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Estimates total phenolic, flavonoid content and *in vitro* antioxidant activity of root of *Suaeda monaica* Forssk ex Gmel (Chenopodiaceae)

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ABSTRACT

Antioxidant activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of the root of mangrove herb *Suaeda monaica* have been tested using various antioxidant model system viz, DPPH, hydroxyl, superoxide, ABTS and reducing power. Methanol extract of *Suaeda monaica* is found to possess higher DPPH and hydroxyl radical scavenging activity. Ethanol and methanol extracts of *Suaeda monaica* exhibited highest superoxide and ABTS radical cation scavenging activity. Methanol extract of root of *Suaeda monaica* showed the highest reducing ability. This study indicates significant free radical scavenging potential of *Suaeda monaica* root which can be exploited for the treatment of various free radical mediated ailments.

Introduction

Suaeda monaica Forssak.ex.Gmel belonging to Chenopodiaceae family is a salt marsh mangrove herb similar to *Suaeda maritima* in appearance. It is a herb, smaller in size. Leaves simple, succulent, linear, young twigs are slender ribbed. The leaves have been used as edible green leaves. Traditionally, the leaf from *Suaeda monaica* is known to use as a medicine for hepatitis and scientifically it is reported to be used as ointment for wounds and possess antiviral activity, because of the presence of triterpenoids and sterols[1,2]. The present attempt has been made to find out the *in vitro* antioxidant efficacy of various extract from root of *Suaeda monaica.*

Oxidative stress caused by reactive oxygen species has been implicated in the etiology of various diseases like cancer, aging, diabetes, autoimmune diseases, cardiovascular diseases and neurological degenerative disorders. The primary oxygen derived free radical are superoxide anion, hydroxyl, hydroperoxyl, peroxyl and alkoxyl radicals and non free radicals are hydrogen peroxide, hypochlorus acid, ozone and singlet oxygen. These reactive intermediate are collectively termed reactive oxygen species (ROS). These reactive oxygen (ROS) such as superoxide anions, hydroxyl radical and nitric oxide inactivate enzymes and damage important cellular components causing injury through covalent binding and lipid peroxidation. Antioxidants are agents which may offer resistance against the oxidative stress by scavenging the free radicals, inhibiting the lipid peroxidation and by the neutralizing the damage due to oxidants by neutralizing the free radicals and thus prevent diseas[3]. However, the use of natural antioxidants is limited by a lack of knowledge about their molecular composition, amount of active ingredients in the source material and the availability of relevant toxicity data[4]. Natural antioxidants tend to be safer and they also possess antiviral, anti-inflammatory, anti-cancer, anti-tumor and hepatoprotective properties[5]. Therefore, the evaluation of antioxidant activity of various plant extract is considered as an important step in the identification of their ability to scavenge the free radicals.

Materials and methods

The root of *Suaeda monaica* were collected from Tuticorin coast, Gulf of Mannar, Tamil Nadu. The collected samples were cut into small fragments and 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 stem samples of *Suaeda monaica* were dried in shade, and then coarsely powdered separately in a wiley 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 though Whatman No.41 filter 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[6]. 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* [7]. An aliquot of 0.5ml of sample (1mg/mL) was mixed with 0.1ml of 10% aluminium chloride and 0.1ml of potassium

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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[8].

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[8]. 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 all extracts in methanol at different concentration (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 for hydroxyl radical was measured according to the modified method of Halliwell[9]. 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 37^0c 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*[10]. 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 (125,250,500 &1000µg/ml), and 0.5 mL Tris – 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^oC 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. All the test were 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* [11]. 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[12]. 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^oC for 20 minutes. Then 5mL of 10% trichloroacetic acid was added and centrifuged at 980 g (10 minutes at 5^oC) 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

Total phenolic content and total flavonoid content

The total phenolic content and total flavonoid content of the methanol extract of *Suaeda monaica* root was found to be 0.88 $g100g^{-1}$ and 0.84 $g100g^{-1}$respectively.

DPPH radical scavenging activity:

DPPH radical scavenging activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of *Suaeda monaica* root 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 *Suaeda monaica* possessed 65.28% scavenging activity on DPPH.

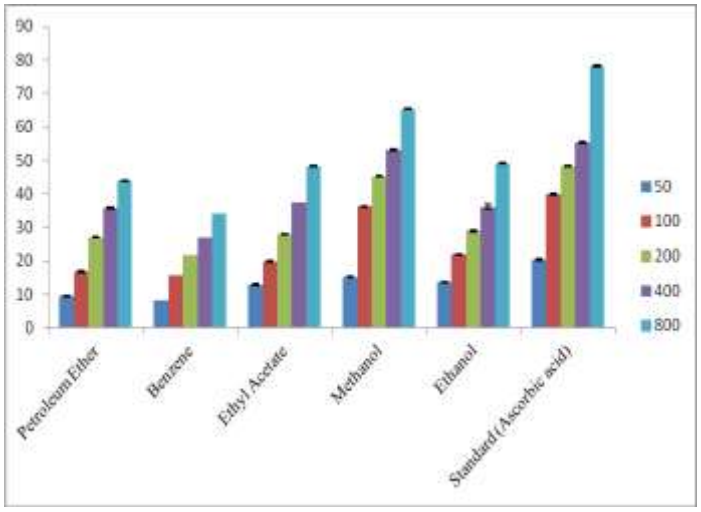

Figure 1. DPPH radical scavenging activity of methanol extract of *Suaeda monaica*

Hydroxyl radical scavenging activity:

Hydroxyl radical scavenging activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of *Suaeda monaica* root was shown in Fig 2. Methanol extract showed very potent activity. At 800µg/mL concentration, *Suaeda monaica* possessed 62.13% scavenging activity on hydroxyl radical.

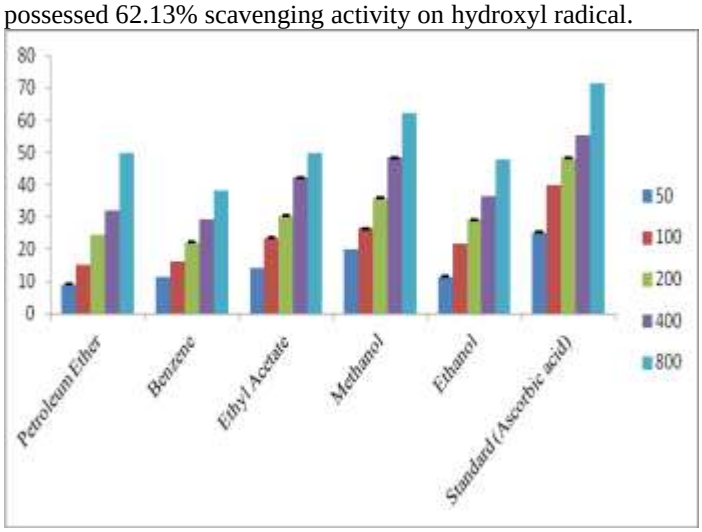

Figure 2. Hydroxyl radical scavenging activity of methanol extract of *Suaeda monaica*

Superoxide radical scavenging activity:

The *Suaeda monaica* root extracts were subjected to the superoxide scavenging assay and the results were shown in Fig 3. It indicates that ethanol extract of *Suaeda monaica* root (800µg/mL) exhibited the maximum superoxide scavenging activity of 94.27% which is higher than the standard ascorbic acid whose scavenging effect is 76.11%.

ABTS radical cation scavenging activity:

The *Suaeda monaica* root extracts were subjected to the ABTS radical cation scavenging activity and the results were presented in Figure 4. The ethanol extract exhibited potent ABTS radical cation scavenging activity in concentration dependent manner. At 800µg/mL concentration, *Suaeda monaica* root possessed 88.22% scavenging activity on ABTS which is higher than the standard trolox whose scavenging activity is 69.04%

Reducing power:

Figure 5 showed the reducing ability of different solvent extracts of *Suaeda monaica* root 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 activity.

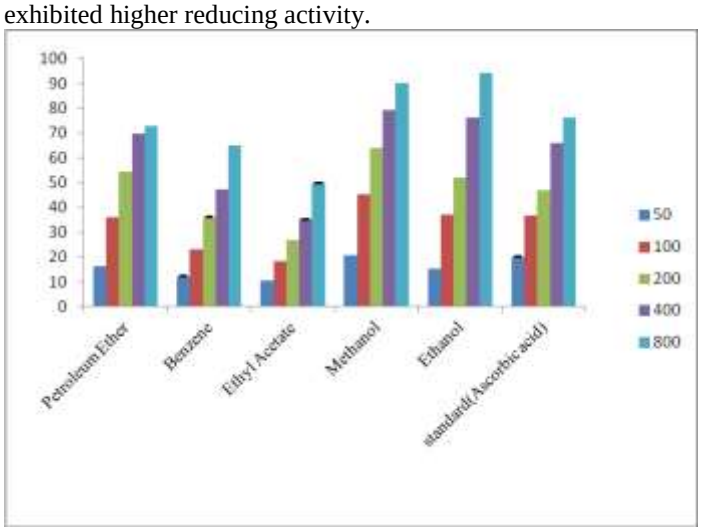

Figure 3. Superoxide radical scavenging activity of methanol extract of *Suaeda monaica*

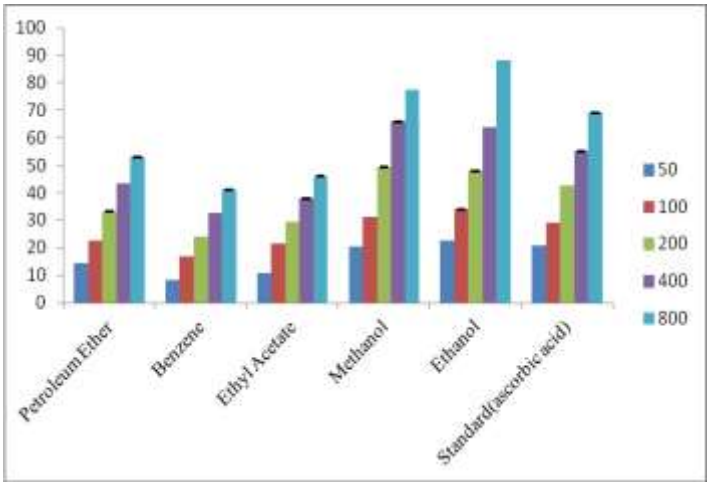

Figure 4. ABTS radical cation scavenging activity of methanol extract of *Suaeda monaica*

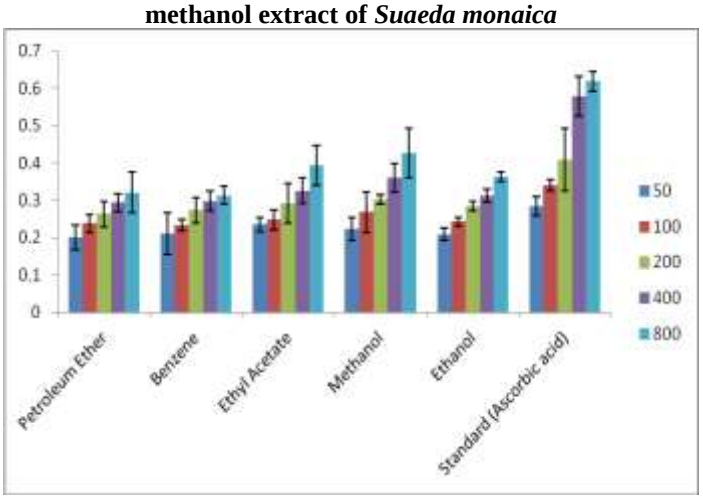

Figure 5. Reducing power ability of methanol extract of *Suaeda monaica*

IC_{50} value:

IC_{50} values of petroleum ether extract of *Suaeda monaica* root and standard ascorbic acid for DPPH, hydroxyl, superoxide radical scavenging and trolox for ABTS radical cation scavenging were found to be 11.24µg/mL and 19.24µg/mL; 16.14µg/mL and 20.63µg/mL; 20.13 µg/mL and 24.04µg/mL and 15.16µg/mL and 20.84µg/mL respectively. IC_{50} values of

benzene extract of *Suaeda monaica* root and standard ascorbic acid for DPPH, hydroxyl, superoxide radical scavenging and trolox for ABTS radical cation scavenging were found to be 10.11µg/mL and 19.24µg/mL; 13.55µg/mL and 20.63µg/mL; 18.48µg/mL and 24.04µg/mL and 13.28µg/mL and 20.84µg/mL respectively. IC_{50} values of ethyl acetate extract of *Suaeda monaica* root and standard ascorbic acid for DPPH, hydroxyl, superoxide radical scavenging and trolox for ABTS radical cation scavenging were found to be 12.84µg/mL and 19.24µg/mL; 15.84µg/mL and 20.63µg/mL; 14.93µg/mL and 24.04µg/mL and 14.61µg/mL and 20.84µg/mL respectively. IC_{50} values of methanol extract of *Suaeda monaica* root and standard ascorbic acid for DPPH, hydroxyl, superoxide radical scavenging and trolox for ABTS radical cation scavenging were found to be 14.84µg/mL and 19.24µg/mL; 20.54µg/mL and 20.63µg/mL; 26.12µg/mL and 24.04µg/mL and 22.94µg/mL and 20.84µg/mL respectively. IC_{50} values of ethanol extract of *Suaeda monaica* root and standard ascorbic acid for DPPH, hydroxyl, superoxide radical scavenging and trolox for ABTS radical cation scavenging were found to be 12.42µg/mL and 19.24µg/mL; 15.28µg/mL and 20.63µg/mL; 28.05µg/mL and 24.04 µg/mL and 23.80µg/mL and 20.84µg/mL respectively.(Table 1.)

Discussion

The systematic literature collection, pertaining to this investigation indicates that the plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical scavengers. Therefore, it is necessary to determine the total amount of phenols and flavonoids in the plant extract chosen for the study. Flavonoids are the most diverse and widespread group of natural compounds and likely to be the most important natural phenolics. These compounds possess a broad spectrum of chemical and biological activities including radical scavenging activity. Flavonoids are important secondary metabolite of plant modulating lipid peroxidation involved in atherogenesis, thrombosis and carcinogenesis. It has been confirmed that pharmacological effect of flavonoids is correlating with their antioxidant activity[13]. Phenolic compounds are considered to be the most important antioxidants of plant materials. They constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators. 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[14]. The presence of these compounds such as total phenolics and flavonoids in *Suaeda monaica* extract may give credence to its local usage for the management of oxidative stress induced ailments. Free radicals are chemical species which contains one or more unpaired electrons. They are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability. They are formed inside the system, and are highly reactive and potentially damaging transient chemical species. These radical are continuously produced in the human body because they are essential for detoxification, chemical signaling, energy supply and immune functions. Free radicals are regulated by endogenous antioxidant enzyme system, but due to over production of free radicals by exposure to environmental oxidant substances such as cigarette smoking, UV radiation etc or a failure in antioxidant defense mechanism or damage to cell structures, the risk increases for many diseases such as Alzhemer's disease, mild congestive impairment, Parkinson's disease, cardiovascular disorders, liver diseases, ulcerative colitis, inflammation and cancer[15,16]. The body possesses defense mechanism against free radical induced oxidative stress, which involve preventive and repair mechanisms. Enzymatic antioxidants such as superoxide dismutase, catalase, glutathione peroxidase etc and non-enzymatic antioxidants such as carotenoids, ascorbic acid, phenolic compounds, flavonoids etc act by one or more mechanisms like reducing activity, free radical scavenging, potential complexing of pro-oxidant metals and quenching of singlet oxygen. It is possible to reduce the risk of chronic diseases and prevent the disease progression by either enhancing the body's natural antioxidant defense or supplementing with proven antioxidants. For this reason, discovery of natural antioxidants is a major thrust area[17]. The antioxidant activity of the petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of *Suaeda monaica* root were investigated against various *in vitro* models.

The 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical is widely used as a model system to investigate the free radical scavenging activities of several plant extracts. DPPH is stable, nitrogen centered free radical which produces violet color in ethanol solution. It was reduced to a yellow colored product, diphenylpicryl hydrazine, with the addition of the extracts. The reduction in the number of DPPH molecules can be calculated with the number of available hydroxyl groups[3]. The DPPH scavenging activity of all the extracts of *Suaeda monaica* root was found to increase in a concentration dependent manner. Among all the five extracts, methanol extract of *Suaeda monaica* recorded the highest scavenging activity of 65.28% at a concentration of 800µg/mL.

The hydroxyl radical scavenging activity is measured as the percentage of inhibition of hydroxyl radicals generated in the Fenton's reaction mixture by studying the competition between deoxyribose and the extract for hydrogen radicals generated from Fe^{3+}/EDTA/H_2O_2 systems. The hydroxyl radicals attack deoxy ribose which eventually results in TBARS formation. In the present study, methanol extract of *Suaeda monaica* exhibited the highest hydroxyl scavenging activity of 62.13% at a concentration of 800µg/mL.

Table 1. IC_{50} values of different solvent extracts of *Suaeda monaica* stem*

Solvent	IC_{50} (µg/mL)			
	DPPH	Hydroxyl	Superoxide	ABTS
Petroleum ether	11.24	16.14	20.13	15.16
Benzene	10.11	13.55	18.48	13.28
Ethyl acetate	12.84	15.84	14.93	14.61
Methanol	14.84	20.54	26.12	22.94
Ethanol	12.42	15.28	28.05	23.80
Ascorbic acid	19.24	20.63	24.04	-
Trolox	-	-	-	20.84

*All values are expressed as mean ± SEM for three determination.

Superoxides are produced from molecules oxygen due to oxidative enzymes[18] of body as well as via non enzymatic reaction such as antioxidation by catecholamines[19]. The decrease in absorbance at 560nm with antioxidants thus indicates the consumption of superoxide anion in the reaction mixture. The superoxide scavenging activity of all the five extracts of *Suaeda monaica* root were found to increase in a concentration dependent manner. The IC_{50} values of ethanol and methanol extracts of *Suaeda monaica* root on superoxide radical scavenging activity were found to be 28.05 and 26.12µg/mL respectively, and that of ascorbic acid was 24.04 µg/mL. the results indicate that the extracts of *Suaeda monaica* have a potent scavenging activity with increasing percentage inhibition. The probable mechanism of scavenging the superoxide anions may be due to the inhibitory effect of the extract towards generation of superoxide in the *in vitro* reaction mixture.

The ABTS assay is based on the inhibition of the absorbance of the radical cation ABTS, which has a characteristic long wavelength absorption spectrum. The scavenging activity of all the five extracts of *Suaeda monaica* root were found to increase in a concentration dependent manner. The IC_{50} values of ethanol and methanol extracts of *Suaeda monaica* root on ABTS radical cation scavenging activity were found to be 23.80 and 22.94 µg/mL respectively, and that of trolox was 20.84µg/mL. The results imply that all the five extracts were found to be very good inhibitors and scavengers of the ABTS radical.

The presence of reductants (antioxidants) in the sample would result in reduction of the FE^{3+}/ferric cyanide complex to the ferrous form. The Fe^{2+} could therefore, be monitored by measuring the formation of Perl's Prussian blue with absorbance at 700nm. The strengthening compounds of reducing power had a stronger peroxide reducing ability[20]. The reductive capabilities of different concentration of all the five extracts of root of *Suaeda monaica* in comparison to the standard, ascorbic acid. It was found that the reducing power increases with increasing concentration of extract. In the present study, methanol extract of root showed the highest reducing ability. However, the activity was lesser than the standard ascorbic acid.

The antioxidant activities of different extracts of *Suaeda monaica* root are in accordance with their amount of phenolics and flavonoids content. Currently, there has been an increased interest worldwide to identify antioxidant compounds from plant sources which are pharmacologically potent and have small or no side effects for use in protective medicine and the food industry. Increasing acquaintance in antioxidant phytoconstituents and including them in daily uses and diet can give sufficient support to human body to fight those diseases. However, further studies are needed to isolate active principles responsible for the overall antioxidant activity of the extract.

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Reference

1. Ravikumar S, Gnanadesigan M, Serebiah JS, Inbaneson SJ. Hepatoprotective effect of an Indian salt marsh herb *Suaeda monaica* Forrsk ex.Gmel against concanavalin-A induced toxicity in rats. Life Sci Med Res. 2010;2:1-9.
2. Ravikumar S, Gnanadesigan M, Inbaneson SJ, Kalaiarasi A. Hepatoprotective and antioxidant properties of suaeda maritime (L.) Dumort ethanolic extract on concanavalin-A induced hepatotoxicity in rats. Indian J Exp.Biol. 2011;49:455-460.
3. Abirami B, Gayathri P, Uma D. *In vivo* antioxidant potential of *Pterocarpus marsupium* bark. Int. J. Chem Pharmaceau Sci. 2012; 3:17-24.
4. Shahidi F, WAnasundara VN, et al. Natural antioxidant from low pungency mustard flour. Food Res. Int.1994;27: 489-493.
5. Lim YY and Murtijaya J. Antioxidant properties of *Phyllanthus amarus* as affected by different drying methods. Lebensm Wiss Technol. 2007;40:1664-1669.
6. Lachman J, Hamouz K, Orsak M, Pivec V. Potato tubers as a significant source of antioxidant human nutrition. Rostl Vyr. 2000; 46: 231-236.
7. Eom SH, Cheng WJ, Hyoung JP, Kim EH, Chung MI, Kim MJ, Yu C, Cho DH. Far infra red ray irradiation stimulates antioxidant activity in *Vitis flexuosa* Thunb. Berries. Kor J Med Crop Sci. 2007; 15: 319-323.
8. Shen Q, Zhang B, Xu R, Wang Y, Ding X, Li P. Antioxidant activity *in vitro* of selenium-contained protein from the se-enriched. *Bifodobacterium animalis* 01.Anaerobe. 2010;16:380-386.
9. Halliwell B, Gutteridge JMC, Aruoma OI. The deoxyribose method: a simple test to be assay for determination of rate constants for reaction of hydroxyl radicals. Ana Biochem. 1987; 165: 215-219.
10. Srinivasan R, Chandrasekar MJN, Nanjan MJ, Suresh B. Antioxidant activity of *Caesalpinia digyna* root. J Ethnopharmacol. 2007;113:284-291.
11. Huang MH, Huang SS, Wang BS, Sheu MJ, Hou WC, *et al.* Antioxidant and anti-inflammatory properties of *Cardiospermum halicacabum* and its reference compounds *ex vivo and in vivo.* J Ethnopharmacol. 2011;133:743-750.
12. Kumar RS, Hemalatha S. *In vitro* antioxidant activity of alcoholic leaf extract and subfractions of *Alangium lamarckii* Thwaites. J Chem Pharm Res. 2011;3:259-267.
13. Shi J, Yu J, Pohorly J, Young C, Bryan M, Wu Y. Optimization of the extraction of polyphenols from grapes seed meal by aqueous ethanol solution. Food Agric.Environ. 2006;1:42-47.
14. Sulaiman SF, Yusoff NAM, Eldeen IM, Seow EM, Sajak AAB, Supriatno OKL. Correlation between total phenolic and mineral content with antioxidant activity of eight Malaysian bananas (*Musa* sp.). J.Food Compost Anal. 2011;24:1-10.
15. Rajkapoor B, Burkan ZE, Senthilkumar R. oxidants and human diseases: role of antioxidant medicinal plants- a review. Pharmacologyonline. 2010;1:1117-1131.
16. Rajkumar V, Cuba G, Kumar RA, Mashew L. Evaluation of antioxidant activities of *Bergenia ciliate* rhizome. Rec Nat Prod. 2010;4:38-48.
17. Senthilkumar R, Rajkapoor B, Perumal P. Antioxidant activities of *Indigofera cassioides* Rottl.ex.DC.using various *in vitro* assay models. Asian Pac.J.Trop.Biomed.2012;2:256-261.
18. Sainani GS, Manika JS, Sainani RK. Oxidative stress: a key factor in pathogenesis of chronic diseases. Medicines update. 1997;1:1.
19. Hemmani T, Parihar MS. Reactive oxygen species and oxidative damage. Indian J Physio and Pharmacol. 1994;42:440.
20. Karthika K, Paulsamy S, Jamuna S. Evaluation of *in vitr0* antioxidant potential of methanolic leaf and stem extracts of *Solena amplexicaulis* (Lam.) Gandhi. J.Chem.Pharmaceu.Res. 2012;4:3254-3258.