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Antioxidant properties, phenolic composition, bioactive compounds and nutritive value of medicinal halophytes commonly used as herbal teas

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ABSTRACT

Halophytes, distributed from coastal regions to inland deserts have traditionally been used for medicinal and nutritional purposes. Living in sub-optimal conditions, these plants synthesize stress associated bioactive molecules, which are still remain largely unexplored. In search of natural antioxidant sources, antioxidant capacity (AC) and total phenolic content (TPC) of 100 medicinal plants (halophytes vs non-halophytes), commonly used as herbal teas, were investigated. Nutrients and phytochemical composition, especially phenolic metabolites in selected medicinal plants with higher AC were also determined. Most of the medicinal plants analysed for the first time showed considerable AC. In general, halophytes displayed higher AC and TPC than non-halophytes. High correlation indicated a major contribution of TPC in AC of these plants. Five medicinal halophytes i.e., *Thespesia populneoides*, *Salvadora persica*, *Ipomoea pes-caprae*, *Suaeda fruticosa*, and *Pluchea lanceolata* displayed significantly higher AC than synthetic antioxidants (BHT and BHA). Presence of bioactive phytochemicals including phenols (42.3–63.9 mg GAE g^{-1}), flavonoids (12.3–37.1 mg QE g^{-1}), tannins (8.7–20 mg TAE g^{-1}), proanthocyanidins (15.8–22.4 mg CE g^{-1}), carotenoids (0.07–0.84 mg g^{-1}), alkaloids (0.64–1.1 mg g^{-1}), and saponins (11.2–28.4 mg DAE g^{-1}) reflected therapeutic benefits of these plants. HPLC analyses showed that the hydrolysed extracts contained chlorogenic acid, gallic acid, catechin, and quercetin as abundant phenolic metabolites which may be responsible for higher AC. These plants were also found to contain suitable amounts of proteins (8.5–17%), carbohydrates (2.6–11.4%), fibre (31.6–41.2%), and minerals (2.1–9.7%) showing their nutritional potential that has already been exploited by rural communities. The present study highlights the potential of medicinal halophytes as a source of natural antioxidants, valuable phytochemicals, and essential nutrients for pharmaceutical, nutraceutical, and chemical industries.

1. Introduction

Reactive oxygen species (ROS), produced during aerobic metabolism, are essential mediators of important functions (Salganik, 2001). However, over-production of ROS results in oxidative damage of macro-molecules. Studies have demonstrated the involvement of ROS in a number of disorders including Alzheimer, atherosclerosis, diabetes, inflammation, and neurodegenerative and cardiovascular diseases. ROS also plays a key role in certain types of cancers and the ageing process. Antioxidants are molecules that neutralize harmful ROS by inhibiting oxidative chain reaction, preventing lipid peroxidation, reducing free radical concentration and chelating metal ions (Zhou and Yu, 2004). It has been recognized that consumption of vegetables and fruits reduce the risk of degenerative diseases, which may be ascribed to their antioxidant compounds (Oueslati et al., 2012). In addition, some commercial antioxidants i.e. butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), which have been widely used in pharmaceuticals and food industry are found to be toxic (Sasaki et al., 2002). The impact of oxidative stress on human health and increasing safety concerns about synthetic antioxidants, shift the focus of the scientific community to search for new sources of safe and feasible natural antioxidants. Vegetables, cereals, fruits, and mushrooms have been screened worldwide; however, medicinal plants are more potent source of natural antioxidants (Cai et al., 2004; Li et al., 2008, 2013; Albouchi et al., 2013; Baba et al., 2015).

Medicinal plants have long been used to treat infections and other human ailments. Medicinal plants share a common origin with edible plants thus it is difficult to separate medicinal plants from foods. For instance, a number of medicinal plants have been used as vegetables or salads and also for colouring, flavouring or spicing agents (Qasim et al., 2011, 2014). In this capacity, medicinal plants can provide basic nutrients and essential minerals. Studies have demonstrated a suitable composition of protein, carbohydrate, fat, fibre and minerals in some medicinal plants, comparable to or even better than common edible plants (Hussain et al., 2010). Beside nutritional importance, health benefits of medicinal plants are associated with their secondary

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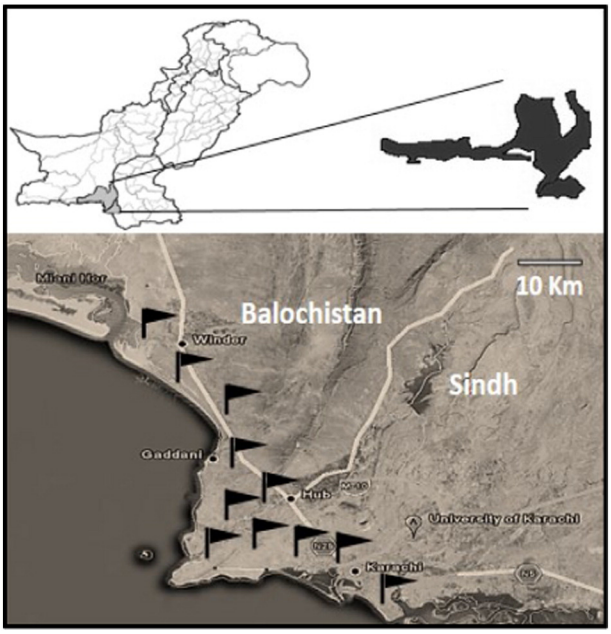

Fig. 1. Map of study area showing site of plant collection from coastal habitats (dark flags).

metabolites (Ksouri et al., 2007, 2008, 2012; Falleh et al., 2013). Medicinal plants contained a variety of secondary metabolites (e.g. phenols, flavonoids, tannins, proanthocyanidins, carotenoids, alkaloids, saponins, etc.) with a broad range of biological and pharmacological properties. Herbal remedies, usually prepared in the form of decoctions, infusions, and tonics, are commonly known as herbal teas (Qasim et al., 2011, 2014). The therapeutic effects of medicinal plants and herbal teas are associated with their antioxidant potentials (Cai et al., 2004; Li et al., 2013). Phenolic compounds were found as major contributors towards antioxidant capacity (AC) of these plants (Li et al., 2008). Interestingly, the synthesis and accumulation of polyphenols and other antioxidant metabolites are enhanced when the plant undergoes biotic/abiotic stress e.g. salinity (Navarro et al., 2006; Meot-Duros et al., 2008). Therefore it is important to characterize salt stressed plants (halophytes) for their antioxidant (Meot-Duros et al., 2008; Lee et al., 2011) and other health related effects (Trabelsi et al., 2010; Oueslati et al., 2012). Some reports are available showing halophytes as sources of polyphenolic antioxidants and other secondary metabolites of high medicinal value (e.g. Ksouri et al., 2007, 2012; Mariem et al., 2014; Stankovic et al., 2015), little is known about the phytochemical constituents and biological potential of these plants. Considering the growing demand for natural products, there is a need to search new candidates among halophytes that can serve as a safe, sustainable and eco-friendly source of natural antioxidants and other bioactive compounds.

A number of coastal plants which has been used in the form of herbal tea against a range of disease conditions was reported earlier (Qasim et al., 2010, 2011, 2014). These plants thrive in harsh environments, especially hyper saline conditions which demand various adaptive mechanisms, for example redox homeostasis. Plants maintain equilibrium between ROS generation and energy consumption in enzymatic and non-enzymatic antioxidant defence to prevent cells from oxidative damage (Noctor and Foyer, 1998; Apel and Hirt, 2004). Therefore, halophytes are expected to produce bioactive compounds with high AC and hence could be better candidates for focus. The present study aimed to determine the AC and polyphenolic content of 100 medicinal plants from coastal areas of Pakistan. Nutrient, bioactive compound and phenolic metabolite contents were also determined in selected species (i.e., *Thespesia populneoides*, *Salvadora persica*, *Ipomoea pes-caprae*, *Suaeda fruticosa*, and *Pluchea lanceolata*) showing high AC. A relationship between AC and salt resistance of medicinal plants was also determined.

2. Materials and methods

2.1. Sample collection and preparation

Medicinal plants were collected from coastal areas of Sindh and Balochistan province of Pakistan (Fig. 1). The study area represents an arid to semi-arid climate with low annual rainfall (<250 mm) and high temperature (~30 °C; Fig. 2). Jafri (1966) and Ali and Qaiser (1995–2015) were used for initial identification of plants, which were further confirmed by Dr. Jahan Alam (Senior Taxonomist), Centre for Plant Conservation (CPC), University of Karachi. Voucher specimens were also deposited in the CPC for later access. At least five replicated samples per plant species were collected and dried under shade. Leaves of medicinal plants were separated and ground to fine powder using a ball mill (Retsch MM-400). Ground material (1.0 g) was extracted in 20 mL of 80% methanol using a shaking water bath (GFL-1092) at 40 °C for 3 h. After extraction, samples were centrifuged at 4000 rpm and the supernatant was recovered for further analyses (Abideen et al., 2015; Qasim et al., 2016).

2.2. List of chemicals used

2,2′-azino-bis3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical cation (PubChem CID: 90658258); Butylated hydroxytoluene (BHT; PubChem CID: 31404); Butylated hydroxyanisole (BHA; PubChem CID: 8456); Caffeic acid (PubChem CID: 689043), Catechin (PubChem CID: 107957), Chlorogenic acid (PubChem CID: 1794427), Coumarin (PubChem CID: Coumarin), 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) free radical (PubChem CID: 2735032); Ferulic acid (PubChem

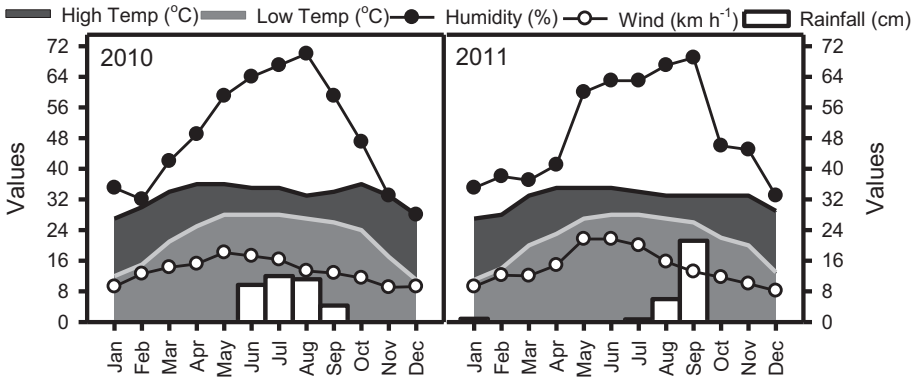

Fig. 2. Two years (2010 and 2011) data of study area comprising mean annual temperature, rainfall, wind velocity and humidity (Pakistan meteorological department).

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Table 1
Plant type, antioxidant capacity (DPPH, FRAP) and total phenolic content (TPC) of 100 medicinal plants. Values are mean (±standard error) of at least 5 replicates.

Family, genus, species and author	Plant type	TPC	DPPH	FRAP
		(mg GAE g^{-1})	IC_{50} (μg ml^{-1})	(mMol Fe^{+2} g^{-1})
Acanthaceae				
Peristrophe paniculata (Forssk.) Brummitt	Xero	1.59 (0.29)	754.22 (56.30)	0.13 (0.02)
Amaranthaceae				
Trianthema portulacastrum L.	Xerohal	3.71 (0.36)	973.26 (25.71)	0.23 (0.01)
Achyranthes aspera L.	Weed	23.45 (1.89)	421.59 (3.26)	3.85 (0.31)
Amaranthus viridis L.	Weed	10.74 (1.32)	698.35 (16.31)	2.40 (0.17)
Arthrocnemum indicum (Willd.) Moq.	Hyphal	22.29 (2.56)	193.39 (6.59)	3.57 (0.31)
Arthrocnemum macrostachyum (Moric.) C. Koch	Hyphal	13.47 (1.23)	698.12 (15.30)	3.59 (0.52)
Atriplex stocksii Boiss.	Xerohal	7.64 (0.32)	701.26 (10.40)	1.19 (0.14)
Chenopodium album L.	Xerohal	32.26 (0.84)	94.23 (10.91)	3.96 (0.54)
Haloxylon stocksii (Boiss.) Benth. & Hook.	Xerohal	18.52 (1.29)	311.15 (15.63)	3.12 (0.01)
Suaeda fruticosa (L.) Forssk.	Xerohal	46.54 (4.32)	33.31 (3.54)	5.32 (0.86)
Aerva javanica (Brum. f.) Juss. Ex J.A. Schultes var. *bovei* Webb.	Xerohal	7.91 (1.17)	798.36 (13.10)	1.34 (0.01)
Aerva javanica (Brum. f.) Juss. ex J.A. Schultes var. *javanica*	Xerohal	21.11 (1.28)	365.35 (10.20)	3.98 (0.05)
Apiaceae				
Ammi visnaga (L.) Lam.	Weed	13.91 (1.56)	651.19 (13.60)	2.64 (0.21)
Asclepiadaceae				
Glossonema varians	Xero	5.54 (0.28)	816.24 (12.30)	0.91 (0.37)
Calotropis procera (Ait.) Ait.	Xero	3.73 (0.41)	725.36 (22.65)	1.86 (0.01)
Leptadenia pyrotechnica (Forssk.) Dcne.	Xero	2.11 (0.86)	991.62 (22.20)	1.69 (0.28)
Asteraceae				
Artemisia scoparia Waldst. & Kit.	Weed	15.13 (1.98)	516.56 (9.65)	1.57 (0.36)
Inula grantioides Boiss	Xerohal	7.31 (0.12)	711.89 (13.11)	1.66 (0.42)
Launaea resedifolia (L.) Kuntze	Xerohal	15.53 (0.12)	512.36 (13.10)	1.75 (0.42)
Pluchea lanceolata (DC.) C. B. Clarke	Xerohal	42.28 (3.58)	38.76 (6.11)	5.21 (0.34)
Tridax procumbens L.	Weed	8.54 (0.84)	621.36 (16.80)	1.86 (0.03)
Avicenniaceae				
Avicennia marina (Forssk.) Vierh	Hyphal	16.18 (1.24)	365.35 (17.78)	2.68 (0.05)
Azioaceae				
Aizoon canariense L.	Xero	1.93 (0.07)	712.36 (10.70)	1.71 (0.02)
Zaleya pentandara (L.) Jeffrey	Xerohal	18.52 (1.29)	311.15 (15.63)	3.12 (0.01)
Boraginaceae				
Heliotropium currassvicum L.	Xerohal	7.26 (0.41)	796.35 (25.70)	1.53 (0.03)
Trichodesma indicum (L.) R. Br.	Xerohal	8.53 (0.13)	689.21 (29.80)	2.87 (0.01)
Brassicaceae				
Farsetia jacquemontii Hook.f. & Thoms.	Xero	9.81 (1.23)	659.12 (19.28)	1.18 (0.05)
Caesalpiniaceae				
Caesalpinia bonduc (L.) Roxb.	Xero	6.62 (0.69)	845.26 (15.69)	1.39 (0.16)
Cassia holosericea Fresen	Xero	15.77 (1.51)	489.65 (39.71)	1.76 (0.11)
Parkinsonia aculeata L.	Xerohal	10.37 (0.32)	1003.1 (15.60)	1.31 (0.15)
Capparidaceae				
Capparis decidua Forssk.	Xero	7.15 (0.85)	881.26 (18.60)	1.31 (0.18)
Cleome brachycarpa Vahl ex DC.	Xero	13.51 (1.35)	621.59 (15.28)	2.46 (0.26)
Cleome viscosa L.	Xero	10.34 (0.11)	721.26 (41.50)	2.02 (0.36)
Convolvulaceae				
Commicarpus boissieri (Heimerl) Cufod.	Xero	12.77 (1.20)	453.12 (12.45)	1.58 (0.03)
Convolvulus arvensis L	Xero	20.78 (2.04)	152.98 (22.50)	3.13 (0.18)
Convolvulus glomeratus Choisy	Xero	10.22 (0.84)	886.24 (21.70)	1.32 (0.29)
Convolvulus prostrates Forssk.	Xero	24.84 (1.14)	243.07 (12.81)	3.63 (0.42)
Cressa cretica L.	Hyphal	9.53 (0.27)	753.65 (31.70)	2.51 (0.03)
Ipomoea pes-caprae (L.) R. Br.	Psamm	54.21 (2.31)	32.11 (3.25)	5.56 (0.15)
Cucurbitaceae				
Citrullus colocynthis (Linn.) Schrad.	Xero	10.61 (0.16)	825.32 (19.80)	1.21 (0.15)
Cyperaceae				
Cyperus rotundus L.	Hyphal	21.77 (2.31)	89.90 (13.27)	3.55 (0.17)
Euphorbiaceae				
Euphorbia caducifolia Haines	Xerohal	3.11 (0.38)	825.32 (40.30)	0.91 (0.08)
Euphorbia hirta L.	Xerohal	3.91 (1.12)	895.12 (12.36)	1.53 (0.08)
Phyllanthus fraternus Webster	Xero	5.21 (0.92)	789.65 (16.40)	1.58 (0.01)
Gentianaceae				
Enicostema hyssopifolium (Willd) Verdoo	Xerohal	7.82 (0.72)	853.26 (18.67)	1.25 (0.01)
Lamiaceae				
Leucas urticifolia (Vahl) R. Br.	Xero	20.34 (2.14)	197.39 (23.40)	4.03 (0.23)

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Table 1 (*continued*)

Family, genus, species and author	Plant type	TPC	DPPH	FRAP
		(mg GAE g^{-1})	IC_{50} (μg ml^{-1})	(mMol Fe^{+2} g^{-1})
Malvaceae				
Abutilon indicum (L.) Sweet, Hort. Brit.	Xero	3.70 (0.15)	854.26 (10.41)	0.93 (0.02)
Digera muricata (L.) Mart.	Xero	5.16 (0.52)	798.36 (11.30)	1.37 (0.01)
Gossypium stocksii Mast.	Xerohal	29.06 (3.29)	189.34 (10.20)	4.07 (0.14)
Hibiscus micranthus L.	Xero	15.85 (0.52)	417.26 (12.81)	3.29 (0.42)
Sida ovata Forssk.	Xero	6.91 (0.10)	950.21 (53.30)	0.53 (0.07)
Sida spinosa L.	Xero	9.62 (1.65)	602.31 (24.80)	1.01 (0.06)
Thespesia populneoides (Roxb.) Kostel.	Hyphal	63.91 (5.28)	16.64 (1.24)	6.54 (0.56)
Meliaceae				
Azadirachta indica Adr. Juss.	Xero	19.04 (1.78)	347.23 (12.10)	3.67 (0.01)
Mimosaceae				
Acacia nilotica (L.) Delile	Xerohal	8.26 (1.89)	765.36 (11.11)	2.28 (0.03)
Acacia senegal L.	Xero	20.36 (2.13)	265.32 (15.23)	3.56 (0.23)
Prosopis cineraria (L.) Druce	Xerohal	37.26 (2.73)	45.23 (2.21)	5.16 (0.11)
Prosopis juliflora (Swartz) DC.	Xerohal	23.82 (2.06)	365.59 (11.70)	4.35 (0.17)
Nyctaginaceae				
Boerhavia diffusa L.	Xero	5.01 (0.32)	991.25 (20.10)	0.48 (0.08)
Papilionaceae				
Alhaji maurorum Medic.	Hyphal	33.23 (2.89)	65.54 (7.87)	4.50 (0.27)
Clitoria ternatea L.	Xero	10.91 (0.24)	921.65 (10.90)	0.91 (0.16)
Indigofera cordifolia Heyne ex Roth	Xero	8.19 (0.13)	665.53 (29.30)	2.64 (0.47)
Indigofera hebepetala Benth. ex Baker var. *hebepetala* Hook.	Xerohal	33.07 (2.12)	88.96 (4.19)	4.24 (0.06)
Indigofera hochstetteri Baker	Xerohal	18.25 (1.39)	305.15 (15.32)	2.24 (0.12)
Indigofera oblongifolia Forsk.	Xerohal	35.83 (4.99)	48.96 (13.41)	4.74 (0.67)
Rhynchosia minima (Linn.) DC.	Xero	15.88 (2.20)	301.29 (22.60)	3.89 (0.01)
Sesbania grandiflora (Linn.) Poir	Xerohal	13.35 (1.14)	402.89 (28.04)	3.53 (0.03)
Tephrosia strigosa (Dalz.) Sant. & Maheshw.	Weed	13.51 (1.25)	345.26 (18.70)	2.91 (0.02)
Tephrosia subtriflora Baker	Weed	12.47 (1.24)	468.26 (15.36)	2.37 (0.01)
Tephrosia uniflora Pers.	Weed	17.18 (1.24)	311.65 (21.10)	3.37 (0.11)
Poaceae				
Aeluropus lagopoides (L.) Trin. ex Thw.	Hyphal	7.58 (0.11)	771.29 (78.40)	2.38 (0.02)
Cenchrus ciliaris Rich.	Weed	8.98 (0.75)	635.26 (32.51)	1.27 (0.01)
Cymbopogon jwarancusa (Jones) Schult.	Xerohal	7.85 (0.23)	782.65 (16.40)	1.23 (0.25)
Dactyloctenium aegyptium (L.) Willd.	Weed	3.31 (0.41)	745.32 (15.99)	0.64 (0.01)
Dactyloctenium scindicum Boiss.	Xerohal	9.41 (0.72)	721.49 (17.51)	2.22 (0.11)
Desmostachya bipinnata (L.) Stapf	Xerohal	7.87 (0.32)	799.36 (15.05)	2.04 (0.24)
Halopyrum mucronatum (L.) Stapf	Psamm	15.84 (0.26)	214.23 (15.41)	2.18 (0.51)
Panicum turgidum Forssk.	Xerohal	16.61 (0.14)	321.26 (18.30)	2.62 (0.11)
Paspalum pasploides (Michex) Scribner	Hyphal	21.29 (1.25)	289.65 (5.19)	3.35 (0.42)
Phragmites karka (Retz.) Trin. ex. Steud.	Hyphal	10.52 (0.98)	600.12 (12.40)	2.42 (0.06)
Sporobolus ioclados (Nees ex Trin.) Nees	Xerohal	6.81 (0.17)	775.21 (14.71)	1.54 (0.02)
Sporobolus tremulus (Willd.) Kunth.	Hyphal	7.11 (0.38)	745.21 (40.30)	2.34 (0.08)
Urochondra setulosa (Trin.) C.E. Hubb.	Xerohal	7.32 (0.27)	702.78 (28.50)	2.46 (0.11)
Portulacaceae				
Portulaca oleracea L.	Xerohal	23.82 (2.13)	109.51 (16.66)	3.35 (0.27)
Rhamnaceae				
Zizyphus nummularia (Burm. f.) Wight and Arn.	Xero	17.65 (2.12)	325.12 (21.36)	1.53 (0.08)
Salvadoraceae				
Salvadora oleoides Dne.	Xerohal	25.71 (0.36)	313.39 (11.70)	2.13 (0.01)
Salvadora persica L.	Xerohal	58.23 (3.54)	20.92 (1.52)	5.96 (0.29)
Solanaceae				
Datura fastuosa L.	Xero	24.42 (2.02)	289.65 (11.87)	3.56 (0.21)
Solanum forskalii Dunal	Xero	7.21 (0.59)	825.32 (21.01)	1.85 (0.04)
Solanum surattense Burm. f.	Xero	22.29 (2.56)	305.15 (16.59)	3.57 (0.31)
Withania sominifera (L.) Dunal	Xero	4.79 (0.22)	711.45 (21.90)	1.72 (0.03)
Sterculaceae				
Melhania denhamii R. Br.	Xero	31.47 (3.15)	113.26 (17.17)	3.29 (0.46)
Tiliaceae				
Corchorus aestuans L.	Xero	23.72 (2.31)	184.29 (8.35)	4.87 (0.32)
Corchorus depressus (L.) Stocks	Xero	5.27 (0.61)	811.29 (15.44)	0.97 (0.23)
Corchorus olitorius L.	Xero	9.80 (1.79)	615.32 (15.54)	2.55 (0.15)
Corchorus tridens L.	Xero	15.31 (1.05)	322.56 (50.70)	3.83 (0.07)
Grewia tenax (Forsk.) Fiori	Xero	7.31 (0.19)	821.36 (13.10)	1.03 (0.01)
Zygophyllaceae				
Fagonia indica ssp. *schweinfurthia* Hadidi	Xero	8.51 (0.13)	689.21 (29.80)	2.87 (0.01)
Tribulus terrestris L.	Xero	21.72 (1.98)	149.29 (4.65)	4.76 (0.65)
Zygophyllum simplex L.	Xerohal	5.21 (0.92)	789.65 (16.40)	1.58 (0.01)

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Table 1 (*continued*)

Family, genus, species and author	Plant type	TPC (mg GAE g^{-1})	DPPH IC_{50} (μg ml^{-1})	FRAP (mMol Fe^{+2} g^{-1})
BHA			42.15 (2.51)	5.32 (0.42)
BHT			35.24 (1.65)	7.13 (0.54)

Key: Hyphal — Hydrohalophyte; Psamm — Psammohalophyte; Weed — Weedy glycophyte; Xerohal — Xerohalophyte; Xero — Xerophyte.

CID: 445858), Gallic acid (PubChem CID: 370), Kaempferol (PubChem CID: 5280863); Naringenin (PubChem CID: 932); Quercetin (PubChem CID: 16212154); Syringic acid (PubChem CID: 10742), 2,4,6-Tripyridyl-s-Triazine (TPTZ; PubChem CID: 77258). High purity HPLC grade chemicals were purchased from Sigma Aldrich especially reference standards and solvents.

2.3. Antioxidant capacity of medicinal plants

Antioxidant capacity (AC) was determined using DPPH (Brand-Williams et al., 1995) and ABTS (Re et al., 1999) radical scavenging tests. Ferric reducing antioxidant power assay (FRAP) was carried out using the method of Benzie and Strain (1996). Total antioxidant capacity (TAC) of plant samples was also evaluated by the phosphomolybdate complex method (Prieto et al., 1999).

2.4. Quantification of bioactive compounds and nutrient content of medicinal plants

Total phenolic content (TPC) was determined using the Folin–Ciocalteu colorimetric method (Singleton and Rossi, 1965). Colorimetric methods were also used to quantify total flavonoids (TFC) (Chang et al., 2002), total proanthocyanidins (PC) (Sun et al., 1998) and total tannins (TTC) (Pearson, 1976). The content of total saponins (TSC) (Makkar et al., 1995), total carotenoids (TCC) (Duxbury and Yentsch, 1956), and total alkaloids (TA) (Harborne, 1973) were also estimated.

Organic content (ashing), carbohydrates (Anthrone reagent), ether extract (Soxhlet extraction), crude protein (Kjeldahl method), and crude fibre (acid base digestion) were determined using official methods described in AOAC (2005).

2.5. High performance liquid chromatographic (HPLC) analyses

Dried samples (0.5 g) were extracted and phenolic glycosides hydrolysed in 40 mL methanol (62.5%) and 10 mL 6 M HCl according to the method described by Proestos et al. (2006). After purging with nitrogen for about 1 min, samples were refluxed for 2 h in a boiling water bath. Mixtures were then filtered and the final volume was adjusted to 100 mL with methanol. Mixtures were again filtered through a 0.45 μm membrane filter (Millex-HV) before injecting into a HPLC system. The HPLC system (Shimadzu LC-20AT) was equipped with LC-Solution software, auto-sampler (SIL-20A), column oven (CTO-20A), and diode array detector (SPD-M20A). Analytical column, Nucleosil C18, 5 μm 100 A° (250 × 4.60 mm, Phenomenex) coupled with a guard column (Phenomenex) was used. Mobile phase was composed of (A) sodium phosphate buffer (50 mM; pH 3.3) in 10% methanol and (B) 70% methanol. The gradient program by Sakakibara et al. (2003) was used with a flow rate of 1 mL min^{-1}. Phenolic compounds were identified by comparing retention time and UV–Vis spectra of

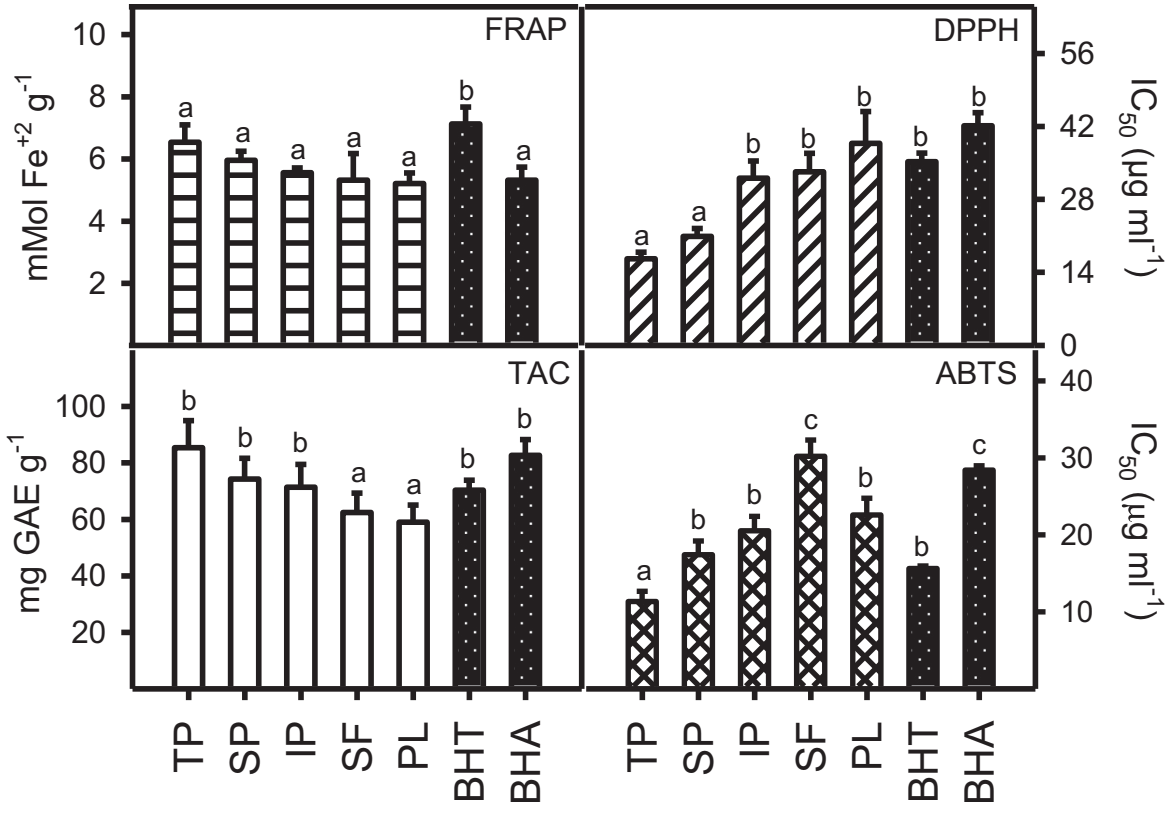

Fig. 3. Antioxidant capacity (DPPH, ABTS, FRAP and TAC) of *Thespesia populneoides* (TP), *Salvadora persica* (SP), *Ipomoea pes-caprae* (IP), *Suaeda fruticosa* (SF) and *Pluchea lanceolata* (PL) in comparison with synthetic antioxidants (BHT and BHA). Similar letters are not significantly different at $p < 0.05$.

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chromatographic peaks with that of authentic reference standards at 280 nm wavelength.

2.6. Statistical analysis

Values are expressed as means (±standard error) of minimum 5 analytical replicates of each plant sample. Pearson's correlation coefficient (r) and the coefficient of determination (R^2) were measured for the antioxidant activities and polyphenols ($p < 0.05$). The post-hoc LSD test was used to compare individual means. SPSS (version 16) and SigmaPlot (version 11) were used for all statistical analyses and graph preparation, respectively.

3. Results and discussion

3.1. Antioxidant capacities of medicinal plants

Antioxidant capacities (AC) of 100 medicinal plants collected from coastal areas of Pakistan were evaluated. In traditional medicinal system, these plants are mostly used as herbal teas against a range of diseases (Qasim et al., 2010, 2011, 2014). Preparing hot or cold tea from herbs is a traditional way to extract medicinal compounds and it is a most common way or consuming herbal remedy in rural communities. In addition, it is also considered that herbs used in such preparations are either free or possess minimal toxic/side effects hence are the suitable candidates for medicinal plant research. For instance, studies have been conducted to characterize the AC and phenolic composition of plant species used in herbal teas and the focus was mostly given to their water extracts (Nagao et al., 2005; Li et al., 2013). On the other hand, several studies showed that maximum yield of phenolic antioxidants has been found in aqueous-methanolic extracts rather than pure water extracts (Li et al., 2008; Abideen et al., 2015; Qasim et al., 2016). Therefore, in this study the aqueous-methanolic extracts were prepared to determine the AC and phenolic constituents of medicinal plants. Most of the plants were analysed for the first time, which in-general, showed considerable AC (Table 1). The DPPH radical scavenging activity (IC_{50}) of 100 medicinal plants ranged between 16.64 (*Thespesia populneoides*) to 1003 μg mL^{-1} (*Parkinsonia aculeata*) indicating a 60 fold variation, where lower DPPH values represent higher AC. The AC using FRAP system showed a 50 fold variation from 0.13 (*Peristrophe paniculata*) to 6.54 mMol Fe^{+2} g^{-1} (*T. populneoides*) (Table 1). Among all plants tested, five antioxidant rich species i.e. *T. populneoides*, *Salvadora persica*, *Ipomoea pes-caprae*, *Suaeda fruticosa*, and *Pluchea lanceolata*, were also subjected to TAC and ABTS activity (Fig. 3). The ABTS (IC_{50}) values ranged from 13.34 (*T. populneoides*) to 30.21 μg mL^{-1} (*S. fruticosa*), while TAC from 85.43 (*T. populneoides*) to 58.97 mg GAE g^{-1} (*P. lanceolata*). These species showed strong radical scavenging and reducing power capacities, which were better than synthetic antioxidants (BHT and BHA; Fig. 3). High AC found in these plants could be related to their biologically active compounds (vide infra). Such compounds make these plants superior to most of the antioxidant rich species including edible plants, herbs, medicinal plants, and some halophytes (Ksouri et al., 2008; Li et al., 2013).

3.2. Total phenolic content and its correlation with antioxidant capacity of medicinal plants

TPC of 100 medicinal plants ranged from 1.59 (*P. paniculata*) to 63.91 mg GAE g^{-1} (*T. populneoides*), showing more than 40 fold variation. Most of the plants had high TPC (≥10 mg g^{-1}) compared to values of some known medicinal plants such as *Diplotaxis harra* and *Diplotaxis simplex* (Falleh et al., 2013). High correlations of TPC of 100 medicinal plants with both DPPH ($R^2 = 0.72$) and FRAP ($R^2 = 0.75$) activities (Fig. 4) indicated the major contribution of phenolic compounds towards AC and associated therapeutic performance analogous to studies on other medicinal plants including halophytes (Cai et al., 2004; Li et al., 2008, 2013; Oueslati et al., 2012; Stankovic et al., 2015). Strong correlation ($R^2 = 0.73$) between DPPH and FRAP suggested radical scavenging and reducing power abilities of plant extracts, respectively (Fig. 4). Correlation (R^2) was also calculated between polyphenols (TPC, TFC, TCT and PC) and AC (DPPH, ABTS, FRAP and TAC) of five selected halophytes (Table 2). The TPC and TFC were strongly correlated with each of the AC measurements (DPPH, ABTS, FRAP and TAC). Polyphenols typically possess one or more phenyl rings and hydroxyl groups and are capable of detoxifying harmful oxidants either by donating hydrogen or electron (Pereira et al.,

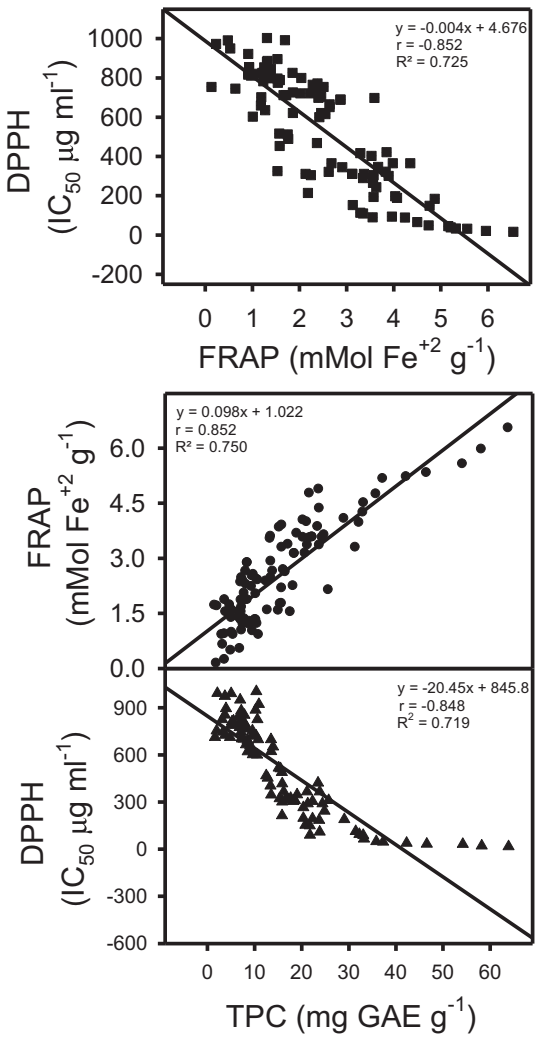

Fig. 4. Correlation of each antioxidant capacity test (DPPH and FRAP) with total phenolic contents (TPC) of 100 medicinal plants.

Table 2
Coefficient of determination (R^2) between polyphenols (TPC, TFC, TTC and PC) and antioxidant capacity (DPPH, ABTS, FRAP and TAC) of five selected halophytes.

	TPC	TFC	TTC	PC	DPPH	ABTS	FRAP
TFC	0.932						
TTC	0.227	0.329					
PC	0.005	0.062	0.645				
DPPH	0.904	0.972	0.404	0.126			
ABTS	0.691	0.519	0.165	0.027	0.613		
FRAP	0.911	0.873	0.301	0.055	0.931	0.771	
TAC	0.973	0.871	0.228	0.002	0.873	0.75	0.954

TPC — Total phenolic content; TFC- Total flavonoid content; TTC — Total tannin content; PC — Proanthocyanidin content; DPPH — DPPH radical scavenging activity; ABTS — ABTS radical scavenging activity; FRAP — Ferric reducing antioxidant power assay; TAC — Total antioxidant capacity.

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Table 3
Phytochemical composition of selected medicinal plants having high antioxidant capacity.

Species	Phenols (mg GAE g^{-1})	Flavonoids (mg QE g^{-1})	Tannins (mg TAE g^{-1})	Proanthocyanidins (mg CE g^{-1})	Carotenoids (mg g^{-1})	Alkaloids (mg g^{-1})	Saponins (mg DAE g^{-1})
T. populnea	63.91 (5.28)	37.13 (4.28)	14.66 (3.24)	20.14 (3.54)	0.72 (0.02)	0.82 (0.03)	28.41 (2.32)
S. persica	58.23 (3.54)	33.65 (3.54)	19.96 (8.43)	22.45 (2.87)	0.84 (0.02)	0.64 (0.02)	22.62 (1.12)
I. pes-caprae	54.21 (2.31)	23.65 (2.31)	11.32 (1.33)	19.67 (2.54)	0.61 (0.01)	1.14 (0.06)	11.25 (1.22)
S. fruticosa	46.54 (4.32)	21.43 (4.32)	8.71 (0.76)	15.76 (1.43)	0.56 (0.01)	0.41 (0.01)	12.62 (0.87)
P. lanceolata	42.28 (3.58)	12.34 (3.58)	13.01 (2.31)	20.52 (4.31)	0.07 (0.02)	0.93 (0.07)	15.43 (1.15)

GAE — Gallic acid equivalent; QE — Quercetin equivalent; TAE — Tannic acid equivalent; DAE — Diosgenin equivalent.

2013). Therefore, in most cases higher TPC have been linked with higher AC (Djeridane et al., 2006; Wong et al., 2006). With over 4000 phenolic compounds identified, any number of compounds either individually or in combination imparting synergistic effects could be responsible for higher AC and desired health benefit of medicinal plants (Williamson, 2001; Arabshahi-Delouee and Urooj, 2007; Rathore et al., 2011).

3.3. Bioactive and nutrient constituents of selected medicinal plants

Efficacy of medicinal plants is a function of their bioactive ingredients. In this study, the TPC, TFC, TTC, PC, TCC, TA, and TSC content were determined in five selected plants (Table 3) indicating their therapeutic benefits. These plants contained TPC (42.28 to 63.91 mg GAE g^{-1}), TFC (12.34 to 37.13 mg QE g^{-1}), TTC (8.71–19.96 mg TAE g^{-1}), and TPC (15.76 to 22.45 mg CE g^{-1}) in high quantities. Other bioactive compounds like TCC (0.07–0.84 mg g^{-1}), TA (0.41–1.14 mg g^{-1}), and TSC (11.25–28.41 mg DAE g^{-1}) were also found in considerable amounts (Table 3). These plant metabolites are reported to have several biological and pharmaceutical effects, including antimicrobial, antimalarial, antiinflammatory, antiviral, hypotensive, hypoglycemic, hepatoprotective, antioxidant and cardiovascular disease protecting activities (Niggeweg et al., 2004; Hirpara et al., 2009; Kasture et al., 2009).

Besides being used as herbal remedies, medicinal plants are also consumed as food due to their nutritional components (Qasim et al., 2011, 2014; Oueslati et al., 2012). The experimental values of nutrients in five selected species of the current study ranged as follows: moisture 68.58–80.21%, dry matter 19.88–31.52%, organic content 91.31–97.94%, ash 2.14–9.69%, proteins 8.54–17.08%, carbohydrates 2.62–11.42%, ether extract 1.03–6.81%, and fibre 31.65–42.17% (Table 4). Results showed that *T. populnea* could be a good source of protein (17.08%), while *S. persica* and *I. pescaprae* were rich in carbohydrates (11.42%) and fibre (42.17%), respectively (Table 4). These plants also have high ash content especially in *S. fruticosa* (9.69%) which could be used as cheap mineral source (Table 4). Results of this study are in line with the traditional use of these plants where *T. populnea*, *S. persica*, and *I. pescaprae* are used as vegetables (Alzoreky and Nakahara, 2003; Burkill, 1995; Narayanan et al., 2011) and *S. fruticosa* as food pickle (Oueslati et al., 2012). Nutritional composition of these plants is comparable to or even higher than some of the common vegetables (Hussain et al., 2010). These plants, in sufficient quantities, can be used as a source of nutrients and essential minerals, therefore, offering a healthcare solution with conceivable dietary balance especially for the rural population of third world countries.

3.4. Phenolic profile using HPLC

Phenolic composition (aglycones) of five selected halophytes were determined after hydrolysis using reference standards of gallic acid, catechin, chlorogenic acid, caffeic acid, syringic acid, ferulic acid, coumarin, naringenin, quercetin, and kaempferol (Fig. 5). All 10 phenolic aglycones were found in *S. persica* and *I. pes-caprae* while 9, 7 and 6 phenolic aglycones were identified in *P. lanceolata*, *T. populnea*, and *S. fruticosa*, respectively (Table 5). *Salvadora. persica* was reported to contain gallic acid, caffeic acid, *trans*-cinnamic acid, chlorogenic acid, resorcinol, kaempferol, quercetin, and rutin (Noumi et al., 2011; Halawany, 2012), whereas derivatives of isochlorogenic acid, isocoumarin and quercetin have been reported in *I. pes-caprae* (Meira et al., 2012). Phenolic compounds were also reported in *P. lanceolata* (pluchoic acid, isorhamnetin, quercitrin, and quercetin-3-rhamnoside; Srivastava and Shanker, 2012), and *T. populnea* (kaempferol, kaempferol 3-glucoside, quercetin, quercetin 3-glucoside, and rutin; Phanse et al., 2013). However, no report on phenolic composition of *S. fruticose* is available. Gallic acid, catechin, and quercetin were found in hydrolysed extracts of all test species. The dominant phenolic aglycones were chlorogenic acid and gallic acid, while catechin and quercetin were major flavonoid aglycones (Table 5). These compounds are reputed for high AC and some of these are used as standards in various antioxidant tests. Chemical structures of these compounds display antioxidant activity by allowing delocalization of electrons, neutralization of free radicals or chelation of metal ions prevent ROS damages (Zhou and Yu, 2004). Bioactive properties of phenolic compounds are known; such as chlorogenic acid displayed anticancer, antiviral, and hepatoprotective activities (Niggeweg et al., 2004), gallic acid is known for antimicrobial, antimalarial, anti-inflammatory, antitumor, and neuroprotective effects (Kasture et al., 2009), catechin bears antiplaque-forming, antiviral, hypotensive, hypoglycemic, and anticancer properties (Nagao et al., 2005)., and quercetin is used to treat hardening of arteries, cardiovascular problems, diabetes, cataracts, peptic ulcer, asthma, and prostate infections (Hirpara et al., 2009). Beside their vast application in medicinal and cosmetic industries, most of these compounds and their derivatives are also used as food additives to improve shelf life by protecting essential nutrients from oxidation and microbial deterioration (Sanches-Silva et al., 2014).

Table 4
Nutrient composition of selected medicinal plants having high antioxidant capacity.

Species	Moisture (% FW)	Dry matter (% FW)	Ash (% DW)	Organic matter (% DW)	Ether Extract (% DW)	Protein (% DW)	Carbohydrate (% DW)	Fibre (% DW)
T. populnea	71.12 (4.32)	28.95 (1.71)	8.73 (0.42)	91.31 (3.24)	4.32 (0.04)	17.08 (0.15)	5.33 (0.05)	31.65 (2.31)
S. persica	80.21 (3.21)	19.88 (1.25)	9.69 (0.26)	90.13 (4.54)	1.03 (0.02)	8.92 (0.02)	2.62 (0.01)	38.22 (3.15)
I. pes-caprae	75.36 (4.27)	24.71 (2.15)	7.29 (0.28)	92.82 (4.42)	6.81 (0.03)	8.54 (0.01)	5.31 (0.03)	42.17 (2.41)
S. fruticosa	73.84 (2.05)	26.29 (0.93)	6.32 (0.15)	93.75 (4.86)	2.33 (0.02)	14.32 (0.07)	11.42 (0.02)	36.62 (1.24)
P. lanceolata	68.58 (3.62)	31.52 (3.11)	2.14 (0.07)	97.94 (5.22)	3.25 (0.02)	10.51 (0.02)	7.36 (0.06)	32.11 (1.35)

FW — Fresh weight; DW — Dry weight.

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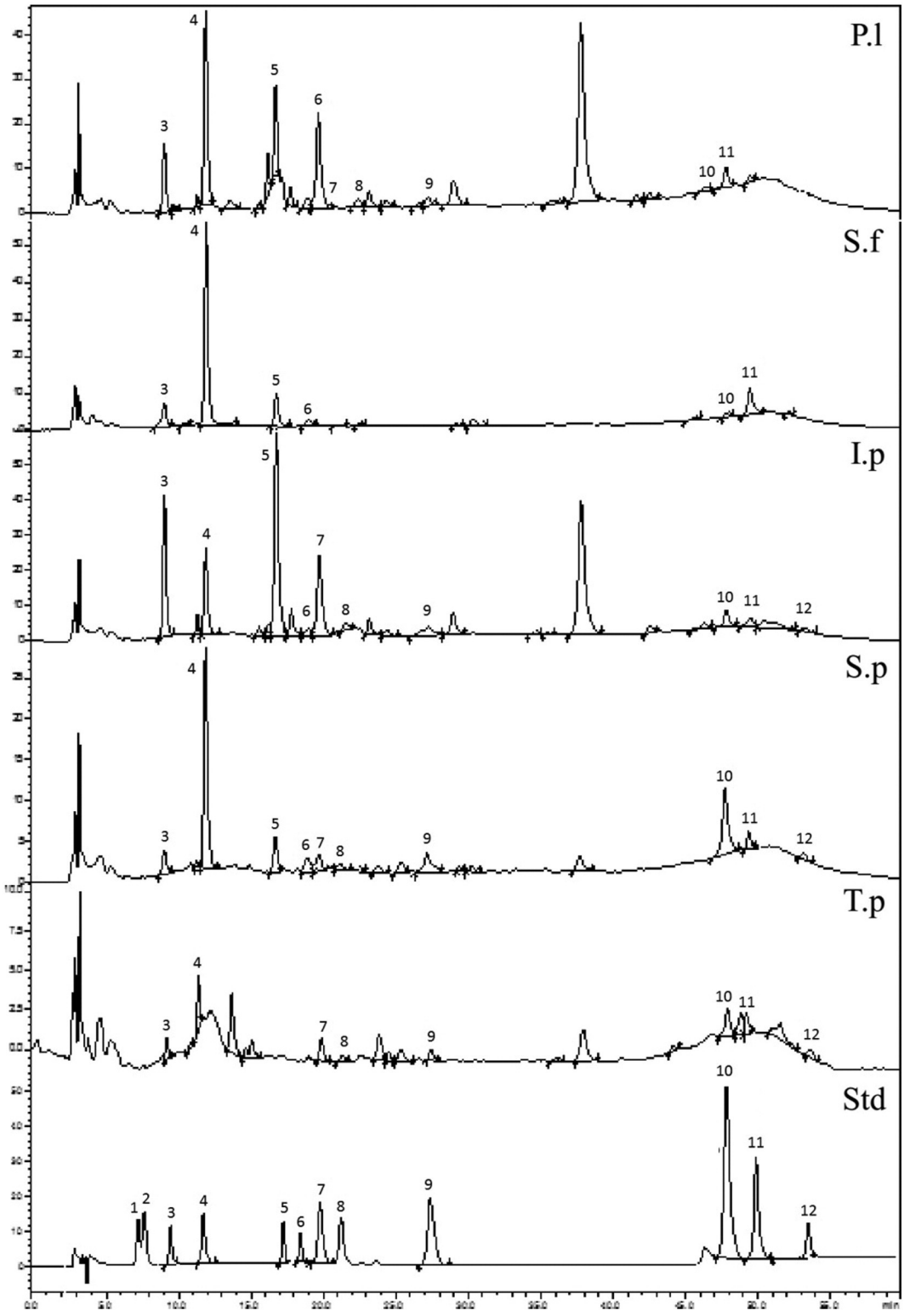

Fig. 5. HPLC chromatograms showing phenolic profile (1 — Pyrogallol; 2 — Hydroquinone; 3 — Gallic acid; 4 — Catechin; 5 — Chlorogenic acid; 6 — Caffeic acid; 7 — Syringic acid; 8 — Ferulic acid; 9 — Coumarin; 10 — Naringenin; 11 — Quercetin and 12 — Kaempferol) of standard compounds (STD) and hydrolysed leaf extracts of *Thespesia populneoides* (TP), *Salvadora persica* (SP), *Ipomoea pes-caprae* (IP), *Suaeda fruticosa* (SF) and *Pluchea lanceolata* (PL).

3.5. Relationship between antioxidant capacity and salt resistance of medicinal plants

Plants produce new metabolites and can also alter composition of existing chemicals to survive in different environmental stresses. For the efficient utilization of unexplored local flora, an eco-physiological approach is needed in medicinal plant research. Keeping this in mind, medicinal plants of this study were analysed on the basis of their salt resistance ability. Halophytes had 37% higher phenolic content and ~25% higher DPPH and FRAP activity than non-halophytes. ANOVA showed a significant effect of salt resistance on polyphenols and AC of medicinal plants (Table 6). Halophytes are naturally designed to grow

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Table 5
Phenolic composition of selected medicinal plants having high antioxidant capacity.

Species	Phenolic compounds (mg g^{-1})									
	Gallic acid	Catechin	Chlorogenic acid	Caffeic acid	Syringic acid	Ferulic acid	Coumarin	Naringenin	Quercetin	Kaempferol
T. populnea	0.391 (0.02)	0.415 (0.03)	nd	nd	0.373 (0.01)	0.333 (0.01)	nd	0.277 (0.01)	0.338 (0.01)	0.357 (0.01)
S. persice	0.269 (0.01)	0.890 (0.04)	0.697 (0.03)	0.373 (0.01)	0.208 (0.01)	0.210 (0.01)	0.172 (0.01)	0.175 (0.01)	0.169 (0.01)	0.190 (0.01)
I. pes-caprae	1.419 (0.07)	0.824 (0.03)	7.369 (0.11)	0.367 (0.01)	0.618 (0.02)	0.241 (0.01)	0.289 (0.01)	0.140 (0.01)	0.191 (0.01)	0.211 (0.01)
S. fruticosa	0.449 (0.02)	1.667 (0.08)	1.268 (0.09)	0.383 (0.01)	nd	nd	nd	nd	0.247 (0.01)	0.176 (0.01)
P. lanceolata	0.652 (0.05)	1.335 (0.12)	1.888 (0.09)	0.436 (0.04)	0.571 (0.06)	0.189 (0.01)	0.251 (0.01)	0.135 (0.01)	0.164 (0.01)	nd

nd — not detected.

and complete their life cycle in harsh saline environments (Alhdad et al., 2013). Under these conditions, the production of ROS, is enhanced many folds which necessitates the role of an efficient antioxidant system. As a result, tolerant plants tend to synthesize bioactive compounds including polyphenolic antioxidants in order to protect their vital metabolic functions from oxidative damage (Falleh et al., 2012, 2013).

Among all plant types, TPC and AC were found higher in hydrohalophytes and xerohalophytes, whereas weedy glycophytes and xerophytes showed relatively lower TPC and AC (Fig. 6). In addition to their strong tolerance to salinity, some halophytes have also adapted to drought and waterlogged conditions. In such habitats, synergistic effects of salinity with drought or flooding amplify the magnitude of applied stress, which in turn increased the synthesis of antioxidant compounds (Alhdad et al., 2013). As a consequence, higher accumulation of polyphenols in leaves of halophytes suggest the fundamental role of these plant metabolites to protect the photosynthetic machinery from excessive light, UV and heat, and stimulates the antioxidant enzyme system (Tattini et al., 2005). For instance, mangroves and mangrove-associated halophytes such as *Aegiceras corniculatum*, *Bruguiera parviflora*, *Salicornia brachiata*, *Suaeda maritima*, and *Tamarix gallica* are also reported to have high AC (Ksouri et al., 2008; Alhdad et al., 2013).

4. Conclusions

The AC and TPC of 100 coastal medicinal plants, used to prepare herbal teas were evaluated in which most of the plants were analysed for the first time. Strong correlation between AC and TPC implied that phenolic compounds are the main contributors to AC. Five widely distributed salt tolerant species i.e. *T. populneoides*, *S. persica*, *I. pes-caprae*, *S. fruticosa*, and *P. lanceolata*, contained considerable amount of bioactive phytochemicals as well as nutrients. Detailed HPLC analyses identified chlorogenic acid, gallic acid, catechin, quercetin and other phenolic compounds which could be responsible for high AC of these plants. Studied plants presents a promising source of natural antioxidants, bioactive compounds, and essential nutrients which can be exploited for multiple industrial and domestic applications. Higher AC and TPC in halophytes than non-halophytes suggest that applying eco-physiological approach in medicinal plant studies would help to find promising candidates with high bioactive properties. Furthermore, these plants do not require good quality soils and fresh water resources but can be grown on vast degraded lands with brackish water irrigation. Sustainable development of saline/marginal lands may provide biomass of high medicinal and edible value resulting in economic gains through safe and environmental friendly measures.

Table 6
Comparison between halophytic and non-halophytic medicinal plants for their total phenolic content (TPC) and antioxidant capacity (DPPH and FRAP). The F values from ANOVA are given and asterisks in superscripts are showing significance level at $p < 0.01$.

	TPC (mg GAE g^{-1})	DPPH (IC_{50} μg ml^{-1})	FRAP (mMol Fe^{+2} g^{-1})
Halophytes	19.31 (2.16)	446.46 (45.94)	2.94 (0.21)
Non-halophytes	12.10 (0.98)	585.74 (34.90)	2.20 (0.16)
ANOVA	9.714**	4.502**	7.774**

GAE — Gallic acid equivalent; DPPH — DPPH radical scavenging activity; FRAP — Ferric reducing antioxidant power assay.

Conflict of interest

Authors declared no conflict of interest.

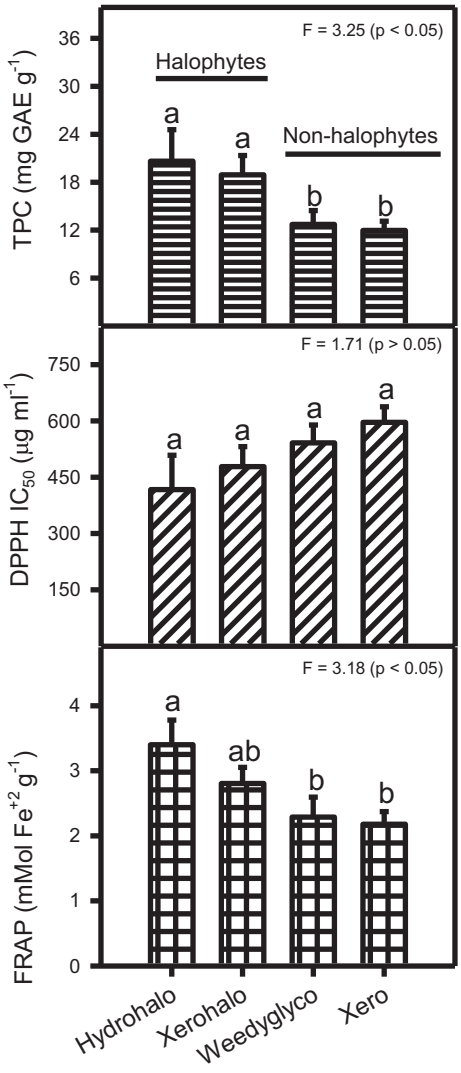

Fig. 6. Comparison of total phenolic content (TPC), DPPH and FRAP among different plant types. Where Hydrohalo, Xerohalo, Weedyglyco and Xero represents hydrohalophytes, xerohalophytes, weedy glycophytes and xerophytes respectively. Similar letters are not significantly different at $p < 0.05$.

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