

ANTIOXIDANT PROPERTIES AND PHENOLIC COMPOSITION OF COASTAL HALOPHYTES COMMONLY USED AS MEDICINE

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ABSTRACT

Halophytes are well adapted in extreme environmental conditions, regulation of secondary metabolism is one of the keys of their success. In this study five coastal halophytes *i.e.* *Atriplex stocksii*, *Cressa cretica*, *Heliotropium bacciferum*, *Ipomea pes-caprae* and *Salsola imbricate*, which are well known for their therapeutic properties were investigated for their antioxidant activity and polyphenol composition. Medicinal halophytes showed considerable antioxidant activity in terms of DPPH (14.4-64.8 I%), ABTS (22.6-230.6 $\mu\text{Mol TE g}^{-1}$), FRAP (0.5-5.6 $\text{mMol Fe}^{+2} \text{g}^{-1}$) and TAC (17.6-36.1 mg AsA g^{-1}). Results indicated that these plants also contained high amount of total phenols (7.7-29.6 mg GAE g^{-1}), flavonoids (4.2-17.3 mg QE g^{-1}) and proanthocyanidins (0.2-0.6 mg CE g^{-1}). Among these, *I. pes-caprae* and *C. cretica* had the highest antioxidant activity and polyphenolic contents. High correlation among antioxidant activity assays ($r = 0.877-0.999$) indicated the radical scavenging and reducing power abilities of these plants. Similarly, strong correlations ($r = 0.654-0.953$) among antioxidant activity measurements and polyphenolic composition suggested that phenolic compounds contributed mainly to the antioxidant activity of these plants. Present study reveals coastal halophytes as rich sources of natural antioxidants, which could be used in herbal formulations, pharmaceuticals/ nutraceuticals, food additives and cure for ailments related to oxidative stress. Furthermore, these plants could be grown using saline resources and provide bioactive raw material with high industrial and economic value.

Keywords: Arabian Sea, Karachi coast, Marginal lands, Medicinal plants, Salt tolerant species, Secondary metabolites

INTRODUCTION

The Karachi coast extends over 100 Km on the Arabian Sea, including several islands and beaches (Shameel and Tanaka, 1992). The diversity of halophyte various sub types (Xerohalophytes, hydrohalophytes) have been established in coastal areas of Pakistan (Khan and Qaiser, 2006; Khan and Gul, 2002). These halophytes are well known for multiple economic usages like fuel wood, food, fodder, medicines, oilseed, landscaping, chemicals (Abideen *et al.*, 2011; Khan *et al.*, 2009; Qasim *et al.*, 2011; Weber *et al.*, 2007) and considered as potential candidates to fulfill as secondary source for basic needs of growing population.

Plants distributed at coastal regions exposed to various abiotic stress such as fluctuating salinity, temperatures, light, nutrient, and water (Ksouri *et al.*, 2010). These stresses can cause over production of reactive oxygen species (ROS). Although ROS, at low concentration, are essential messengers for vital plant functions (Salganik, 2001), their higher quantities can trigger cell and tissue injuries by damaging membranes and molecules (Abdi and Ali, 1999; Karuppanapandian *et al.*, 2011; Zhu, 2001). However, tolerant plants like halophytes are adapted to harsh environments and can protect deleterious effects of ROS by enzymatic and non-enzymatic antioxidant systems (Gill and Tuteja 2010). For instance, production of antioxidant compounds contribute significantly to plant stress resistance and is often associated with plant survival (Gupta and Huang, 2014). Halophytes can produce large quantities of secondary metabolism including phenols, flavonoids, proanthocyanidins, tannins and other antioxidant compounds (Alhdad *et al.*, 2013; Dixon and Paiva, 1995; El Shaer, 2010). Hydrogen atom or electron donation capacity/ metal chelating ability of polyphenols is associated with their antioxidant activity (Anchana *et al.*, 2005; Tsao and Deng 2004). Besides providing antioxidant defense, these compounds also possess a broad range of biological and Bouayed pharmacological potential and can be used to treat health care issues based herbal formulations (Qasim *et al.*, 2011, 2014). In addition, phenolic compounds are also known for their beneficial health effects for humans (Cicerale *et al.*, 2010; Huang *et al.*, 2009) including anti-cancerous, anti-coagulant and hypoglycemic properties (Meira *et al.*, 2012) and are used in different food, pharmaceutical and cosmetic products (Maisuthisakul *et al.*, 2007). Recently, interest is shifted towards find natural herbal product alternate to synthetic antioxidants known to possess harmful health effects (Hu *et al.*, 2000). Moreover, natural compounds have better antioxidant activity and lesser side effects than synthetic ones (Maisuthisakul *et al.*, 2007).

Numerous studies have been conducted related to taxonomy, distribution, and morpho-ecology of coastal plants, but data about their active compounds and secondary metabolite constituents is scanty (Rizvi and Shameel, 2001). Few reports, which incorporated eco-physiological approach in medicinal plant studies, suggested that in search of natural sources of antioxidant compounds, halophytes are the better candidates to focus (Abideen *et al.*, 2015; Qasim *et al.*, 2017). Keeping in mind the above mentioned scenario, this study evaluated the antioxidant activity of five coastal halophytes *i.e.* *Atriplex stocksii*, *Cressa cretica*, *Heliotropium bacciferum*, *Ipomea pes-caprae* and *Salsola imbricate*, that are commonly used in traditional medicines. Composition of polyphenols including total phenols, flavonoids and proanthocyanidins and their correlation with antioxidant activity of medicinal halophytes was also determined.

MATERIALS AND METHODS

Collection of plant material

Leaves of five medicinal halophytes *i.e.* *Atriplex stocksii*, *Cressa cretica*, *Heliotropium bacciferum*, *Ipomea pes-caprae* and *Salsola imbricate*, were collected from their natural habitats along the coast of Karachi. Taxonomic description of selected halophytes is given in Table 1. Mean monthly temperatures (maximum 28-36 °C, minimum 9-29 °C), precipitation (0-9.9 cm) and humidity (25-64%) during 2014 (Fig. 1).

Sample preparation

Leaves were dried under shade condition and ground to finely powdered using ball mill (Retsch MM-400). 25 mg of powdered plant material was extracted in 80% methanol (10 mL) at 40 °C for 3 h using a shaking water bath (GFL-1092; Abideen *et al.*, 2015; Qasim *et al.*, 2016). Extracts were centrifuged and supernatant was recovered for further analysis.

Quantification of secondary metabolites

Estimation of secondary metabolites was based on calorimetric method, total phenolic content (TPC) was carried out using the Folin–Ciocalteu method (Singleton and Rossi, 1965). Calorimetric methods of Chang *et al.* (2002) and Sun *et al.* (1998) were also used for the quantification of total flavonoids (TFC) and proanthocyanidins (PC), respectively.

Determination of Antioxidant capacity

Four different tests were employed to determine the antioxidant activity of medicinal halophytes. DPPH (Brand-Williams *et al.*, 1995) and ABTS (Re *et al.*, 1999) assays are based on radical scavenging ability while, ferric reducing antioxidant power (FRAP; Benzie and Strain, 1996) and total antioxidant capacity (TAC; Prieto *et al.*, 1999) methods were used to determine the reducing potential of plant extracts.

Soil analysis

Soil samples were collected from the root zone (up to 12 cm deep) of medicinal halophytes and analyzed for moisture content, electrical conductivity (EC) and pH (AOAC, 2005).

Statistical analyses

The study was based on a minimum of 5 biological replicates using 5 technical replicates. SPSS (version 20) was used for LSD post-hoc test and Pearson Correlation Coefficient (r). All graphs were plotted using Sigma Plot (version 12.5). Results are presented as means (\pm standard error).

RESULTS AND DISCUSSION

Antioxidant activity and polyphenolic composition of five medicinal halophytes distributed in coastal areas of Karachi were studied. Selected species were either perennial shrubs or herbs, belongs to 3 botanical families (Table 1). Data reveals that the rhizosphere soil was mostly saline (22-27 dSm⁻¹) and dry (0.6-3.5% moisture) with pH ranging 6.8-8.3 (Table 2), which reflects in the xerohalophytic nature of most of the plants (Table 1). Traditionally these plants are used as a folk remedy in nearby communities and some of them are also reported with profound biological activities (Table 2).

Antioxidant activity relies not only on the constituents present in the plant extract but also on the testing method. Hence, cannot be completely assessed by single method rather multiple antioxidant assays are used to harness various antioxidant action mechanisms (Wong *et al.*, 2006). Antioxidant activity of medicinal halophytes

were evaluated using four antioxidant assays *i.e.* DPPH, ABTS, FRAP and TAC. The DPPH and ABTS estimates the radical scavenging ability of plant extracts using 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) free radicals, respectively. The ferric-reducing antioxidant power (FRAP) and total antioxidant capacity (TAC) methods estimate the ability of plant extract to reduce iron and molybdenum ions, respectively. These are simple, reliable, less expensive and easily reproducible methods which reflect the different rationales of antioxidant activity measurements (Cai *et al.*, 2004; Li *et al.*, 2008).

A wide variation among antioxidant activity of five selected species was recorded. It is important to notify that all test species were collected from the same study area to ensure similar edaphic, climatic, and developmental influences on the growth and antioxidant activity. DPPH radical scavenging activity (% inhibition) of curative halophytes ranged between 14.3% (*H. bacciferum*) to 64.7% (*C. cretica*) indicating a 3.5 fold variation. The antioxidant activity using FRAP system (mMol Fe⁺² g⁻¹) showed a 13 fold variation from 0.48 (*H. bacciferum*) to 5.6 (*I. pescaprae*). The ABTS (μMol TE g⁻¹) values ranged from 22.6 (*A. stocksii*) to 230.6 (*C. cretica*), while TAC values (mg AsA g⁻¹) ranged from 17.6 (*H. bacciferum*) to 36.1 (*I. pescaprae*). In general, medicinal halophytes represent powerful radical scavenging and reducing oxidant activities (Fig. 2), which is related to their bioactive secondary metabolites which make them better than antioxidant rich plants like herbs, medicinal plants, edible plants, and some halophytes (Bourgou *et al.*, 2008). Our results verify previous studies based on equivalent or higher antioxidant activity of coastal medicinal plants (Falleh *et al.*, 2012; Medini *et al.*, 2014; Qasim *et al.*, 2017).

Table 1. Taxonomic detail of medicinal halophytes used in this study.

Species	Families	Habit	Plant type	Flowering period
<i>Atriplex stocksii</i> Boiss	Amaranthaceae	Shrub	Xerohalophytes	December-January
<i>Cressa cretica</i> L.	Convolvulaceae	Herb	Hydrohalophyte	Year round
<i>Heliotropium bacciferum</i> Forssk.	Boraginaceae	Shrub	Xerohalophyte	July-September
<i>Ipomoea pes-caprae</i> (L.) R. Br.	Convolvulaceae	Herb	Psammophyte	July-September
<i>Salsola imbricata</i> Forssk.	Amaranthaceae	Shrub	Xerohalophyte	August-October

Table 2. Soil EC, pH and moisture content collected from rhizosphere of medicinal halophytes.

Species	EC (dS m ⁻¹)	pH	Moisture (%)
<i>Atriplex stocksii</i>	27.04 ± 1.341	7.6 ± 0.01	0.93 ± 0.02
<i>Cressa cretica</i>	27.12 ± 3.671	6.8 ± 0.07	3.54 ± 0.42
<i>Heliotropium bacciferum</i>	24.85 ± 2.431	6.9 ± 0.33	2.85 ± 0.65
<i>Ipomoea pes-caprae</i>	25.52 ± 2.511	8.3 ± 0.23	0.56 ± 0.26
<i>Salsola imbricata</i>	22.03 ± 1.351	7.1 ± 0.35	1.36 ± 0.56

Table 3. Medicinal uses of halophytes used in this study.

Species	Common name	Plant part	Preparation	Medicinal uses*
<i>Atriplex stocksii</i>	Phurki val	Leaf	Infusion	Fever, jaundice, dropsy, liver disease
<i>Cressa cretica</i>	Bukkan	Whole plant	Decoction, paste	Antiinflammatory, Antioxidant, Antiviral and for treatment of Sores
<i>Heliotropium bacciferum</i>	Markondi	Leaf	Decoction	Antihyperlipidemic, antitumor, antidiabetic, antioxidant, and antimicrobial
<i>Ipomea.pes caprae</i>	Beach Morning Glory	Leaf	Decoction	Diarrhea, pains, vomiting, inflammation of legs, piles
<i>Salsola imbricata</i>	Lana	Fresh twig	Infusion	Insecticidal, vascular hypertension

*Qasim *et al.*, 2011, Qasim *et al.*, 2014, Ahmad *et al.*, 2014

Table 4. Correlation coefficient (r) of different antioxidant parameters studied.

	TPC	TFC	PC	DPPH	ABTS	FRAP	TAC
TPC	1						
TFC	0.610	1					
PC	0.643	0.865	1				
DPPH	0.740	0.839	0.852	1			
ABTS	0.654	0.735	0.862	0.930	1		
FRAP	0.710	0.950	0.936	0.957	0.888	1	
TAC	0.721	0.953	0.942	0.950	0.877	0.999	1

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) free radicals, ferric-reducing antioxidant power (FRAP), total antioxidant capacity (TAC), total phenolic content (TPC), total flavonoids (TFC) and proanthocyanidins (PC)

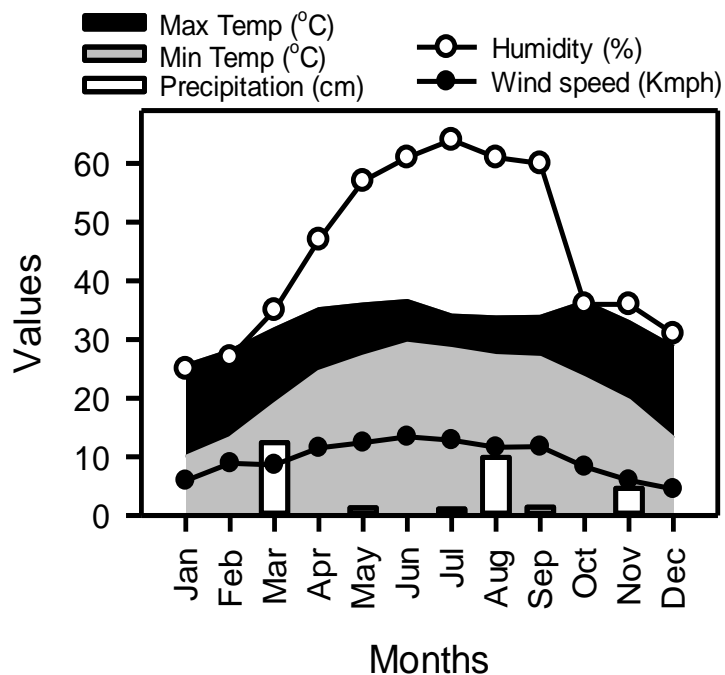


Fig. 1. Mean annual temperatures, rainfall, humidity and wind speed of study area 2014 (Pakistan Meteorological Department).

Polyphenols are biologically active molecules with high antioxidant activity. Polyphenolic analysis of the studied plants showed considerable variation in total phenol (7.6-26.6 mg GAE g⁻¹), total flavonoids (1.3-17.3 mg QAE g⁻¹) and proanthocyanidin (0.2-0.6 CE g⁻¹) contents (Fig. 3). Among all test species, the highest collective polyphenolic content was 42.6 mg g⁻¹, which was found in *I. pes-caprae* (TPC 24.7 mg GAE g⁻¹ + TFC 17.3 mg QE g⁻¹ + PC 0.6 mg CE g⁻¹) followed by 40.6 mg g⁻¹ in *C. cretica* (TPC 29.6 mg GAE g⁻¹ + TFC 10.5 mg QE g⁻¹ + PC 0.5 mg CE g⁻¹). *A. stocksii* (26.7 mg g⁻¹), *H. bacciferum* (20.1 mg g⁻¹), and *S. imbricata* (12.2 mg g⁻¹) had relatively lower polyphenols and generally had non-significant differences in TPC, TFC, and PC contents (Fig. 3). Polyphenols of studied medicinal halophytes are comparable to or even higher than antioxidant rich medicinal plants such as *Cetraria islandica* and halophytes like *Mesembryanthemum edule*, *Cakile maritima*, and *Limonium delicatulum* (Falleh et al., 2012, 2013; Ivanova et al., 2005; Ksouri et al., 2007; Medini et al., 2014).

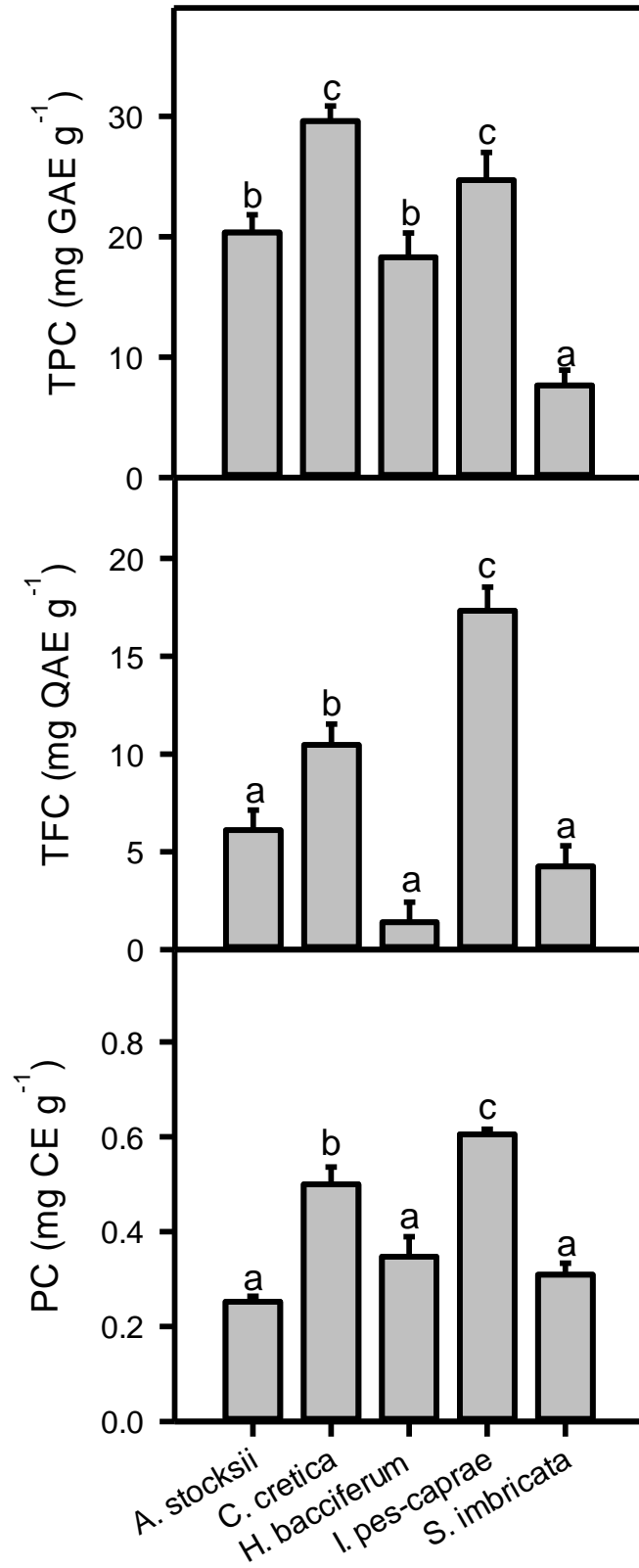


Fig. 2. Analysis of secondary metabolites total phenol (TPC), total flavonoids (TFC) and proanthocyanidin content (P.C) in leaves of medicinal halophyte species.

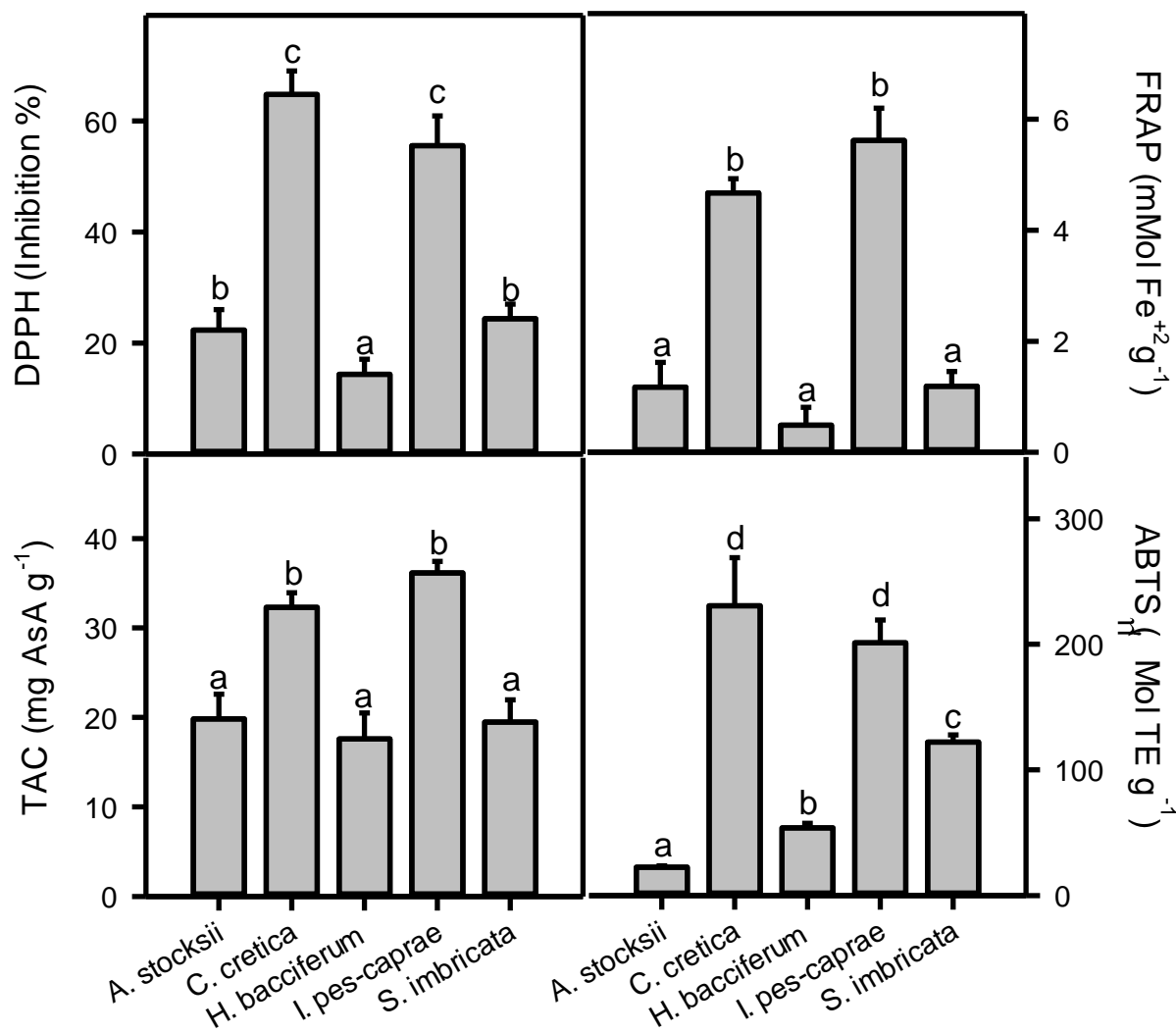


Fig. 3. Variation in antioxidant activity (DPPH, ABTS, FRAP, TAC) of medicinal halophytes.

The correlation (r) of polyphenols (TPC, TFC and PC) and antioxidant activity (DPPH, ABTS, FRAP and TAC) of medicinal halophytes is presented in Table 4. The TPC, TFC and PC showed strong positive correlation with antioxidant activity measurements (DPPH, ABTS, FRAP and TAC). A positive correlation between the antioxidant assays also indicated that extracts of medicinal halophyte contained broad spectrum antioxidant compounds which enables free radicals scavenging and reducing harmful oxidants ability. Our results taken together with previous studies indicating phenolic compounds are the significant contributors to the antioxidant activity of medicinal plants (Boulanouar *et al.*, 2013; Djeridane *et al.*, 2006, Lizcano *et al.*, 2010, Ouchemoukh *et al.*, 2012.). Antioxidant activity of phenolic compounds relies on number and orientation of OH groups, which either donate hydrogens or electrons (Bouayed *et al.*, 2011a, b; Rice-Evans *et al.*, 1996) to neutralize free radicals or chelation metal ions (Li *et al.*, 2008; Shan *et al.*, 2005). Several studies indicate positive association between TPC and higher antioxidant activity (Kim *et al.* 2003; Skotti *et al.*, 2014). Several phenolic compounds have been reported, either individually or aggregated with profound antioxidant activity / health benefits (Lee and Lee, 2010; Owen *et al.*, 2000). Beside strong antioxidant activity, phenolic compounds are reported as antimicrobial, antiviral, antimalarial, anti-inflammatory, antiplaque-forming, hypotensive, hypoglycemic, hepatoprotective, antitumor, anticancer, and neuro and cardio protective effects (Nagao *et al.*, 2005; Niggeweg *et al.*, 2004).

Interestingly, studied medicinal halophytes that showed high antioxidant activity are being commonly used among local populations along coastal areas of Karachi and vicinity. For examples, *I. pes-caprae* is used as herbal tea against several health care issues like common cold and fever (Qasim *et al.*, 2017). Leaf extract is used for treatment of jellyfish sting. This plant also possess antispasmodic, anticancer, antinociceptive, antihistaminic, insulogenic and hypoglycemic activities (Premanathan *et al.*, 1996; Wasuwat, 1970). Higher radical scavenging and reducing power activity of *I. pes-caprae* demonstrated in this study agrees with the previous reports (Banerjee *et al.*, 2008; Thimnavukkamsu *et al.*, 2010). The presence of catechin, gallic acid, chlorogenic acid, caffeic acid, syringic acid, ferulic acid, coumarin, naringenin, kaempferol, and derivatives of quercetin, isocoumarin, and isochlorogenic acid, supports its high antioxidant potency (Meira *et al.*, 2012; Qasim *et al.*, 2017).

Cressa cretica is another important plant used as herbal remedy against several diseases and disorders (Qasim *et al.*, 2017). It is a popular medicinal halophyte, used in folklore medicine for asthma, ulcers, diabetes, stomachic, expectorant, anthelmintic, and aphrodisiac purposes. It has properties to enrich blood, and very helpful in leprosy, constipation, and urinary problems (Priyashree *et al.*, 2010). *In-vivo* and *in-vitro* biological testing highlights its efficacy as a potent antimicrobial, antituberculosis, antitussive, antibilious, anti-inflammatory, and anticancer agent (Priyashree *et al.*, 2010; Rizk and El-Ghazaly, 1995). Animal trials proven that *C. cretica* extracts improves sexuality and testicular functions in rats (Priyashree *et al.*, 2010). Our results revealed the powerful antioxidant nature of this plant are in line with previous studies (Pryanika *et al.*, 2015; Sunita *et al.*, 2011). Presence of scopoletin, syringaresinol, dicaffeoylquinic acid, creticanone, cressatetracontanoic acid, cressatetracontanone, cressatetracosanoate, cressanaphthacene, flavonol glycosides, chlorogenic acids, rutin, and derivatives of quercetin and kampferol in *C.cretica* could be are related to its antioxidant and other biological activities (Abdallah *et al.*, 2017; Priyashree *et al.*, 2010).

Conclusions

The antioxidant activity and polyphenolic contents of five coastal halophytes, which are commonly used in traditional herbal remedies, were evaluated. In general, medicinal halophytes showed strong antioxidant activity with considerable amount of phenols, flavonoids, and proanthocyanidins. Among these plants, *I. pes-caprae* and *C. cretica* were enriched in natural antioxidants, both in extraction as well as in antioxidant potential. A significant relationship between polyphenols and antioxidant activities indicated that phenolic compounds are the major antioxidants in these plants. A strong correlation between antioxidant activity assays also implied that extracts of medicinal halophytes were capable of reducing oxidants and quenching free radicals. This study highlights the importance of medicinal halophytes as promising sources of natural antioxidants, which can be used for multiple domestic and industrial applications. Moreover, these plants are adapted to saline coast lands and do not require prime agricultural lands and fresh water resources to grow. Sustainable development of saline/marginal lands with these medicinal resources can provide industrial raw material of bioactive natural products, which can be used to replace harmful synthetic derivatives from food, pharmaceutical and cosmetic industries.

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