

Anti Oxidant Activity of Selected Coastal Medicinal Plants

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Abstract: Antioxidant activity of defatted ethanol extract of some coastal plant leaves were studied for its free radical scavenging property on different *in vitro* models eg 1,1-diphenyl-2-picrylhydrazyl (DPPH), Superoxide and total phenolic content were analyzed. The extracts showed good dose-dependent free radical scavenging property in all samples. In this model high SOD and radical scavenging value indicated in *Citrullus colocynthis* and low SOD and radical scavenging value was indicated in *Sesuvium portulacastrum*. Although the highest total phenolic content was found in *Suaeda maritime* extract, there is no positive correlation between evaluated antioxidant activities and the phenolic contents of examined the coastal plants.

Key words: *In vitro* • Pichavaram • DPPH • SOD • Phenolic compound • Free radicals

INTRODUCTION

Free radicals are responsible for aging and causing various human diseases. A study shows that antioxidant substances which scavenge free radicals play an important role in the prevention of free radical-induced diseases. By donating hydrogen radicals, the primary radicals are reduced to non radical chemical compounds and are then converted to oxidize antioxidant radicals [1, 2]. This action helps in protecting the body from degenerative diseases. Epidemiological studies have shown the beneficial effects of diets rich in vegetables, fruits and grain products in reducing the risk of cardiovascular disease and certain cancers [3]. The principal agents responsible for the protective effects could be the presence of antioxidant substances that exhibit their effects as free radical scavengers, hydrogen-donating compounds, singlet oxygen quenchers and metal ion chelators [4]. Medicinal components from plants play an important role in conventional as well as western medicine. Plants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm the activity and to ascertain the parameters associated with it. The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world [5, 6]. Much work has been done on ethno medicinal plants in India [7-9].

Halophytes plant are considered to be rare plant forms that arose separately in unrelated plant families

during the diversification of angiosperms [10]; halophytes are traditional human foods [11], most of the research has concentrated on their value in animal feeding systems. Animal feeds that can be produced from halophytes include forage from dried plant shoots (hay), oil, seed meal and grains. Halophytes have mixed characteristics as forages. On the positive side, they generally have high protein content, ranging from 10 to 20% of dry matter [12].

In the present study anti oxidant activity of halophytes was investigated.

MATERIAL AND METHODS

All chemicals used were of analytical grade, 1,1-diphenyl-2-picryl hydroxyl (DPPH) was obtained from Sigma Chemicals, USA. Sodium nitroprusside, sulphanimide, o-phosphoric acid, naphthyl ethylene diamine dihydrochloride, nitroblue tetrazolium (NBT), reduced nicotinamide adenine dinucleotide (NADH), phenazine methosulphate (PMS), 2-Deoxy-D-ribose, hydrogen peroxide, ascorbic acid, ferric chloride (FeCl₃), ferrous sulphate (FeSO₄), trichloroacetic acid (TCA), Thiobarbituric acid (TBA), potassium chloride (KCl), potassium ferricyanide [K₃Fe(CN)₆], Ethylene diamine tetra acetic acid (EDTA), tris hydrochloride buffer, Folin-Ciocalteu phenol reagent (FCR) and all solvents were obtained from SISCO Research Laboratories Pvt. Ltd, Mumbai, India.

The five types of coastal plants leave, *Suaeda monoica*, *Suaeda maritime*, *Citrullus colocynthis*, *Ipomoea pes caprae* and *Sesuvium portulcasturm* were collected the Pichavaram mangrove forest in Tamilnadu, india. The plants were cleaned in tap water and air-dried at room temperature in the dark separately. Dried leaves of each selected plant (3 gram) by quantity were ground to poduce fine homogenous powders. The fine plant powder was soaked in 40 ml of 95% ethanol at room temperature for 72 hrs in the dark. The solution was then filtered through Whatman (Whatman international maidsone and evaporated to dryness using a rotary evaporator at a temperature below 40°C. A stick solution (100mg/ml) of the plant extracts was prepared in 5% Tween 80 dissolved in isotonic phosphate buffer (IPB), pH 7.04 and kept at 4°C until required for experiments. The working solution (10 mg/ml) for assays was made by diluting the stock solution with IPB.

Antioxidant Activity Evolution Methods

Free Radical Scavenging Assay: This assay was base on the method described by Bozin *et al.* [13] with some modifications. Briefly, 950µl of 90 µM 2,2-diphenyl-1-picrylhydrazil (DPPH)solution was added to 50µl of the working samples and made up to a final volume of 4ml with 95% ethanol. After the mixtures were vigorously shaken, they were incubated at room temperature in the dark for 2 hours. The reduction of solution colour caused by scavenging of the free radicals (DPPH) was measure at 515 nm using a spectrophotometer. The capability of samples to scavenge DPPH was obtained by comparisons of sample colour reduction effect with the control (mixture without working solutio9n using the following equation and expressed as percentage values.

Scavenging effect (%): EC50 value was determined from the plotted graph of scavenging activity versus the Concentration of coastal plant extracts, which is defined as the amount of antioxidant necessary to decrease the initial DPPH radical concentration by 50%. Triplicate measurements were carried out and their activity was calculated by the percentage of DPPH scavenged.

$$\text{Percentages inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of test})}{\text{Absorbance of control}} \times 100$$

Superoxide Radical Scavenging Activity Assay: Super oxide anion scavenging activity of *Suaeda monoica* ethanol extract was measured according to the method of Robak and Gryglewski [14] with some modification. All the solutions were prepared in 100 mM phosphate buffer (pH

7.4). 1 ml of NBT (156 µM), 1 mall of NADH (468 µM) and 3ml of MEDM to produce final concentration of 3-110 µg/ml) were mixed. The reaction was started by adding 100ul of phenazine methosulphate (PMS) (60µM) and the mixture then incubated at 25C for 5 min followed by measurement of absorbance at 560 nm. The percentage inhibition was calculated from the formula[1].

Determine the total phenolic compounds [15, 16]. The content of total phenolic compounds in *Suaeda monoica* ethanolic extract was determined by using Folin-Ciocalteu's phenol reagent (FCR) and determining absorbance at 760 nm according to the method of Slinkard and Singleton, [15]. The content was expressed as equivalent of pyrocatechol(ug) by using the following equation, which was obtained from a standard pyrocatechol graph Absorbance = 0.001 × pyrocatechol (ug)+ 0.0033.

RESULTS

DPPH Free Radical Scavenging Activity: Decolouration of the stable free DPPH radical by antioxidant in samples was measured spectrophotometrically. According to the results (Table 1) the highest free radical scavenging potential was obtained from *Citrullus colocynthis* extract (81.91%) followed by *Ipomoea pes caprae* (80.66%), *Suaeda maritime* (79.87%) and *Sesuvium portulcasturm* (53.76%). The DPPH radical scavenging capacities of all examined extracts were significantly (p<0.05) lower than the applied positive Control (BHT).

SOD Activity: The result of Superoxide Dismutase (SOD) activity assay showed *Citrullus colocynthis* (97.46%), obtained the highest activity followed by *Suaeda monoica* (91.25%), *Ipomoea pes caprae* (80.66%) and *Sesuvium portulcasturm* (53.76%). SOD activities of all extracts are significantly (p<0.05) different and only *C. colocynthis* extract capacity is not significantly different with positive control (ascorbic acid).

Total Phenolic Content: The calculation of total phenolic content of plant extracts was carried using the standard curve of gallic acid and presented as gallic acid equivalents (GAE) per gram Suaeda maritime extracat casontained ther highest amount of phenolic compounds and the lowest amount is present in *Ipomoea pes caprae* extract. All examined plant extracts had significantly(p<0.05) different contents of phenolics except *Suaeda monoica* which was not significantly different with *Sesuvium portulcasturm* and *Citrullus colocynthis*.

Table 1: DPPH Radical Scavenging Activity of coastal medicinal plants

Plant Samples	DPPH Radical Scavenging Activity(%)
BHT	93.43±0.10
<i>Suaeda monoica</i>	76.56±0.51
<i>Suaeda maritime</i>	79.87±0.21
<i>Citrullus colocynthis</i>	81.91±1.40
<i>Ipomoea pes caprae</i>	80.66±1.30
<i>Sesuvium portulcasturm</i>	53.76±0.26

Table: 2 SOD Activity of the examined sample are presented as inhibition rate

Plant Samples	DPPH Radical Scavenging Activity(%)
Ascorbic Acid (Positive Control)	98.30±0.40
<i>Suaeda monoica</i>	91.25±0.51
<i>Suaeda maritime</i>	86.30±0.54
<i>Citrullus colocynthis</i>	97.46±0.61
<i>Ipomoea pes caprae</i>	60.97±0.72
<i>Sesuvium portulcasturm</i>	57.45±0.60

Table: 3 Total phenolic content of local plant leaves extract

Plant Samples	Total Phenolic Contents (mg of GAE/g)
<i>Suaeda monoica</i>	56.72±2.75
<i>Suaeda maritime</i>	66.64±3.67
<i>Citrullus colocynthis</i>	61.51±4.35
<i>Ipomoea pes caprae</i>	55.33±4.56
<i>Sesuvium portulcasturm</i>	65.49±6.24

DISCUSSION

Free radicals are involved in many disorders like neurodegenerative diseases, cancer and AIDS. Antioxidants through their scavenging power are useful for the management of those diseases. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts [17].

In the study, the antioxidant activities of five local coastal plant usually consumed as salad vegetables were evaluated. Due to the significant contribution to antioxidant activity by phenolic compounds in plants, the total phenolic content of the ethanolic extracts of these five plants, were measured. The results of both DPPH free radical scavenging and SOD assays showed that *Citrullus colocynthis* extract has the highest antioxidant activity followed by *Ipomoea pes caprae*, *Suaeda maritime* showed the lowest antioxidant potential in both assays. Although DPPH radical scavenging activity of *Suaeda monoica* extract was higher than *Sesuvium portulcasturm* it showed the reverse in SOD assay result. These different results indicate that one method alone is not reliable to evaluate the antioxidant activities.

DPPH is a stable free radical and is widely used to assess the radical Scavenging activity of antioxidant compounds. This method is based on the reduction of DPPH in ethanol solution in the presence of hydrogen donation antioxidant due to the formation of the non radical form DPPH-H [18]. The present investigation indicate higher DPPH scavenging activity for *Citrullus colocynthis* (81.91%) at the concentration then *Sesuvium portulcasturm*(57.45%). This is in accordance with the results of Heo *et al.* [19]. These results confirmed the previous studies [20, 21] Which showed the amount to total phenolic in ethanolic extracts of coastal plants.

The enzyme superoxide dismutase(SOD) is believed to be present in the oxygen-metabolizing cells presumably because its physiological function to provide a defense against the potentially damaging superoxide radical generated by aerobic metabolic reaction [22]. The SOD enzyme is necessary for survival of all oxygen-metabolizing cells [23]. The present investigation indicate higher DPPH scavenging activity for *Citrullus colocynthis* (81.91%) at the concentration then *Sesuvium portulcasturm* (57.45%). When compare to control of Ascorbic acid. The plant extracts from *Tephrosia purpurea* increase the activity of SOD by quenching free radicals [24]. A similar activity observed in the present study with the coastal plants extracts.

Phenolic compounds are hydroxyl compounds which are widely distributed in higher plants, being mostly found in fruits, Vegetable and seeds [25]. These polyphenolic compounds are also commonly found in edible and non edible parts of fruits and plants and have been reported to have multiple biological effects including activity [25]. The present investigation indicated higher DPPH scavenging activity for *Suaeda maritime* (66.64%) then *Ipomoea pes caprae* (55.33%). Thus, the antioxidant potential of ethanol extract of coastal plants extracts may be due to the presence of polyphenolic compounds, which needs further analysis.

CONCLUSION

The result of the present study showed that the ethanolic extract of Coastal medicinal plants, which contain highest amount of and phenolic compounds, exhibited the greatest antioxidant activity. The high scavenging property of coastal medicinal plants may be due to hydroxyl groups existing in the phenolic compounds' chemical structure that can provide the necessary component as a radical scavenger.

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