RESEARCH ARTICLE

Total Phenolic, flavonoid contents and *in vitro* antioxidant activity of leaf of *Sesuvium portulacastrum*. L (Aizoaceae)

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ABSTRACT

Antioxidant activity of the leaf of *Sesuvium portulacastrum* was studied for its free radical scavenging property on different *in vitro* models eg DPPH, hydroxyl, superoxide, ABTS radical cation scavenging and reducing power by using different solvents. Total phenolic and flavonoid contents were estimated. The methanol extract showed promising free radical scavenging activity in dose dependent manner. This antioxidant potency may be related to the presence of antioxidant phenolic and flavonoid compounds present in the extract. These results clearly indicate that *S.portulacastrum*leaf is effective against free radical mediated disease.

Keywords: Mangrove, *Sesuviumportulacastrum*, Methanol, DPPH, ABTS, Reducing power.

INTRODUCTION

Free radicals are produced continually in various metabolic processes and exist in biological systems. They are important for maintaining normal physiological function. Reaction oxygen species (ROS) (eg. Superoxide anion, hydroxyl radical and hydrogen peroxide) increases the possibility of a wide range of common degenerative diseases. The free radical may cause lipid peroxidation, aggregation of protein and degradation of DNA, protein and polysaccharide, which eventually destroys cell membranes and kills cells. [1] Many plants often contain substantial amounts of antioxidants including vitamin C and E, carotenoids, flavonoids and tannins etc., and thus can be utilized to scavenge the excess free radicals from human body [2]

Sesuvium portulacastrum(Aizoaceae) is commonly known as Sea Purslane. It is a frequent pioneer species in the backshore zone of coastal branches, where sand movement is influenced by prevalent winds near the born coast. [3] *S.portulacastrum* has a long history of use in folk medicine where, in Zimbabwe and South Africa use the plant to treat various infections and kidney problems. [4] Kompfer*et al*[5]stated a gram- staining positive coccus, belonging to genus *Salinicoccus,* was isolated from the rhizosphere of *Sesuviumportulacastrum.* Michael*et al*[6] used hydrodistilationto extract the essential oil from the fresh leaves of *S.portulacastrum*, and the essential oil exhibited antibacterial, antifungal and antioxidant activity.[6]Chandrasekaran*et al*[7] expressed the fatty acid methyl esters (FAME extract) from *S. portulacastrum* can be used in traditional medicine as a potential antimicrobial agent. But, the studies revealed with the *in vitro* antioxidant activities from mangrove associated plants are two limited. Hence, the present studies were made and attempt to evaluate the *in vitro* antioxidant activities from mangrove associated plants (*Sesuvium portulacastrum*) using different models viz, DPPH, Hydroxyl, Superoxide and ABTS. The present attempt has been made to find out the *in vitro* antioxidant efficacy of various extract from the leaf of *Sesuvium portulacastrum.*

MATERIALS AND METHODS

The leaf of *S. portulacastrum* was collected from Tuticorin coast, Gulf of Mannar, Tamil Nadu. The collected samples were cut into small fragments and

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shade dried until the fracture is uniform and smooth. The dried plant material was granulated or powdered by using a blender, and sieved to get uniform particles by using sieve No. 60. The final uniform powder was used for the extraction of active constituents of the plant material.

Preparation of Plant extract

Freshly collected leaf samples of *S. portulacastrum* were dried in shade, and then coarsely powdered separately in a wily mill. The coarse powder (100g) was extracted successively with petroleum ether, benzene, ethyl acetate, methanol and ethanol, each 250 ml in a Soxhlet apparatus for 24 hrs. All the extracts were filtered thoughWhatman No.41 filters paper. . All the extracts were concentrated in a rotary evaporator. The concentrated extracts were used for *in vitro* antioxidant activity. The methanol extract was used for the estimation of total phenolics and flavonoids.

Estimation of Total Phenolic content

Total phenolic content was estimated using the Folin-Ciocalteu method.[8] Samples (100µL) were mixed thoroughly with 2 ml of 2% Na_2CO_3. After 2 min. 100 µL of Folin-Ciocalteu reagent was added to the mixture. The resulting mixture was allowed to stand at room temperature for 30 min and the absorbance was measured at 743 nm against a blank. Total phenolic content was expressed as gram of gallic equivalents per 100 gram of dry weight (g $100g^{-1}$DW) of the plant samples.

Estimation of Flavonoids

The flavonoids content was determined according to Eom*et al*[9]. An aliquot of 0.5ml of sample (1mg/ml) was mixed with 0.1ml of 10% aluminium chloride and 0.1ml of potassium acetate (1M). In this mixture, 4.3ml of 80% methanol was added to make 5ml volume. This mixture was vortexed and the absorbance was measured spectrophotometrically at 415nm. The value of optical density was used to calculate the total flavonoid content present in the sample.

DPPH radical scavenging activity

The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant component. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of the non radical form DPPH-H[10]

The free radical scavenging activity of all the extracts was evaluated by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) according to the previously reported method. [10] Briefly, an 0.1mM solution of DPPH in methanol was prepared, and 1ml of this solution was added to 3 ml of the solution of various concentration of extracts and the reference compound (50,100,200,400 & 800µg/ml).The mixtures were shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance was measured at 517 nm using a UV-VIS spectrophotometer (Genesys 10S UV: Thermo electron corporation). Ascorbic acid was used as the reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability to scavenging the DPPH radical was calculated by using the following formula.

DPPH scavenging effect (% inhibition) = $\{(A_0 - A_1)/A_0)*100\}$

Where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged

Hydroxyl radical scavenging activity

The scavenging capacity forhydroxyl radical was measured according to the modified method of Halliwell*et al*[11]. Stock solutions of EDTA (1mM), FeCl3 (10mM), Ascorbic Acid (1mM), H_2O_2 (10mM) and Deoxyribose (10 mM), were prepared in distilled deionized water.

The assay was performed by adding 0.1ml EDTA , 0.01ml of $FeCl_3$**,**0.1ml H_2O_2, 0.36ml of deoxyribose, 1.0ml of the extract of different concentration (50,100,200,400 &800µg/ml)dissolved in distilled water,0.33ml of phosphate buffer (50mM , pH 7.9), 0.1ml of ascorbic acid in sequence . The

mixture was then incubated at 37^{0}c for 1 hour. 1.0ml portion of the incubated mixture was mixed with 1.0ml of 10%TCA and 1.0ml of 0.5% TBA (in 0.025M NaOH containing 0.025% BHA) to develop the pink chromogen measured at 532nm. The hydroxyl radical scavenging activity of the extract is reported as % inhibition of deoxyribose degradation is calculated by using the following equation

Hydroxyl radical scavenging activity= $\{(A_0 - A_1)/A_0)*100\}$

Where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged.

Superoxide radical scavenging activity

The superoxide anion scavenging activity was measured as described by Srinivasan*et al.*[12]The superoxide anion radicals were generated in 3.0 ml of Tris – HCL buffer (16 mM, P^H 8.0), containing 0.5 ml of NBT (0.3mM), 0.5 ml NADH (0.936mM) solution, 1.0 ml extract of different concentration (50,100,200,400 & 800µg/ml), and 0.5 mlTris – HCl buffer (16mM, P^H 8.0). The reaction was started by adding 0.5 ml PMS solution (0.12mM) to the mixture, incubated at 25^{o}C for 5 min and the absorbance was measured at 560 nm against a blank sample, ascorbic acid. The percentage inhibition was calculated by using the following equation

Superoxide radical scavenging activity= $\{(A_0 - A_1)/A_0)*100\}$

Where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance in presence of all of the extract samples and reference. The entire test was performed in triplicates and the results were averaged.

Antioxidant Activity by Radical Cation (ABTS. +).

ABTS assay was based on the slightly modified method of Huang *et al.*[13] ABTS radical cation (ABTS+) was produced by reacting 7mM ABTS solution with 2.45 mM potassium persulphate and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. The ABTS + Solution were diluted with ethanol to an absorbance of 0.70+0.02 at 734 nm. After addition of 100µL of sample or trolox standard to 3.9 ml of diluted ABTS+ solution, absorbance was measured at 734 nm by Genesys 10S UV-VIS (Thermo scientific) exactly after 6 minutes. Results were expressed as trolox equivalent antioxidant capacity (TEAC).

ABTS radical cation activity = $\{(A_0 - A_1)/A_0)*100\}$

Where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged.

Reducing Power

The reducing power of the extract was determined by the method of Kumar and Hemalatha,.[14] 1.0 ml of solution containing 50,100,200,400 &800µg/ml of extract was mixed with sodium phosphate buffer (5.0 ml, 0.2 M, pH6.6) and potassium ferricyanide (5.0 ml, 1.0%): The mixture was incubated at 50^{o}C for 20 minutes. Then 5ml of 10% trichloroacetic acid was added and centrifuged at 980 g (10 minutes at 5^{o}C) in a refrigerator centrifuge. The upper layer of the solution (5.0 ml) was diluted with 5.0 ml of distilled water and ferric chloride and absorbance read at 700 nm. The experiment was performed thrice and results were averaged.

Statistical analysis

Antioxidant activities like DPPH radical scavenging activity, hydroxyl radical scavenging activity, superoxide radical activity, ABTS radical cation scavenging activity and reducing powers were estimated in triplicate determinations. Data were analyzed using the statistical analysis system SPSS (SPSS software for windows release 17.5; SPSS Inc., Chicago IL, USA) Estimates of mean, standard error for aforesaid parameters were calculated.

RESULTS AND DISCUSSION

Total phenolic content and total flavonoid content:

The total phenolic content and total flavonoid content of the methanol extract of *Sesuvium portulacastrum* leaf was found to the 0.89 $g100g^{-1}$ and 0.15 $g100g^{-1}$ respectively. Phenolics are secondary metabolites that play a role in the maintenance of the human body. The presence of phytoconstituents, such as phenols, flavonoids and tannin in plants, indicates the possibility of antioxidant activity and this activity will helps in preventing a number of diseases through free radical scavenging activity. Phenolic compounds are commonly found in plants and have been reported to have several biological activities including antioxidant properties. Many studies focused on the biological activities of phenolic compounds, which have potential antioxidants and free radical scavenger. [15]

Phenolic compounds are well known as antioxidant and scavenging agents free radicals associated with oxidative damage. Phenolic compounds have attracted much interest recently because *in vitro* studies suggest that they have a variety of beneficial biological properties like anti-inflammatory, antitumor and antimicrobial activities. Studies have attributed that antioxidant properties are due to the presence of phenols and flavonoids.[16-17]

Antioxidant activity of phenolic compounds is based on their ability to donate hydrogen atoms to free radicals. In addition, they possess ideal structural properties for free radical scavenging properties. Flavonoids are important secondary metabolites of plant modulating lipid peroxidation involved in atherogenesis, thrombosis and carcinogenesis. It has been confirmed that pharmacological effects of flavonoids is correlating with their antioxidant activities. [18]

DPPH radical scavenging activity:

Free radicals and other reactive species are thought to play an important role in many human diseases. Radical scavenging activities are very important due to the deleterious role of free radicals in biological systems. Many secondary metabolites which include flavonoids, phenolic compounds etc, serve as sources of antioxidants and do scavenging activity. [19] In this study, it is evident that the extract of the study species *S.portulacastrum* leaf possess effective antioxidant activity. *In vitro* antioxidant activity of the petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of *S.portulacastrum* leaf was investigated in the present study by DPPH, hydroxyl, superoxide and ABTS radical cation scavenging activities and their reducing ability. These methods have proven the effectiveness of the extracts in comparison to that of reference standard antioxidant, ascorbic acid and trolox.

The radical scavenging activity of different extracts was tested using the stable free radical DPPH. Unlike laboratory generated free radical such as the hydroxyl radical and superoxide anion, DPPH has the advantage of being unaffected by certain side reactions such as metal ion chelation and enzyme inhibition brought about by various additives. A freshly prepared DPPH solution exhibits a deep purple colour generally fades/disappears when an antioxidant present in the medium. Thus, antioxidant molecule can quench DPPH free radicals and convert them to a colourless product resulting in a decreasing absorbance at the 517 nm.

DPPH radical scavenging activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of *S.portulacastrum* leaf was shown in Fig 1. The scavenging effect increases with the concentration of standard and samples. Among the solvent tested, methanol extract exhibited highest DPPH radical scavenging activity. At 800µg/ml concentration, methanol extract of *S.portulacastrum* possessed 121.63% scavenging activity on DPPH. From the result, in the present study, the extracts have shown about equivalent free radical scavenging activity with dose dependent manner. The IC_{50} value of ascorbic acid was 19.11µg/ml where as methanol extract was found to be 31.53µg/ml.(Table 1)

Hydroxyl radical scavenging activity:

Hydroxyl radical is highly reactive oxygen centered, radical formed from the reaction of various hydroperoxides transition metal ion. It attacks protein, DNA, polyunsaturated fatty acids in membranes and most biological molecule it contacts and is known to the capable of abstracting hydrogen atoms from membrane lipids and brings about peroxidic reaction of lipids. [20] The present extract exhibited concentration dependent scavenging activity against hydroxyl radical generated in a Fenton reaction system. Hydroxyl radical scavenging activity ofpetroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of *S.portulacastrum*leaf was shownin Fig 2. Methanol extract showed very potent hydroxyl radical scavenging activity. At 800µg/ml concentration methanol extract of*S.portulacastrum* possessed 72.74% scavenging activity on hydroxyl radical.The scavenging activity may be due to the presence of various phytochemicals including polyphenols and flavonoids in *S.portulacastrum* leaf extracts.The IC_{50} value of ascorbic acid was 20.84µg/ml where as methanol extract was found to be 19.17µg/ml.

Superoxide radical scavenging activity:

Superoxide anions damage biomolecules directly or indirectly by forming H_2O_2, OH, peroxy nitrite or singlet oxygen during aging and pathological events such as ischemic reperfusion injury. Superoxide has also been observed to directly initiate lipid peroxidation [21].The *S.portulacastrum* leaf extracts were subjected to the superoxide scavenging assay and the results were shown in Fig 3. It indicates that ethanol extract of *S.portulacastrum* leaf (800µg/mL) exhibited the maximum superoxide scavenging activity of 98.11% which is higher than the standard ascorbic acid whose scavenging effect is 98.11%.The IC_{50} value of ascorbic acid was 23.79µg/ml where as methanol extract was found to be 27.52µg/ml.

ABTS radical cation scavenging activity:

ABTS radical scavenging activity is relatively recent one, which involves a more drastic radical, chemically produced and is often used for screening complex antioxidant mixtures such as plant extracts, beverages and biological fluids. The ability in both the organic and aqueous media and the stability in a wide pH range raised the interest in the use of ABTS for the estimation of antioxidant activity. [13] The*S.portulacastrum* leaf extracts were subjected to the ABTS radical cation scavenging activity and the results were presented in Fig 4. The methanol extracts exhibited potent ABTS radical cation scavenging activity in concentration dependent manner. At 800µg/ml concentration of *S.portulacastrum* possessed 94.70% scavenging activityon ABTS which is higher than the standard Trolox whose scavenging activity is 70.29%.The methanol extract showed potent antioxidant activity in ABTS method, which is higher than that of standard trolox. Here, the *S.portulacastrum* leaf extracts radical scavenging activity showed a direct role of its phenolic compounds in free radical scavenging.The IC_{50} value of ascorbic acid was 21.18µg/ml where as ethanol extract was found to be 22.89µg/ml.

Reducing power:

The reducing properties of the plant extracts are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom. [22-24] Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation. The data obtained in the present study suggest that it is likely to contribute significantly towards the observed antioxidant effects. Like the antioxidant activity, the reducing power of the extract increase with increasing concentration. Figure 5 showed the reducing ability of different solvent extracts of *S.portulacastrum* leaf compared to ascorbic acid. Absorbance of the solution was increased when the concentration increased. A higher absorbance indicates a higher reducing power. Among the solvent tested, methanol extract exhibited higher reducing ability.

CONCLUSION

The results from various free radicals scavenging systems reveal that all the extracts of *S.portulacastrum* leaf have significant antioxidant activity. The extracts are found to have different levels of antioxidant activity in all the methods tested. IC_{50} values obtained were comparable to that of the standards used i.e, ascorbic acid and trolox. The overall antioxidant activity of the *S.portulacastrum* leaf extracts might be attributed to the presence of phytochemical constituents. The findings of the present study suggest that *S.portulacastrum* leaf could be a potential source of natural antioxidant that could have great importance as therapeutic agent in preventing or slowing the oxidative stress related degenerative diseases. Furthermore, the *in vitro* antioxidant activity of this extract needs to be assessed prior to clinical use.

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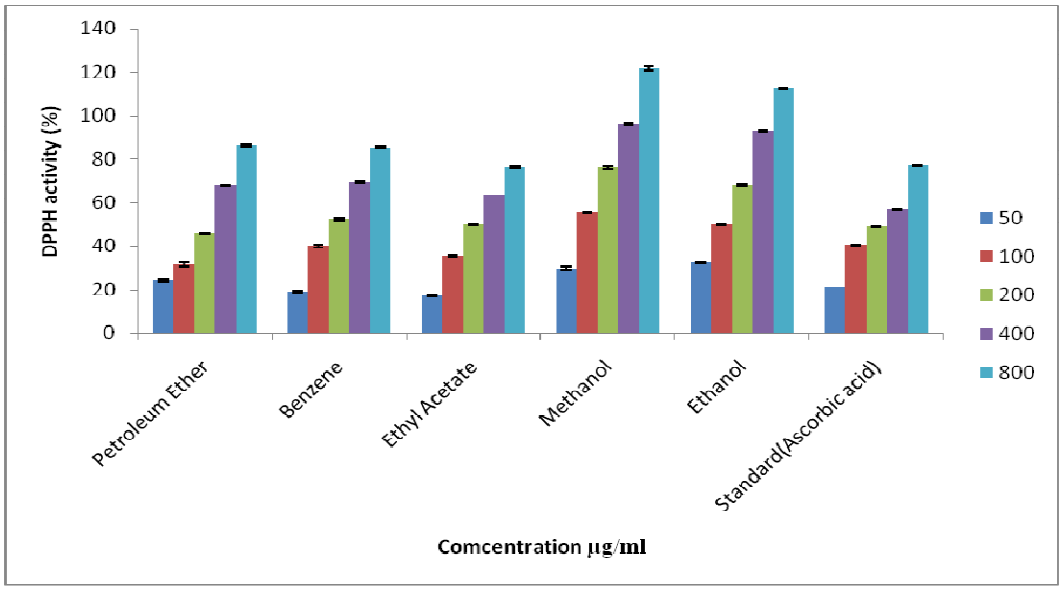

Fig 1: DPPH radical scavenging activity of different extracts of *Sesuvium portulacastrum* leaf.

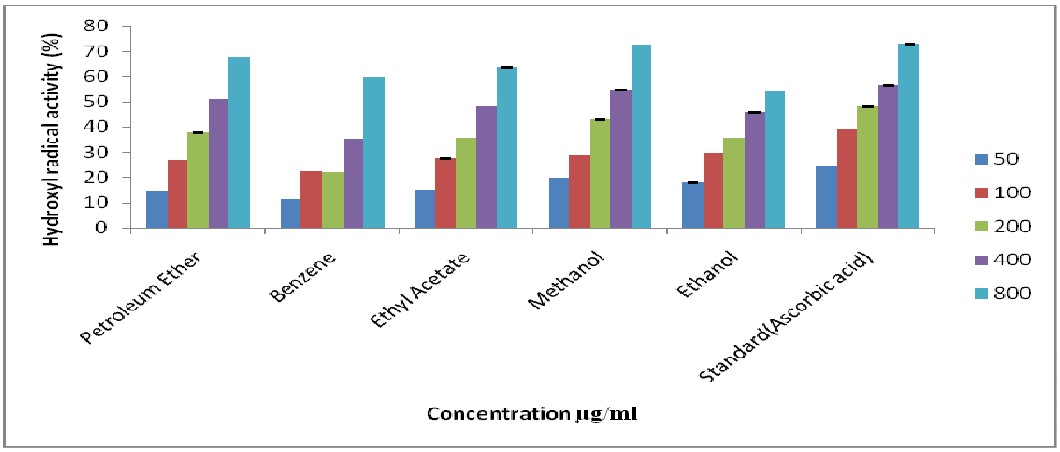

Fig 2: Hydroxyl radical activity of different extracts of *Sesuvium portulacastrum*leaf.

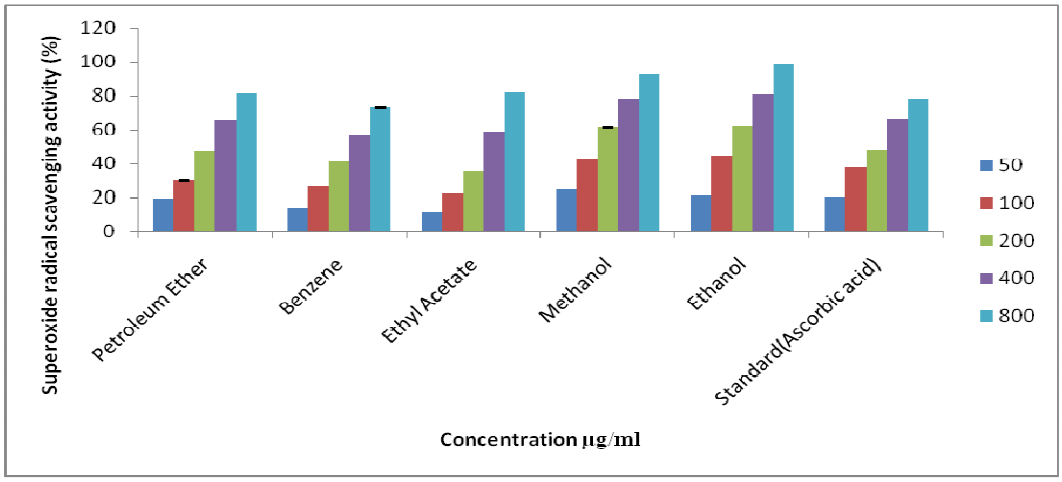

Fig 3: Superoxide radical scavenging activity of different extracts of *Sesuvium portulacastrum* leaf.

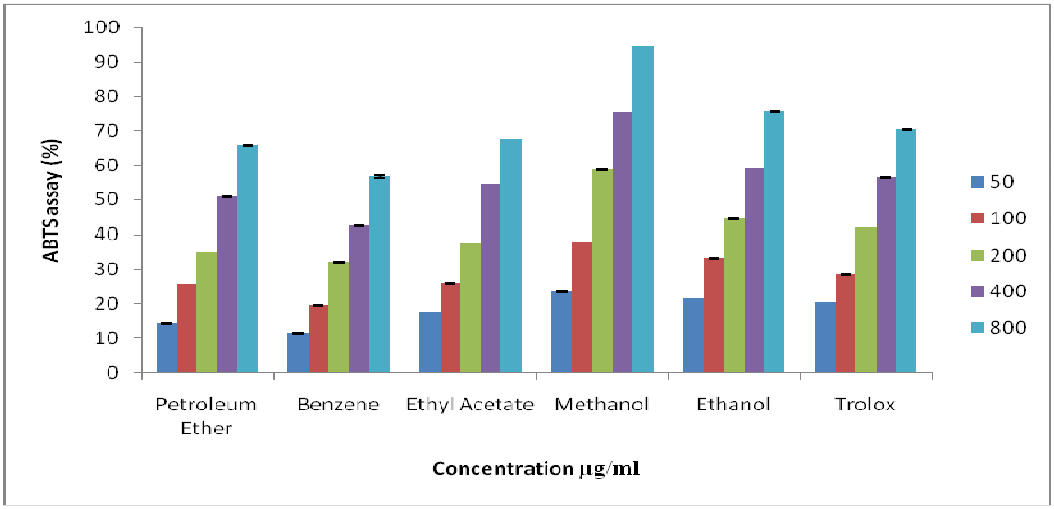

Fig 4: ABTS radical cation scavenging activity of different extracts of *Sesuvium portulacastrum* leaf.

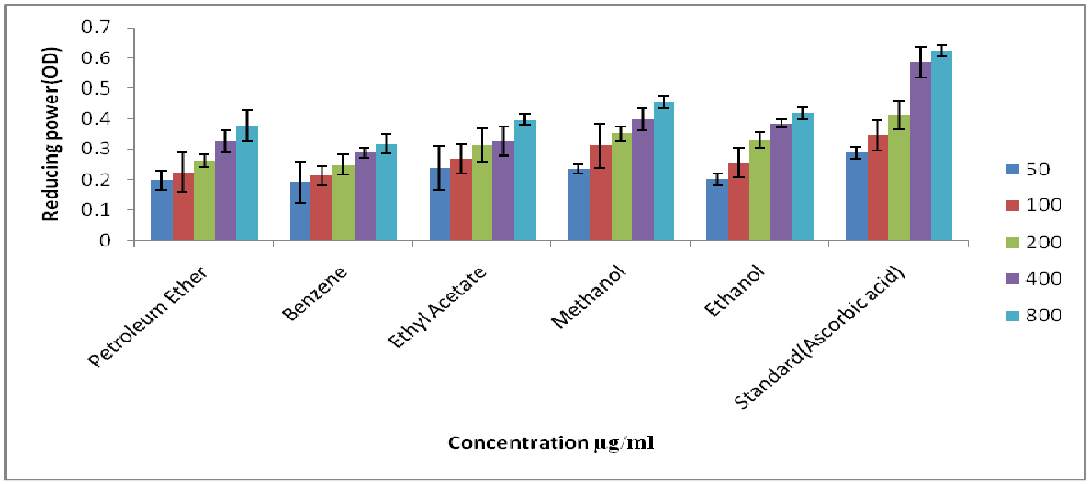

Fig 5: Reducing power ability of different extract of*Sesuvium portulacastrum* leaf

Table 1: IC_{50} values of different solvent extracts of *Sesuviumportulacastrum*leaf *

Solvent	IC_{50} (µg/ml)			
	DPPH	Hydroxyl	Superoxide	ABTS
Petroleum ether	20.99	17.92	21.26	16.94
Benzene	20.87	16.40	19.89	15.37
Ethyl acetate	18.73	17.49	22.01	17.34
Methanol	31.53	19.17	24.65	22.89
Ethanol	29.38	16.49	27.52	19.20
Ascorbic acid	19.11	20.84	23.79	-
Trolox	-	-	-	21.18

All the values are mean by triplicate determines*

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