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Halophytes of Chenopodiaceae and Aizoaceae from South-East Coast of India as Potential Sources of Essential Nutrients and Antioxidants

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Abstract In this study, we assessed the antioxidant efficacy and nutritional value of the halophytic plants in order to find possible sources for future novel antioxidants in nutraceutical and pharmaceutical formulations. The lyophilized samples of the five tropical halophytes (Chenopodiaceae and Aizoaceae), namely, *Salicornia brachiata*, *Arthrocnemum indicum*, *Suaeda maritima*, *Suaeda monoica*, and *Sesuvium portulacastrum* from the southeast coast of India were analyzed for total digestible protein, amino acid and fatty acid composition using spectrophotometric, HPLC and GC methods, respectively. The aqueous and ethyl acetate extracts of these samples were studied for its free radical scavenging activity using DPPH· radical scavenging assay and total phenolic content (mg GAE/g) using Folin–Ciocalteu method. Protein content of *S. brachiata* (4.6 g/100g) and *S. maritima* (4.0 g/100g) were higher than that of others. A good ratio of essential/ non-essential (E/NE) amino acids in all species (>1.0) indicated them as sources of well balanced and high-quality proteins. High DHA (1.33%) and EPA (1.26%) in *S. maritima* resulted in having a higher *n*-3:*n*-6 ratio (0.24) than in other halophytes (0.09-0.16). The PUFA/ SFA ratio was found to be significantly higher in *S. brachiata* (1.16) due to high 18:2*n*-6 (16.9%) and 18:3*n*-6 (21.9%) ($p < 0.05$), suggesting its ability to thrive under stress conditions, which was supported by the high phenolic contents (557 mg GAE/g) and antioxidant activity against DPPH radical (IC_{50} 0.90 mg/mL) of its EtOAc extract. The presence of high titer of amino acids, fatty acids, nutritional antioxidants (phenolics) and free radical quenching potential of these underutilized species indicate their potential towards human health applications.

Keywords: *halophytes, fatty acids, antioxidants, phenolics, amino acids*

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1. Introduction

Halophytes or salt tolerant plants are able to grow in saline to extremely saline habitats and have particular characteristics which enable them to evade and/or tolerate salinity by various eco-physiological mechanisms. These plants are naturally grown or cultivated in salt-affected lands such as in saline semi-deserts, mangrove swamps, marshes, sloughs, degraded soils and seashores [11,16]. Amino acids are important osmoregulatory metabolites in halophytes. Earlier reports indicated that the accumulation of amino acid analogues of proline, glycine-betaine and salt stress proteins under stress conditions is related to salt tolerance of plants [2]. Amino acids and amides have been reported to accumulate in higher plants under salinity stress [14]. The response to salinity is reflected in the amino acid pool in halophytes. It has long been recognized that environmental conditions play a major role in determining the quantity and quality of amino acids produced by halophytes. The fatty acids, especially, polyunsaturated fatty acids (PUFAs) are important biochemical indicators of marine plants and large variety of organisms [4]. It was reported that saturated and monounsaturated fatty acids are synthesized in the body, but essential PUFAs cannot be synthesized *de novo* due to the lack of essential enzymes required to synthesize the PUFAs in adequate levels from precursor fatty acids, and therefore, must be externally supplied in the diet in human beings. The halophytes can be the candidate sources to explore these essential nutrients for well being of human populations.

The reactive oxygen species (ROS) are physiological metabolites formed during aerobic life as a result of the metabolism of oxygen. DNA, cell membranes, proteins and other cellular constituents are target site of the degradation processes, and consequently induce different kinds of serious human diseases such as chronic inflammation, atherosclerosis, cancer, cardiovascular disorders, and ageing [21]. Oxidative reactions induced by ROS have potential to affect biomolecules including lipids, carbohydrates and proteins. Halophytes are known for their ability to withstand and quench these ROS, since they are equipped with a powerful antioxidant system that

includes enzymatic and non-enzymatic components [12]. Since there is an increased interest in the antioxidants of natural origin in recent times due to their safety concerns, considerable interest has arisen in finding alternative sources of antioxidants [5]. Therefore, it is rational to explore the underutilized plants such as halophytes for their application in food and pharmaceutical applications.

The nutritional usefulness and antioxidant potential of the halophytes from South Eastern coast of India has been rarely studied. In the present framework, tropical halophytes, namely, *Salicornia brachiata* Miq., *Arthrocnemum indicum* Nels., *Suaeda maritima* L. Dumort, *Suaeda monoica* Forssk ex JF Gmel. from Chenopodiaceae; and *Sesuvium portulacastrum* L. from Azoaceae, abundantly available along the South East coast of the Indian subcontinent were selected for nutritional evaluation with respect to protein, amino acids and fatty acids to make available appropriate nutritional labeling. This study further envisages the antioxidant potential of the aqueous and ethyl acetate extracts of these halophytes with respect to phenolic content and free radical scavenging activity. This information is essential in the search for additional healthy food sources for use as antioxidants, functional foods as well as potential candidates in treatment of cancers and inflammatory diseases.

2. Materials and Methods

2.1. Chemicals and Reagents

All solvents were of analytical or high performance liquid chromatography (HPLC) grade as required (E-Merck, Darmstadt, Germany). All glasswares were rinsed with $CHCl_3/CH_3OH$ (2: l v/v), and dried under N_2. All other reagents were of analytical grade, and purchased from E-Merck. DPPH (1, 1-diphenyl-2-picrylhydrazyl), Folin–Ciocalteau reagent, bovine serum albumin, trichloroacetic acid and gallic acid are obtained from HiMedia. Standards of fatty acid methyl esters (Supelco™ 37 Component FAME Mix, Catalog No. 47885-U), standards of amino acids (PIERCE amino acid standard H), TBHQ, BHT and boron trifluoride/methanol (14% BF_3/CH_3OH, w/v) were procured from Sigma-Aldrich Chemical Co. Inc. (St. Louis, MO).

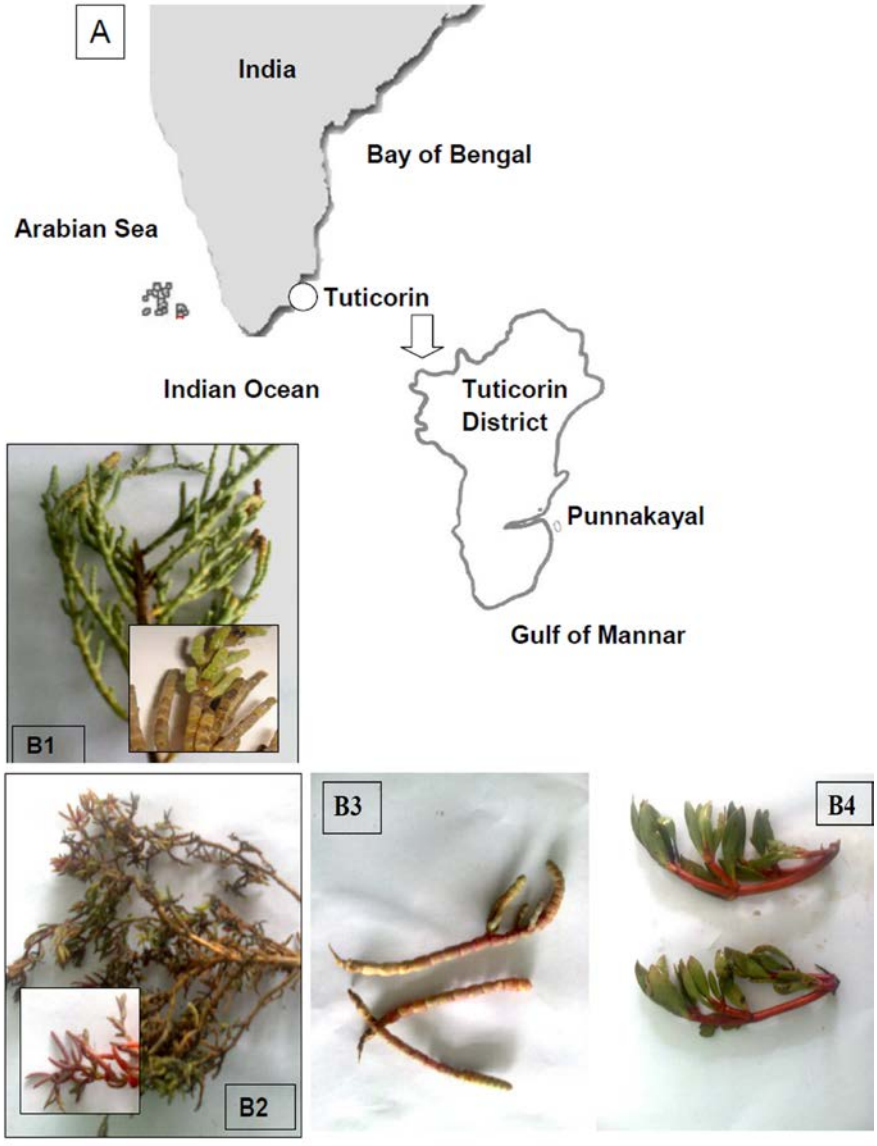

Figure 1. (A) Sampling site of the halophytes; (B) Photographs of (B1) *Salicornia brachiata* (B2) *Suaeda maritima*, (B3) *Arthrocnemum indicum* and (B4) *Sesuvium portulacastrum*

2.2. Samples

Five halophyte species based on their abundance in nature and their sustainable utilization were collected from Punnakayal, situated about 26 km Southeast of Tuticorin in the Gulf of Mannar region on the Southeast coast of India (Lat 8º48' N; Long 78º11' E) **(**Figure 1A**).** The indicative photographs of the halophytic plants, *Salicornia brachiata* and *Suaeda maritima* are shown in Figure 1B1 and Figure 1B2, respectively. The samples were collected in triplicate, stored in separate sterilized polythene bags, and transported to the laboratory for further processing.

2.3. Preparation of Aqueous and Ethyl Acetate Extracts of Halophytes

One kilogram of each halophyte was cleaned with running water to remove extraneous materials, ground in a mixer grinder and lyophilized (24 h) in a laboratory lyophilizer (Alpha 1-4 LD plus, Germany) to obtain the lyophilized halophyte samples (LHS; 800g each). One portion of LHS (100 g each) was refluxed (80-90°C) with double distilled water (1 L X 3), centrifuged in a benchtop refrigerated centrifuge (Thermo Scientific, USA), and the upper layer was lyophilized to furnish aqueous extracts of halophytes (AEH). Another portion of LHS (100 g each) was extracted with EtOAc (1 L X 3), filtered through Whatman No.1 filter paper, and evaporated (50°C) *in vacuo* (Heidolph, Germany) to furnish EtOAc extracts of halophytes (EEH). The extracts were stored at 4°C until further analysis.

2.4. Nutritional Analysis of the Lyophilized Halophyte Extracts

Total proteins, amino acid, and fatty acid profiles of freeze dried extracts of halophytes were determined as detailed below:

2.4.1. Determination of Total Digestible Protein

The total digestible protein content of LHS was estimated by the established method [13] with slight modifications. The absorbance of the protein aliquot was measured at 660 nm in a UV-VIS spectrophotometer (Varian Cary 50). The protein content of the samples was calculated from the standard curve of bovine serum albumin (BSA, 4 mg/ml in distilled water), and expressed as g/100g LHS.

2.4.2. Amino Acid Analyses

Estimation of amino acids in lyophilized halophyte samples (LHS) was carried out using the Pico - Tag method as described earlier [10] with slight modifications. In brief, 0.1g of the samples was dissolved in 10ml of 6 N HCl, digested at 110°C in sealed glass tubes for 24 h. The solution was cooled and filtered through GF/A filter paper to obtain the filtrate, which was evaporated *in vacuo* (Heidolph, Germany), using distilled water (20 ml X 3) to remove the acid. The acid-free sample was then made up to 5 ml with HCl (0.05 N), and filtered through a nylon filter syringe (0.2 µ). The aliquot containing hydrolyzed amino acids was treated with redrying reagent (methanol 95%: water: triethylamine, 2:2:1 v/v/v), and thereafter pre-column derivatization of hydrolyzable amino acids was performed with phenylisothiocyanate to form phenylthiocarbamyl amino acids (PTC). The reagent was freshly prepared, and the composition of derivatising reagent (methanol 95%: triethylamine: phenylisothiocyanate, 7:1: l v/v/v, 20µL). The contents were thoroughly mixed and allowed to stand at room temperature, and thereafter removed under vacuum. The derivatized sample (PTC derivative, 20 µL) was diluted with sample diluent (20 µL, 5 mM $NaHPO_4$ buffer, pH 7.4: acetonitrile 95:5 v/v) before being injected into reverse-phase binary gradient HPLC (Waters Corp., Milford, Massachusetts, USA), fitted with a packed column (dimethylocatadecylsilyl- bonded amorphous silica; Nova-Pak C_{18}, 3.9 X 150 mm) maintained at 38 ± 1°C in a column oven to be detected by UV (λ_{max} 254 nm, Waters 2487 dual absorbance detector). The mobile phase eluents used were eluents A and B (A: sodium acetate trihydrate (0.14 M, 940 ml, pH 6.4) containing triethylamine (0.05%), mixed with acetonitrile (60 ml); B: acetonitrile: water 60:40, v/v). A gradient elution program, with increasing eluent B was employed. The standard was run before each sample injection in triplicate, and the output was analyzed using BREEZE software. Amino acid content was expressed as mg /100g protein.

2.4.3. Fatty Acid Composition Analysis by Gas-Liquid Chromatography

The fatty acid composition of the total lipids of the lyophilized halophyte samples (LHS) were determined as described elsewhere [5,17] with slight modifications. In brief, the triglycerides were saponified with alkaline reagent (3 ml, 0.5 N KOH/MeOH). The saponificable materials were thereafter reacted with a methylating mixture (14% BF_3/CH_3OH) yielding fatty acid methyl esters (FAME) that was later extracted with *n*-hexane/H_2O (1:2, v/v). The *n*-hexane layer was suitably dried to be reconstituted in petroleum ether (40-60°C), and stored at -20°C until required. The gas chromatograph (Perkin Elmer, USA; HP 5890 Series II) was equipped with a SP 2560 (crossbond 5% diphenyl- 95% dimethyl polsiloxane) capillary column (100 m X 0.25 mm i.d., 0.50 µm film thickness, Supelco, Bellfonte, PA) using a flame ionization detector (FID) equipped with a split/splitless injector, which was used in the split (1:15) mode. The oven temperature ramp program: 140°C for 1 min, rising at 30°C / min to 250°C, where it was held for 1.0 min, followed by an increase of 25°C /min to 285°C, where it was held for 2.0 min, until all peaks had appeared. The injector and detector were held at 285 and 290°C, respectively. Nitrogen (> 99.99%) was used as the carrier gas at 25 cm/s flow rate. The injection volume was 0.02 µL. FAMEs were identified by comparison of retention times with known standards. Results were expressed as percent weight of total fatty acids (% TFA).

2.5. Evaluation of Antioxidant Activity

The antioxidant capacities of the aqueous (AEH) and EtOAc (EEH) extracts of the halophytes were determined, by the total phenolic contents (TPC) and DPPH radical scavenging assay as illustrated below.

2.5.1. Total Phenolic Content

Total phenolic content in AEH and EEH were determined by an established method [15] with slight

modification. The samples (5 mg/ml) in MeOH were added to the Folin Ciocalteu reagent (5 ml, diluted ten fold with distilled water) and Na_2CO_3 (0.7 M, 4 ml). Gallic acid was used as standard, and a calibration curve was plotted using serial dilution of gallic acid (0.2, 0.1, 0.05, 0.005 mg/ml) in distilled water. The absorbance of the aliquot was measured at 760 nm after incubating the sample for 2 h at room temperature. The total phenolic content was expressed as milligram gallic acid equivalent (mg GAE)/g extract.

2.5.2. 2, 2-Diphenyl -1- Picryl Hydrazyl (DPPH) Radical Scavenging Activity

$DPPH^{\cdot}$ radical scavenging activity of AEH and EEH were carried out following established method [22] with slight modification. Briefly, different concentrations of the extracts in MeOH (ranging from 0.1 to 1.0 mg/ml) were prepared and each solution (2 ml) was mixed with 0.16 mM $DPPH^{\cdot}$ solution (2 ml in MeOH). The mixtures were shaken vigorously and maintained for 30 min at ambient temperature (30°C) in the dark. The absorbance of mixtures was measured at 517 nm against a reagent blank by using UV–VIS spectrophotometer (Varian, Cary 50), and from the plot of scavenging activity on $DPPH^{\cdot}$ IC_{50} value (concentration of the sample to scavenge 50% of the DPPH radicals; mg/ml) was calculated. The analyses were performed in triplicate.

2.6. Statistical Analysis

One-way analysis of variance (ANOVA) was carried out with the Statistical Program for Social Sciences 13.0 (SPSS, USA, ver. 13.0) to assess for any significant differences between the means. Differences between means at the 5% ($p < 0.05$) level were considered significant. The mean variance in the data set was detected using principal component analysis (PCA). The selected variables for PCA were total phenolic contents (TPC, mg GAE/g) DPPH scavenging activity, total PUFA and total amino acids (TAA) of aqueous and EtOAc extracts.

Table 1. Total digestible protein and amino acid composition of lyophilized halophyte samples (LHS)

Total digestible protein	4.62 ± 0.006[a]	3.93 ± 0.02[b]	4.02 ± 0.08[b]	3.78 ± 0.22[b]	3.43 ± 0.02[c]
			Amino acid (mg/100 g protein)		
Essential amino acids	*S.brachiata*	*A.indicum*	*S.maritima*	*S.portulacastrum*	*S. monoica*
His (1.9 mg/100g)	9.62 ± 0.06[a]	3.44 ± 0.01[b]	3.21 ± 0.01[b]	3.44 ± 0.02[b]	2.53 ± 0.03[c]
Arg	49.0 ± 0.5[a]	14.3 ± 0.1[b]	13.5 ± 0.18[b]	12.4 ± 0.16[b]	8.96 ± 0.46[c]
Thr (3.4 mg/100g)[p]	33.9 ± 0.4[a]	10.1 ± 0.1[b]	10.5 ± 0.1[b]	9.4 ± ± 0.2[b]	6.22 ± 0.03[c]
Val (3.5 mg/100g)[p]	51.5 ± 0.24[a]	14.0 ± 0.2[b]	13.1 ± 0.11[c]	12.4 ± 0.05[d]	8.18 ± 8.18[e]
Met[p]	11.6 ± 0.18[a]	3.16 ± 0.09[b]	3.04 ± 0.08[b]	3.24 ± 0.22[b]	2.09 ± 0.03[c]
Ile (2.8 mg/100g)[p]	46.0 ± 0.23[a]	11.2 ± 0.06[b]	10.5 ± 0.05[b]	9.56 ± 0.12[b]	6.34 ± 0.01[c]
Leu (6.6 mg/100g)[p]	70.0 ± 1.0[a]	17.4 ± 0.05[b]	16.4 ± 0.17[b]	15.6 ± 0.5[b]	10.2 ± 0.03[c]
Phe[p]	30.5 ± 0.25[a]	7.92 ± 0.07[b]	7.49 ± 0.04[bc]	7.21 ± 0.08[c]	5.44 ± 0.2[d]
Lys (5.8 mg/100g)[p]	89.9 ± 0.2[a]	27.6 ± 0.6[b]	26.6 ± 0.3[bc]	25.6 ± 0.2[bc]	24.6 ± 1.1[c]
Σ Essential amino acid	392.02[a]	109.12[b]	104.34[b]	98.85[b]	74.56[c]
Non-essential amino acids					
Asp	86.3 ± 1.3[a]	23.8 ± 0.8[b]	22 ± 1 ± 0.4[b]	21.9 ± 0.4[b]	15.6 ± 0.4[c]
Glu	124.2 ± 2[a]	32.1 ± 0.4[b]	30.2 ± 0.3[b]	29.6 ± 1[b]	19.7 ± 1.2[c]
Ser	29.9 ± 1.4[a]	8.75 ± 0.25[b]	8.04 ± 0.17[bc]	8.49 ± 0.03[b]	6.42 ± 0.08[c]
Gly	19.4 ± 0.2[a]	5.34 ± 0.13[b]	5.0 ± 0.01[b]	5.14 ± 0.02[b]	4.26 ± 0.01[c]
Ala	44.2 ± 0.08[a]	11.4 ± 0.16[b]	9.0 ± 0.2[b]	9.45 ± 0.2[b]	7.28 ± 0.03[c]
Pro	14.1 ± 0.4[a]	3.67 ± 0.11[b]	3.56 ± 0.09[b]	3.49 ± 0.01[b]	3.63 ± 0.01[b]
Tyr (6.3 mg/100g)	17.2 ± 0.2[a]	5.05 ± 0.07[b]	4.49 ± 0.04[b]	4.14 ± 0.06[b]	2.32 ± 0.01[c]
Cys (2.5 mg/100g)	3.25 ± 0.01[a]	0.97 ± 0.02[b]	0.75 ± 0.03[b]	0.88 ± 0.07[b]	1.25 ± 0.03[b]
Σ Non-essential amino acid	338.35[a]	91.08[b]	83.04[b]	83.09[b]	60.46[c]
ΣAmino acid	730.37[a]	200.2[b]	187.38[b]	181.94[b]	135.02[c]
E/NE ratio	1.16[a]	1.20[a]	1.26[a]	1.19[a]	1.23[a]

[p]Essential amino acid for humans. Total digestible protein represented in g/ 100g LHS. Amino acid composition represented in mg/ 100g protein. FAO/WHO reference pattern (1990) for evaluating proteins (mg/ 100g) were indicated in parentheses (FAO/WHO, 1990). Tryptophan was not determined.

3. Results and Discussion

3.1. Total Digestable Protein Content in Halophyte Samples

The total digestible protein contents of the lyophilized halophyte samples (LHS) are presented in Table 1. Among the different species, *S. brachiata* exhibited significantly higher protein content than others ($p < 0.05$). The lowest digestible protein content was recorded in *S. monoica*. However, no significant differences in protein were apparent in *S. maritima*, *A. indicum*, and *S. portulacastrum* ($p > 0.05$). Halophyte *Atriplex* spp were reported to have high protein content (12-22%) [23], whereas the protein content of *Salicornia bigelovii* found to be only 4 to 6% [9].

3.2. Amino Acid Content in Halophytes

The essential, non-essential amino acid compositions and the ratio of essential to non-essential (E/NE) amino acids of lyophilized halophyte samples (LHS) are recorded in Table 1.

3.2.1. Essential Amino Acids

The essential amino acids, *viz.*, histidine, arginine, threonine, valine, methionine, leucine, isoleucine, phenyl alanine and lysine were found to be present in the halophytes. Among the five species analyzed significantly higher ($p < 0.05$) amount of total amino acids were found in *S. brachiata* (730.4 mg/100g protein) followed by *A. indicum*, *S. maritima*, *S. portulacastrum*, and the lowest was found in *S. monoica* (Table 1). In general, lysine, which is absent in cereals, constitutes a major share to the total essential amino acid pool in halophytes. The amount of lysine was in the order: *S. brachiata* > *A. indicum* > *S. maritima* > *S. portulacastrum* > *S. monoica*. Threonine, another essential amino acid deficient in cereals, was found to be more in *S. brachiata* (33.9 mg/100g protein) as compared to the other halophytes (6 - 10.5 mg/100g protein). Leucine, valine, arginine, and isoleucine were the other predominant essential amino acids found in *S. brachiata*. It was reported that valine, lysine, histidine, and arginine were observed under conditions of hyposalinity (10%) and in hypersalinity (65%) [20]. Threonine, phenylalanine, methionine, and histidine constitute a minor share of total essential amino acid (< 34%) in *S. brachiata*. The amino acid profile of these salt stressed plants showed that all essential amino acids were significantly higher in concentrations, when compared with the reference pattern [8], which implied that the proteins present had a high biological value, and are therefore called complete proteins.

3.2.2. Non-Essential Amino Acids

Among the non-essential amino acids, negatively charged amino acid glutamate was observed to be the predominant amino acid, in all the species and was in the order: *S. brachiata* > *A. indicum* > *S. maritima* > *S. portulacastrum* > *S. monoica* (Table 1). The second most non-essential amino acid was found to be aspartate in all the five species (15.6-86.3 mg/100g). These reports are in agreement with the present study that these amino acids occupy a major share in total non essential amino acid pool under salinity stress [20]. Since negatively charged ions (aspartate and glutamate) play a significant role in osmoregulation, the change in amino acid constituents in response to salinity is considered an important factor. Earlier reports indicated that raising the salinity stimulated an immediate increase in the amino acids aspartate, glutamate, glycine, histidine, lysine, and arginine [20]. The present study indicated that the secondary amino acid proline, which was known to be present as low as 1-2% in non-saline crops was found abundant in all the halophytes, especially *S. brachiata*. Earlier studies revealed that the appearance of proline at hypersalinity is supportive of the fact that it acts as an osmoticant [20]. Table 1 shows the amino acid pattern of halophytes as compared to the FAO/WHO [8] for evaluating proteins [8]. Glycine was found to constitute a major share in *S. brachiata* that was significantly higher than those in other species (4.3 - 5.3 mg/100g protein), thereby signifying the potential capability of this species to withstand salinity and adverse stress conditions. It may be possible that the amino acid glycine or its conjugate (glycine betaine) that was earlier reported to have unique osmotic property [6] helps to protect the cells of halophytes against osmotic injury. It is apparent that the osmotic activity of amino acids is due to its dipolar zwitterion characteristics, and solubility in water.

3.2.3. Essential to Non-Essential Amino Acid Ratio (E/NE Ratio)

The present study indicated a reasonably good ratio of essential to non-essential amino acids (E/NE, mg/100 g protein) for all species (1.16 in *S. brachiata* to as high as 1.23 in *S. monoica*) (Table 1). The results obtained from this study showed that *Suaeda* sp possesses well-balanced and high-quality protein source in the respect of E/NE ratio (1.26). Any ratio of E/NE amino acids higher than 1.0 is considered to be excellent [8], and therefore it can be concluded that all the studied halophytes are sources of well balanced proteins and high-quality protein source in respect of E/NE ratio.

3.3. Fatty Acid Composition in Halophytes

The different groups of fatty acids, *viz.*, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and PUFAs in LHS of *Salicornia brachiata*, *Suaeda maritima* and *Suaeda monoica* are illustrated under the following sections.

3.3.1. Saturated Fatty Acids (SFAs)

The composition of SFAs reflected in the three candidate halophytes are indicated in Table 2. The fatty acid composition in *S. brachiata* exhibited the lowest content of SFAs (38.1% TFA) among the three candidate species thereby indicating its superior nutritional quality with respect to SFA (Table 2). The percentage of SFAs in the halophytes was in the following descending order: *S. monoica* > *S. maritima* > *S. brachiata*. Among SFAs 16:0 was found to be prominent (16-21% TFA) contributing 21.1% of TFAs in *S. brachiata* and *S. maritima*. However, *S. monoica* exhibited the lowest level of this fatty acid among others (16.1% TFA). An earlier report indicated that the lipids in the seeds of halophytic plants

Arthrocnemum indicum, *Cressa cretica*, *Halopyrum mucronatum*, *Haloxylon stocksii* and *Suaeda fruticosa* were found to contain four major saturated fatty acids, including 16:0 [24]. Among other SFAs, 14:0, 18:0, and 22:0 contributed a major share in the present study. *S. brachiata* and *S. monoica* showed significantly high 18:0 content (> 5.5%) compared with *S. maritima* ($p < 0.05$) (< 5.0%) (Table 2).

Table 2. Fatty acid composition (% TFA) of lyophilized halophyte samples (LHS) *S. brachiata S. maritima* and *S. monoica*

	Fatty acid (% total fatty acids)		
Fatty acids	*S. brachiata*	*S. maritima*	*S. monoica*
	Saturated		
12:0	0.62 ± 0.01[a]	0.47 ± 0.01[b]	0.54 ± 0.02[a]
14:0	2.08 ± 0.02[a]	4.24 ± 0.04[b]	4.36 ± 0.11[b]
15:0	0.92 ± 0.04[a]	0.47 ± 0.02[a]	8.11 ± 0.41[c]
16:0	21.1 ± 0.4[a]	21.1 ± 0.1[a]	16.08 ± 0.24[b]
17:0	0.23 ± 0.02[a]	3.14 ± 0.11[b]	5.36 ± 0.07[b]
18:0	5.55 ± 0.3[a]	4.08 ± 0.07[b]	5.9 ± 0.01[a]
20:0	0.46 ± 0.01[a]	0.55 ± 0.04[a]	1.61 ± 0.02[b]
22:0	4.16 ± 0.04[a]	6.51 ± 0.18[a]	4.29 ± 0.03[a]
24:0	2.93 ± 0.02[a]	5.57 ± 0.02[b]	4.62 ± 0.06[ab]
Σ SFA	38.13 ± 1.02[a]	46.12 ± 1.32[b]	50.86 ± 2.42[b]
	Monounsaturated		
14:1*n*-7	0.39 ± 0.03[a]	6.2 ± 0.12[b]	0.54 ± 0.01[a]
15:1*n*-7	3.08 ± 0.02[a]	1.26 ± 0.04[b]	2.35 ± 0.01[ab]
16:1*n*-7	0.46 ± 0.01[a]	0.78 ± 0.04[a]	1.41 ± 0.01[a]
18:1*n*-7	0.23 ± 0.02[a]	0.39 ± 0.06[a]	0.54 ± 0.02[a]
18:1*n*-9	7.55 ± 0.32[a]	3.69 ± 0.13[b]	4.82 ± 0.14[b]
20:1*n*-9	2.23 ± 0.01[a]	3.22 ± 0.17[a]	2.88 ± 0.06[a]
22:1*n*-9	2.08 ± 0.04[a]	4.08 ± 0.04[b]	3.75 ± 0.05[ab]
24:1*n*-9	0.31 ± 0.02[a]	1.33 ± 0.01[b]	0.2 ± 0.001[a]
Σ MUFA	16.33 ± 1.02[a]	20.94 ± 2.02[b]	16.48 ± 1.72[a]
	Polyunsaturated		
16:2*n*-4	0.77 ± 0.06[a]	2.04 ± 0.01[b]	2.28 ± 0.16[b]
16:3*n*-4	0.23 ± 0.028[a]	2.75 ± 0.03[b]	3.62 ± 0.03[c]
18:2*n*-6	16.8 ± 0.8[a]	12.6 ± 0.6[b]	14.0 ± 0.2[ab]
18:3*n*-6	21.8 ± 0.5[a]	6.35 ± 0.10[b]	5.96 ± 0.7[b]
18:3*n*-3	2.23 ± 0.02[a]	2.35 ± 0.01[a]	2.35 ± 0.15[a]
20:2*n*-6	0.62 ± 0.01[a]	0.63 ± 0.06[a]	0.34 ± 0.02[b]
20:3*n*-6	0.15 ± 0.03[a]	0.63 ± 0.02[b]	0.47 ± 0.01[c]
20:4*n*-6	0.23 ± 0.01[a]	0.39 ± 0.015[a]	0.27 ± 0.07[a]
20:5*n*-3	0.46 ± 0.04[a]	1.26 ± 0.03[b]	0.34 ± 0.08[a]
22:6*n*-3	0.92 ± 0.04[a]	1.33 ± 0.03[a]	0.6 ± 0.1[ab]
Σ PUFA	44.3 ± 2.02[a]	30.3 ± 1.82[b]	30.2 ± 1.12[b]
∑*n*-3	3.61 ± 0.06[a]	4.94 ± 0.12[a]	3.29 ± 0.05[a]
∑*n*-6	39.7 ± 1.01[a]	20.6 ± 0.98[b]	21.0 ± 1.05[b]
n-3/*n*-6	0.09 ± 0.02[a]	0.24 ± 0.07[b]	0.16 ± 0.02[ab]
∑ PUFA/∑ SFA	1.16 ± 0.02[a]	0.66 ± 0.03[b]	0.59 ± 0.05[b]

ΣSFA: total saturated fatty acids; ΣMUFA: total monounsaturated fatty acids; ΣPUFA: total polyunsaturated fatty acids, data presented as mean values of three samples (mean ± standard deviation). These values do not total 100% because minor fatty acids are not reported.

3.3.2. Monounsaturated Fatty Acids (MUFAs)

Among MUFAs, 18:1*n*-9 was found to be the prominent fatty acid in *S. brachiata* and *S. monoica* whereas 14:1*n*-7 dominated in *S. maritima*. The highest levels of MUFAs were found in *S. maritima* (21.0% TFA), which was mostly contributed by 14:1*n*-7 (6.2% TFA), 18:1*n*-9 (3.7 % TFA), 20:1*n*-9 (3.2% TFA), and 22:1*n*-9 (4.1% TFA). Significant differences ($p < 0.05$) were observed for 14:1*n*-7 in *S. maritima* (6.20% TFA) and that in *S. brachiata* and *S. monoica* (0.4-0.5% TFA). No significant differences in 16:1*n*-7 between the candidate holophytic species were observed (0.5-1.4% TFA, $p > 0.05$) (Table 2).

3.3.3. Polyunsaturated Fatty Acids (PUFAs)

PUFAs are considered as single most important nutritional indicator in these candidate halophytes. The total PUFA content of the candidate halophytes is presented in Table 2. The highest PUFA was observed in *S. brachiata* (44.3% TFA) followed by *S. maritima* (30.3% TFA) and *S. monoica* (30.2% TFA), thereby revealing their use as high value nutritional supplements. These results are similar to those reported in an earlier study, reporting that the halophytes, *viz*., *Arthrocnemum indicum*, *Cressa cretica*, *Halopyrum mucronatum*, *Haloxylon stocksii* and *Suaeda fruticosa* were found to possess potentially high unsaturated fatty acids (65–74%) [24]. The eicosapentaenoic acid (EPA, 20:5*n*-3) and docosahexaenoic acid, DHA (22:6*n*-3) are two important *n*-3 PUFAs required essentially for human beings. Among *n*-3 fatty acids, EPA was found to be the most abundant in *S. maritima* (1.3% TFA) than in *S. brachiata* (0.5% TFA), and *S. monoica* (0.3% TFA). The high DHA and EPA (both 1.3% TFA) levels in *S. maritima* caused it to have a higher *n*-3: *n*-6 ratio (0.24) than in other species (0.09-0.16) (Table 2). The fatty acids EPA and DHA was found to be significantly low in *S. brachiata* and *S. monoica* apparently due to the absence of specific elongases and Δ^5-desaturases responsible to biosynthesize 20:5*n*-3 from its precursor molecule (18:3*n*-3). The C_{18} PUFAs were present in significantly higher amounts in *S. brachiata* (40.1% TFA), although significant C_{16} PUFAs *viz*., 16:2*n*-4 (2.3% TFA) and 16:3*n*-6 (3.6% TFA) was apparent in *S. monoica*. *S. monoica* and *S. maritima* were recorded to contain C_{16} PUFAs in significantly higher amount (5.9 and 4.8%, respectively) than in *S. brachiata* (1%, $p < 0.05$). No significant differences were apparent in the percentage of 20:2*n*-6, 20:3*n*-6, and 20:4*n*-6 in these candidate halophytes ($p>0.05$). *S. brachiata* exhibited maximum PUFA/SFA ratio than in the other two species. The PUFA/SFA ratio was found to be significantly high in *S. brachiata* apparently due to the significantly higher value of 18:2*n*-6 (16.8%) and 18:3*n*-6 (21.8%) ($p < 0.05$) among all the fatty acid (Table 2). Apparently, *S. brachiata* recorded the significantly high *n*-6 fatty acids ($p < 0.05$), whereas *S. maritima* exhibited maximum *n*-3 fatty acids (4.9% TFA) than others. Higher content of *n*-6 fatty acids in *S. brachiata*, suggests its ability to thrive under high salinities.

3.4. Yield of Aquous and Ethylacetate (EtOAc) Extracts of Halophytes

The percent yield (% w/w of LHS) of the aqueous extracts (AEHs) of *A. indicum* and *S. maritima* were found to be significantly higher ($p < 0.05$) (9.9 and 9.0%, respectively) than recorded in *S. brachiata* (5.14%), *S. portulacastrum* (6.5%), and *S. monoica* (4.0%). Among EtOAc extracts (EEHs), *S. brachiata* and *S. portulacastrum* recorded maximum yield (0.5 and 0.6%, respectively) compared with *S. maritima* (0.36%) and *A.indicum* (0.34%) and *S. monoica* (0.31%).

3.5. Antioxidant Activities of Aquous and EtOAc Extracts of Halophytes

The antioxidant activities of the aqueous and EtOAc extracts of halophytes (AEH and EEH, respectively) were determined, by the total phenolic content (TPC) and DPPH radical scavenging activity assays as illustrated below.

3.5.1. Total Phenolic Content

Polyphenolics are popularly termed as nutritional antioxidants that were measured in this study in order to understand the antioxidant defenses in the aqueous and ethyl acetate extracts of the experimental halophytes. The aqueous extracts of *S. maritima* and *S. portulacastrum* showed total phenolic content as 67.8 and 76.5 mg GAE/g in *S. monoica*. The observations are in accordance with the earlier report [1] who reported that dried aqueous and acetone extract of the leaves of *S. maritima* had significant differences in phenolic contents. The total phenolic content of the aqueous extracts of the experimental halophytes followed the descending order: *S. portulacastrum* > *S. maritima* > *A. indicum* > *S. brachiata* > *S. monoica* (Figure 2A). The local inhabitants from Gulf of Mannar area of south India consume cooked leaves of *S. portulacastrum* as a food item in their diets, apparently due to high phenolic compounds, which induce flavor in addition to high antioxidant activities. An earlier study reported the phenolic content of *S. portulacastrum* as 55.1 mg GAE/g in aqueous extracts [1]. Earlier studies also revealed that holophytic algal species possess polyphloroglucinol phenolics (phlorotannins) as phenolic compounds (Nakamura et al., 1996). EtOAc extracts of *S. brachiata* and *S. maritima* recorded significant difference (557 and 491 mg GAE/g, respectively) in total phenolic contents (Figure 2A). EtOAc extracts of other halophytes followed the order: *S. monoica* > *S. portulacastrum* > *A. indicum*. In general, the total phenolic contents of EtOAc extract were significantly higher than those of the aqueous extracts (Figure 2A) ($p < 0.05$). An earlier report indicated that EtOAc extracts of *S. brachiata* were characterized by higher polyphenol contents (557 mg of GAE/g DW), as compared to the aqueous extracts (54.2 mg of GAE/g DW) [11]. The higher total phenolic contents in these halophytes may possibly be related to the presence of amino acids *viz.*, aspartate, proline, lysine, and glycine, which synthesize numerous substances including phenolics by involving themselves in protein or energy metabolism, and /or transmethylation reactions. Amounts of phenolic compounds were reported to vary greatly among different halophytic *Mesembryanthemum* spp. *M. edule* had the highest total phenolic contents (70 mg GAE /g DW), whereas *M. nodiflorum* and *M. crystallinum* exhibited no significant differences in their phenolic contents [7].

3.5.2. Free Radical Scavenging Activity

The aqueous extract of *A. indicum* was found to exhibit reasonably strong antioxidant activity (IC_{50} 1.04 mg/ml) and thereby proved to be endowed with strong antioxidant activity (Figure 2B). No significant difference ($p> 0.05$) was apparent in the scavenging activities exhibited by aqueous extracts of the other four species. Interestingly, the aqueous extract of *S. brachiata* recorded the lowest DPPH radical scavenging activity (IC_{50} 1.89 mg/ml) (Figure 2B). Phenolic compounds, including procatechuic acid, ferulic acid, caffeic acid, quercetin, and isorhamnetin, were isolated from *S. herbacea*, and found to be responsible for radical scavenging properties [19]. The

antioxidant activities of the related species (*A. indicum* and *S. brachiata*) may be due to the analogues of these compounds. The antioxidant activities of the aqueous extracts of *Salicornia* spp may be due to the combined effects of estrogen-like compounds that could play a protective role in overiectomic conditions against free radical production [19]. No significant difference ($p>0.05$) in DPPH radical scavenging activity (IC_{50} 0.89 - 0.96 mg/ml) were evident in the EtOAc extract of the experimental halophytes and was in the order: *S. maritima* > *S. brachiata* > *A.indicum* > *S. monoica* > *S. portulacastrum* (Figure 2B). The DPPH radical scavenging activity of EtOAc extract (IC_{50} < 1 mg/ml) was found to be significantly stronger than those of the aqueous extracts (IC_{50} >1 mg/ml) (Figure 2B). Therefore, compounds in the EtOAc extracts of halophytes appeared to be the reservoir of compounds responsible to scavenge free radicals, and seem to play an important role in the anti-oxidative capacity. It is apparent that the compounds that are soluble in EtOAc may not be soluble in water, and, therefore differential activities of EtOAc and aqueous extracts in halophytes were recorded. In addition, the organic polar solvent extracts (EtOAc) of halophytes may contain polyphenolic compounds capable of deactivating DPPH free radical. These observations clearly depict that the antioxidant properties exhibited by potential halophytic species in the present study may be due to the presence of phlorotannins and polyphenolic compounds, or any other potential antioxidants present with them. It can be generalized that EtOAc extract of *S. brachiata* and *S. maritima* possess reasonably good polyphenolic content with free radical scavenging activity, and are the candidate halophytic species to further explore the compounds responsible for radical scavenging activities.

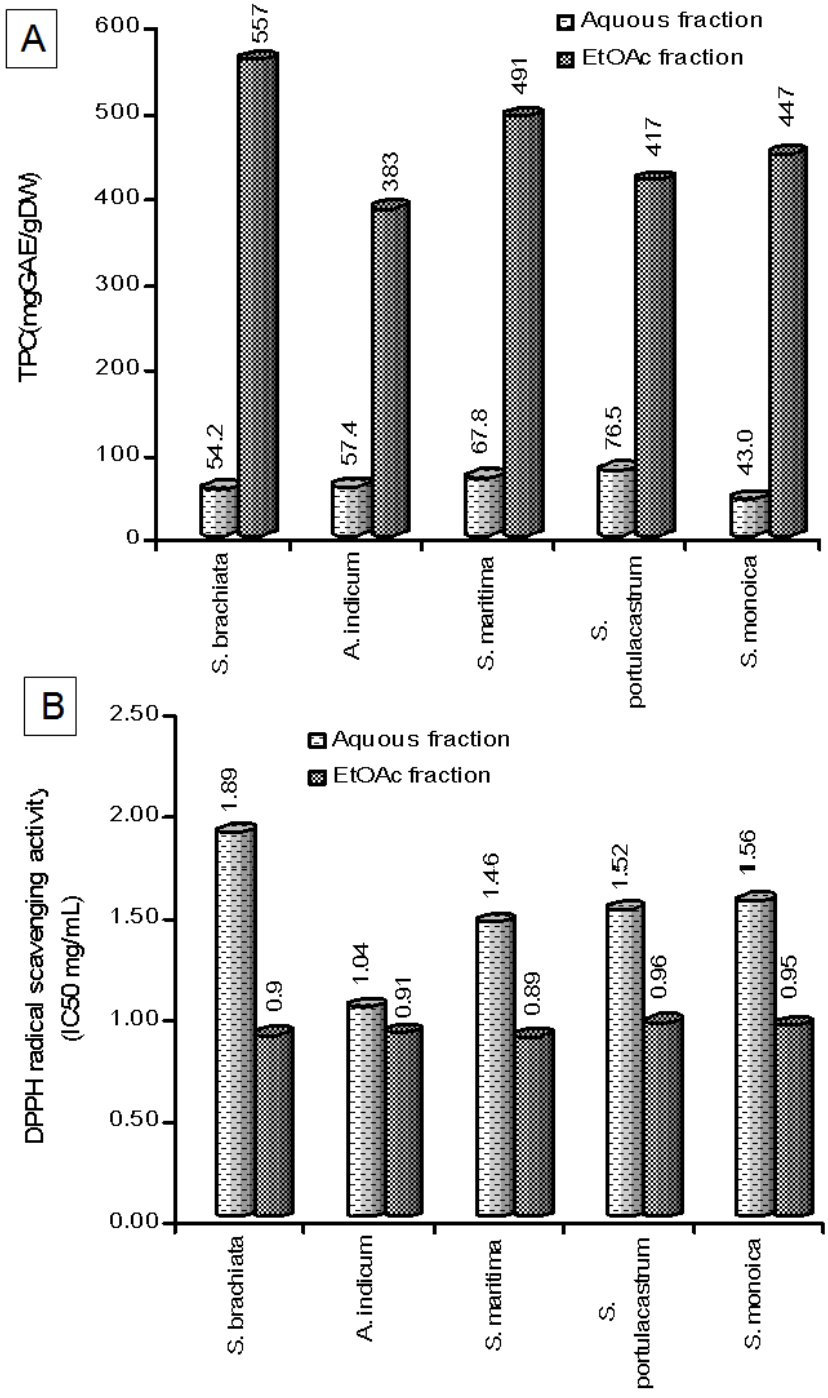

Figure 2. (A) Total phenolic content and (B) DPPH radical scavenging activities of halophyte extracts

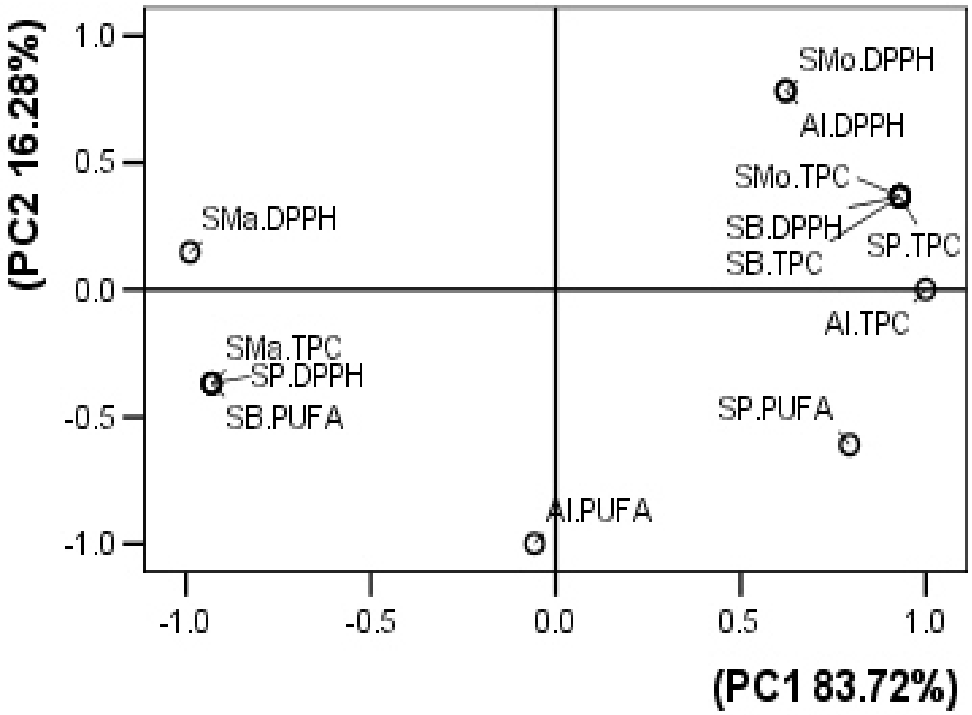

Figure 3. Loading plot of total phenolic content, DPPH· scavenging activities and total PUFA of EtOAc extracts

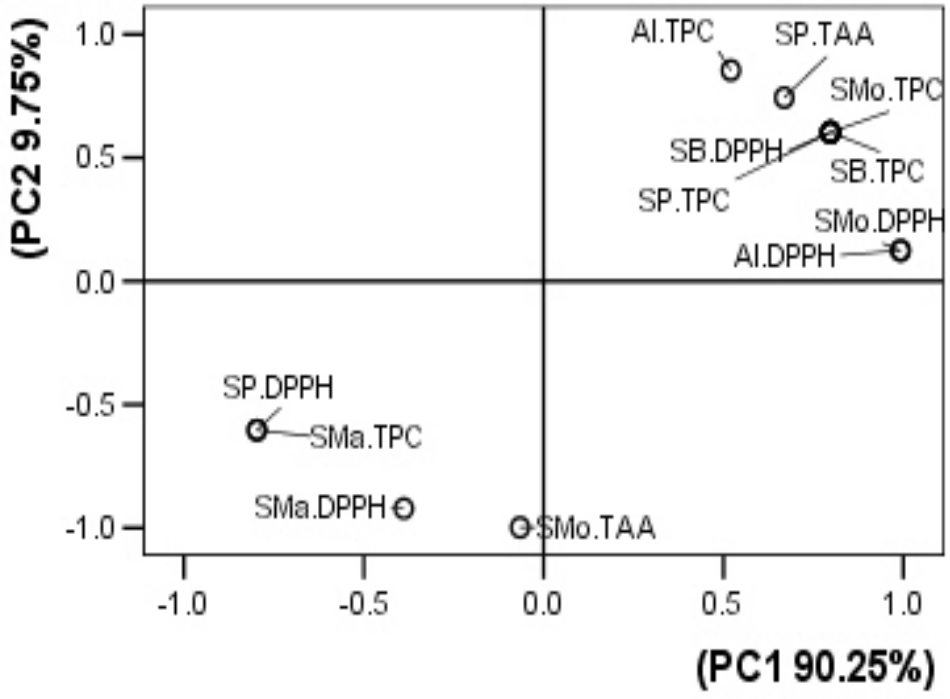

Figure 4. Loading plot of total phenolic content, DPPH· scavenging activities and TAA of aqueous extracts

3.6. Correlations between Antioxidant Activities of EtOAc and Aqueous Extracts of Halophytes

The similarities and differences among EtOAc and aqueous extracts of the five selected halophyte species, *S. brachiata* (SB), *A. indicum* (AI), *S. maritima* (SMa), *S. portulacastrum* (SP), and *S. monoica* (SMo), and the relationships among total phenolic content (TPC), DPPH radical scavenging activity, and nutritional parameters were statistically analyzed using PCA. With respect to EtOAc extracts, the first two principal components explained 100% (PC 1 –83.72%; PC 2-16.28%) of the total variance in the data set (Figure 3). The first two principal components explained 100% (PC 1 –90.25%; PC 2-9.75%) of the total variance in the data set for EtOAc extract (Figure 4). A significant correlation was observed between TPC and DPPH radical scavenging assays ($p < 0.001$) of EtOAc extract between *S. brachiata* (SB), *A. indicum* (AI), *S. portulacastrum* (SP), and *S. monoica* (SMo), thereby indicating the role of phenolic compounds

to impart antioxidant activities. However, a negative correlation between DPPH and total phenolic contents was apparent for the EtOAc extract of *S. maritima* (SMa) demonstrating the presence of other non-phenolic compounds possessing antioxidant activities. In an aqueous extract too the total phenolic content and DPPH radical scavenging activities were deduced to be positively correlated with *S. brachiata* (SB), *A. indicum* (AI), *S. portulacastrum* (SP), and *S. monoica* (SMo) as in it's EtOAc extract, excepting *S. maritima* (SMa) thereby indicating the presence of non-phenolic compounds responsible for antioxidant activities in the later (Figure 5). No significant correlation between the fatty and amino acid contents *vis-à-vis* TPC and antioxidant activities could be deduced from PCA analyses. The Pearson correlation of the aqueous and EtOAc extracts were shown to be not correlated which proved that the activity of these different extracts may be due to different sets of compounds, for aqueous extracts, polar high molecular weight compounds; and less polar phenolic compounds for EtOAc extracts (Figure 5).

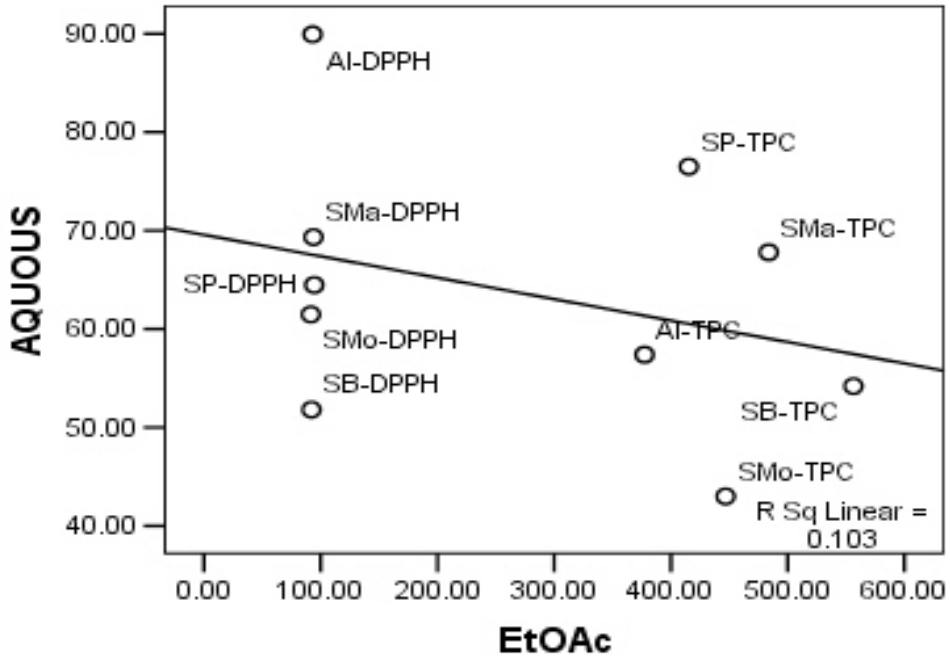

Figure 5. Correlation plot between aqueous and EtOAc extracts towards TPC and DPPH scavenging activities

4. Conclusions

The present study provides valuable information regarding the potential of halophytes as natural sources for antioxidants, high value protein, balanced amino acids and fatty acids. The results emerged from the present study validate the potential use of renewable sources like these candidate halophytes to offer excellent nutritional and health package for use in food supplements in nutraceutical formulation, and as health food for human consumption. Considering the underutilization of salt marsh land in coastal habitats, development of any biologically useful products from halophytes has dual benefits-as health products and their commercial farming in coastal habitats, resulting in C- sequestration and C-budgeting in a scenario where climate change may pose a serious threat in the future. Development of value-added products from these underutilized species will also promote their farming in coastal habitats, which has not been seriously explored earlier due to the lack of knowledge about their commercial importance.

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Statement of Competing Interests

The authors declare that they have no competing interests including any financial, personal or other relationships with other people or organizations that could inappropriately influence, or be perceived to influence, the present work.

List of Abbreviations

DPPH - 1, 1-diphenyl-2-picrylhydrazyl; GAE - gallic acid equivalent; EPA - Eicosapentaenoic acid ; DHA - docosahexaenoic acid; SFA – Saturated fatty acid; MUFA – Monounsaturated fatty acid; PUFA – Polyunsaturated fatty acid; EtOAc – Ethyl acetate ; ROS – Reactive oxygen species; OH – hydroxyl; BHT – butylated hydroxytoluene; BHA - butylated hydroxyanisole; TBHQ - tert-butylhydroquinone; LHS - lyophilized halophyte samples; AEH - aquous extracts of halophytes; EEH - EtOAc extracts of halophytes; PTC - phenylthiocarbamyl ;

HPLC – high performance liquid chromatography; SB – *Salicornia brachiata*; AI - *Arthrocnemum indicum* ;SMa - *Suaeda maritima* ; SP - *Sesuvium portulacastrum* ; SMo - *Suaeda monoica*; Total phenolic content – TPC; TAA – Total amino acids

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