented in

Table 6. Superoxide anion radical scavenging activity of Methanolic extract of Alangium salvifolium

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	Standard (Quercetin)
1	125	64.41 ± 0.50	49.44 ± 0.48
2	250	68.22 ± 0.11	61.88 ± 0.49
3	500	81.10 ± 0.31	78.39 ± 0.18
4	1000	84.41 ± 0.03	89.28 ± 0.09
		IC_{50} =85 µg/ml	**IC_{50} = 145 µg/ml**

* Values are expressed as mean ± SEM of 3 observations

Table 7: Total antioxidant activity of Petroleum ether extract of *Alangium salvifolium*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Petroleum ether extract)	Standard (Ascorbate)
1	125	18.26±0.09	26.87 ± 0.08
2	250	24.39±0.19	30.30 ± 0.05
3	500	33.42±0.27	60.64 ± 0.02
4	1000	39.41±0.14	55.23 ± 0.01
		IC_{50} = 1320µg/ml	**IC_{50} = 410 µg/ml**

*Data presented as the mean ± SEM for three measurements

Table 8: Total antioxidant activity of Ethyl acetate extract of Alangium salvifolium

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetate extract)	Standard (Ascorbate)
1	125	13.36±0.16	26.87 ± 0.08
2	250	17.71±0.50	30.30 ± 0.05
3	500	40.12±0.43	60.64 ± 0.02
4	1000	45.49±0.16	55.23 ± 0.01
		IC_{50} = 1090µg/ml	**IC_{50} = 410 µg/ml**

*Data presented as the mean ± SEM for three measurements

Table 9: Total antioxidant activity of Methanolic extract of Alangium salvifolium

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	Standard (Ascorbate)
1	125	39.20±0.02	26.87 ± 0.08
2	250	49.73±0.29	30.30 ± 0.05
3	500	61.29±0.70	60.64 ± 0.02
4	1000	69.65±0.05	55.23 ± 0.01
		IC_{50} = 255µg/ml	**IC_{50} = 410 µg/ml**

*Data presented as the mean ± SEM for three measurements

Table 10: The total Phenolic content of various extracts of Alangium salvifolium

S.No	Extracts	Total phenol content (mg/g of Catechol) (±SEM)*
1.	Petroleum ether extract of *Alangium salvifolium*	1.943 ± 0.022
2.	Ethyl acetate extract of *Alangium salvifolium*	2.604 ± 0.073
3.	Methanolic extract of *Alangium salvifolium*	9.604 ± 0.025

*All values are expressed as mean ± SEM for three determinations

Table 11: The total flavonoids content of various extracts of Alangium salvifolium

S.No	Extracts	Total flavonoids content (mg/g) (±SEM)*
1.	Petroleum ether extract of *Alangium salvifolium*	0.977 ± 0.006
2.	Ethyl acetate extract of *Alangium salvifolium*	1.770 ± 0.09
3.	Methanolic extract of *Alangium salvifolium*	5.637 ± 0.093

*All values are expressed as mean ± SEM for three determinations

Table 4. A maximum scavenging activity of petroleum ether extract and Quercetin at 1000

µg/ml was found to be 39.14% and 89.28% respectively. IC50 value of petroleum ether extract on superoxide radical scavenging activity was found to be 1365 µg/ml, whereas the IC50 value of Quercetin was found to be 145µg/ml.

The percentage of superoxide anion scavenging activity of ethyl acetate extract of Alangium salvifolium was presented in Table 5. A maximum scavenging activity of ethyl acetate extract and Quercetin at 1000 µg/ml was found to be 49.42% and 89.28% respectively. IC50 value of ethyl acetate extract on superoxide radical scavenging activity was found to be 1005 µg/ml, whereas the IC50 value of Quercetin was found to be 145µg/ml.

The percentage of superoxide anion scavenging activity of methanolic extract of Alangium salvifolium was presented in Table 6. A maximum scavenging activity of methanolic extract and Quercetin at 1000 µg/ml was found to be 49.42% and 89.28% respectively. IC50 value of ethyl acetate extract on superoxide radical scavenging activity was found to be 85µg/ml, whereas the IC50 value of Quercetin was found to be 145µg/ml.

On the superoxide anion radical scavenging activity, the methanolic extract of Alangium salvifolium had significant when compared to standard Quercetin. Similar result was not obtained into other two extracts. IC50 value of methanolic extract of Alangium salvifolium and Quercetin was found to be 85µg/ml and 145µg/ml respectively.

Determination of Total antioxidant activity (Phosphomolybdic acid method)

The percentage of total antioxidant activity of petroleum ether extract of Alangium salvifolium was depicted in Table 7. The petroleum ether extract of Alangium salvifolium exhibited a maximum total antioxidant activity of 39.41% at 1000 µg/ml whereas for ascorbate (standard) was found to be 55.23 % at 1000 µg/ml. IC50values of the petroleum ether extract of Alangium salvifolium and ascorbate were found to be 1320µg/ml and 410µg/ml respectively.

The percentage of total antioxidant activity of ethyl acetate extract of Alangium salvifolium was presented in Table 8. The ethyl acetate extract of Alangium salvifolium exhibited a maximum total antioxidant activity of 45.49% at 1000 µg/ml whereas for ascorbate (standard) was found to be 55.23 % at 1000 µg/ml. The IC50 of the ethyl acetate extract of Alangium salvifolium and ascorbate were found to be 1090µg/ml and 410µg/ml respectively.

The percentage of total antioxidant activity of methanolic extract of Alangium salvifolium presented in Table 9. The methanolic extract of Alangium salvifolium exhibited a maximum total antioxidant activity of 69.65% at 1000 µg/ml whereas for ascorbate (standard) was found to be 55.23 % at 1000 µg/ml. The IC50 of the methanolic extract of Alangium salvifolium and ascorbate were found to be 255µg/ml and 410µg/ml respectively.

Based on the above data result clearly indicated the methanolic extract of Alangium salvifolium had more effective antioxidant activity when compared to standard ascorbate. Similar results were not revealed in other two extracts. IC50 values of the methanolic extract of Alangium salvifolium and Ascorbate were found to be 255µg/ml and 410µg/ml respectively.

Total phenolic content

Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups24. The phenolic compounds may contribute directly to antioxidative action25. The content of phenolic compounds (mg/g) in various extracts of Alangium salvifolium was presented in Table 10. The methanolic extract of Alangium salvifolium was found higher content of phenolic components than that extracts. These results suggestthat the high levels of antioxidant activity were due to the presence of phenolic components. From all these observations it can be concluded that the plant extracts with high level of polyphenolic compounds show good antioxidant activity in vitro systems.

Total flavonoids content

The total amount of flavonoids content of various extract of Alangium salvifolium was summarized in Table 11. Flavonoids present in food of plant origin are also potential antioxidants26, 27. The higher content of flavonoids was found in methanolic extract of Alangium salvifolium than that of other extracts.

CONCLUSION:

In the present investigation, we demonstrated that methanolic extract of whole plant of Alangium salvifolium contained higher

levels of total phenolic and flavonoid compounds and was capable of inhibiting, quenching free radicals to terminate the radical chain reaction, and acting as a reducing agent. Significant antioxidant activity of methanolic extract of Alangium salvifolium provides a scientific validation for this plant as an accessible source of natural antioxidants with consequent health benefits. Further work on isolation and identification of active compounds and their efficacy needs to be done.

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