Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology
Official Journal of the Societa Botanica Italiana

ISSN: 1126-3504 (Print) 1724-5575 (Online) Journal homepage: https://www.tandfonline.com/loi/tplb20

Phytotoxic analysis of coastal medicinal plants and quantification of phenolic compounds using HPLC

Muhammad Qasim, Yoshiharu Fujii, Muhammad Zaheer Ahmed, Irfan Aziz, Kazuo N. Watanabe & M. Ajmal Khan

PLANT BIOSYSTEMS - AN INTERNATIONAL JOURNAL DEALING WITH ALL ASPECTS OF PLANT BIOLOGY
https://doi.org/10.1080/11263504.2018.1549607

Taylor & Francis
Taylor & Francis Group

Check for updates

Phytotoxic analysis of coastal medicinal plants and quantification of phenolic compounds using HPLC

Muhammad Qasim[a,b,c], Yoshiharu Fujii[b], Muhammad Zaheer Ahmed[a], Irfan Aziz[a], Kazuo N. Watanabe[c] and M. Ajmal Khan[a]

[a]Institute of Sustainable Halophyte Utilization (ISHU), University of Karachi, Karachi, Pakistan; [b]Tokyo University of Agriculture and Technology, Fuchu City, Japan; [c]Gene Research Center, Graduate School of Life and Environmental Sciences, University of Tsukuba, Ibaraki, Japan

ABSTRACT
Medicinal plants are rich sources of bioactive phytochemicals. Production of such chemicals usually increased under unfavorable conditions. This study investigated the phytotoxic potential of 105 medicinal plants distributed in arid/semiarid areas along Arabian Sea coast of Pakistan. Total polyphenols, flavonoids, tannins and composition of individual phenolic compounds were also determined in species which showed high phytotoxic potential. Sandwich method was used to determine the phytotoxicity of medicinal plants on the growth of lettuce seedlings. Radicle growth was inhibited more than the hypocotyl growth. In general, halophytes showed higher phytotoxic potential than non-halophytes. *Capparis cartilaginea*, *Indigofera hochstetteri*, *Parkinsonia aculeata* and *Prosopis glandulosa* showed highest degree of inhibition. Higher amount of total phenols (16.35–25.33 mg GAE g^{-1}), flavonoids (3.32-6.41 mg QE g^{-1}) and tannins (1.54–2.54 mg TAE g^{-1}) were found in these species. Pyrocatechol, quercetin, gallic, hydroxybenzoic and ferulic acids were detected as major phytotoxins, of which, gallic acid, pyrocatechol and quercetin were most abundant. These phytochemicals could be used for the production of natural, safe, healthy and eco-friendly agro-chemicals. Furthermore, these plants can be grown without encroaching agricultural lands, and can convert vast areas of arid/saline lands into economically viable resources, which also helps to halt climate change and desertification.

ARTICLE HISTORY
Received 19 April 2018
Accepted 29 October 2018

KEYWORDS
Allelochemicals; halophytes; hypocotyl; leaf litter leachates; radicle; sandwich method

Introduction

Plants release chemicals which may directly or indirectly influence the growth and survival of other species. Some of these chemicals could be used as a potential source of natural herbicides/weedicides (Fujii et al. 1990). In this context, medicinal plants have been screened worldwide due to their diverse chemical nature and potent bioactivities. Crude extracts of some of species are widely used as efficient bioherbicides (Bais et al. 2003; Fujii et al. 2003; Gilani et al. 2010; Alqarawi et al. 2018; Zribi et al. 2018). Putative chemicals such as polyphenols, coumarins, terpenoids, alkaloids, cyanogenic glycosides and steroids are known to inhibit seed germination and growth of other plants hence, could be used to manage weeds in agricultural settings (Chung et al. 2001). On the other hand, synthetic herbicides are the major pollutants of soil and water and are toxic for plants, animals, and humans. Some of these herbicides are banned by the regularity authorities due to their health risks, toxicities and cancer causing effects (Gasnier et al. 2009). Plants can offer a variety of ingredients, which can be used to develop natural, safe, healthy and environment friendly herbicides (Islam et al. 2018). Since, the concept of organic farming has grown rapidly, the farmers are prompted to produce contaminant free crops and cultivation of several crops like sugar cane, maize and some vegetables has been started using natural herbicides.

Quantity and quality of phytochemicals may vary with plant species and their habitat conditions. Plants distributed in extreme environments usually produce secondary metabolites in higher quantities. Recently, these metabolites and their enhanced production under stress conditions is gaining interest among researchers. For instance *Cynodon dactylon*, *Desmostachya bipinnata*, *Leptochloa fusca*, *Prosopis juliflora*, *Sporobolus arabicus*, *Suaeda fruticose* and some other salt and drought tolerant plants were found to have potent phytotoxic effects (Mahmood et al. 1993; Noor and Khan 1994; Mo and Hangqing 2001; Li et al. 2004). These plants produce higher quantities of phytotoxins to get competitive advantage over other plants. Therefore, investigation among stress tolerant plants may extend the pool of natural products, which may provide alternative to harmful synthetic agrochemicals.

Coastal area of Pakistan is representing an arid/semiarid region, providing habitat for about 40% of local halophytic flora (Khan and Qaiser 2006). Some plants from this region

CONTACT Muhammad Qasim mqasim@uok.edu.pk Institute of Sustainable Halophyte Utilization (ISHU), University of Karachi, University road, Karachi 75270, Pakistan

Supplemental data for this article can be accessed at https://doi.org/10.1080/11263504.2018.1549607.

Published online 15 Jan 2019

have been identified for their distinct and potent phytochemicals and bioactivities (Abideen et al. 2015; Qasim et al. 2017; Nazir et al. 2018). Local dwellers have a thorough knowledge about variety of edible and medicinal plants, which are commonly used for primary health care (Qasim et al. 2012, 2014, 2017; Toqeer et al., 2018). However, little is known about their phytotoxic potential. The aim of this study was to evaluate the phytochemical properties of 105 medicinal plants of this region. Considering the salt and drought resistance abilities of halophytes, studied medicinal plants were categories to check weather halophytes have higher alleopathic potential than non-halophytes. Content and composition of some putative bioactive chemicals mainly polyphenolics in selected/promising candidate species were also analyzed using HPLC.

Materials and methods

Plant collection and sample preparation

Plants were collected from coastal areas of Pakistan (Figure 1) in 2010 and 2011, during which high temperatures (minimum 23 °C and maximum 33 °C), and humidity (50%) with low rainfall (~4 cm) were recorded (Pakistan Meteorological Department, Karachi; Figure 2). For phytotoxic study; samples were oven dried at 70 °C for 24 h and for polyphenolic content, plant samples were extracted with 80% methanol for 3 h at 40 °C and their supernatants were used for analyses (Qasim et al. 2016; 2017). For extraction and sample preparation for HPLC analysis, methods described by Zahra et al. (2017) and Ahmed et al. (2017) were used, respectively.

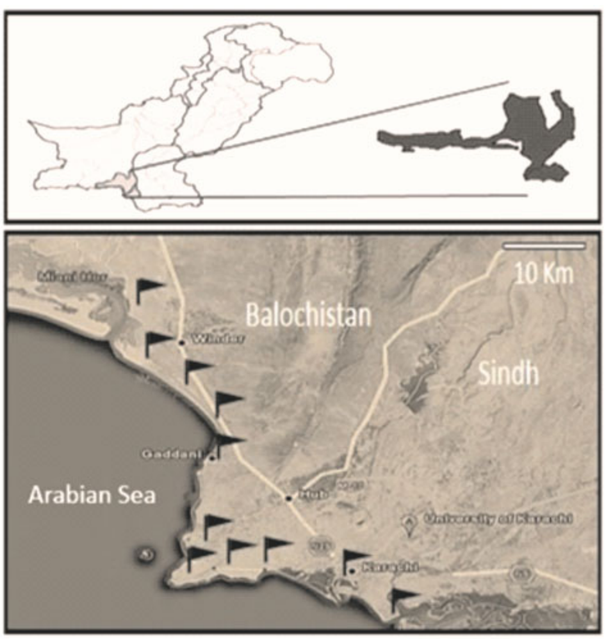

Figure 1. Map of study area showing location of plants collected from coastal habitats (dark flags).

Phytotoxic potential by sandwich method

Sandwich method which is based on the standard estimated values of average annual litter fall per unit area was used for phytotoxic analysis (Fujii et al. 2003). Briefly, 5 mL of 0.75% (w/v) pre-heated agar solution (Nakalai Tesque, Tokyo Japan, gelling temperature 30–31 °C) was poured in six-welled (10 cm^2 area) plastic plates. After solidification of agar, 10 and 50 mg of dried leaves from each species were placed over solidified layer and was covered with another layer of agar (5 mL). *Lactuca sativa* (*L. sativa* Great Lakes No. 366, Takii Seed Co. Ltd., Japan) was used as a test plant because of its high sensitivity to chemicals (Shao et al. 2013). Five lettuce seeds were placed in each well after solidification of agar gel. Three separate plates were used as technical replicates of each plant treatment (10 and 50 mg). Each plate contained five wells for treatment (biological replicates) and one well for its respective control (agar gel without dried leaves). Finally, plates were sealed with plastic tape and incubated for 72 h at 24 °C in dark. Seed germination and length of seedlings (radicle and hypocotyl) were measured after the incubation period.

Content and composition of phenolic compounds

Total phenolic (Singleton and Rossi 1965), flavonoid (Chang et al. 2002) and tannin (Sun et al. 1998) contents were determined in selected species. The HPLC system (Shimadzu LC-20AT) including auto-sampler (SIL-20A), photo-diode array detector (SPD-M20A), column oven (CTO-20A), Nucleosil C18 column (5 µm 100A, 250 × 4.60 mm, Phenomenex), guard column (KJO-4282, Phenomenex) and LC Solution software was used to quantify individual phenolic compounds. Mobile phase consisted of sodium phosphate buffer (50 mM; pH 3.3) and 70% methanol. Gradient program by Qasim et al. (2017)

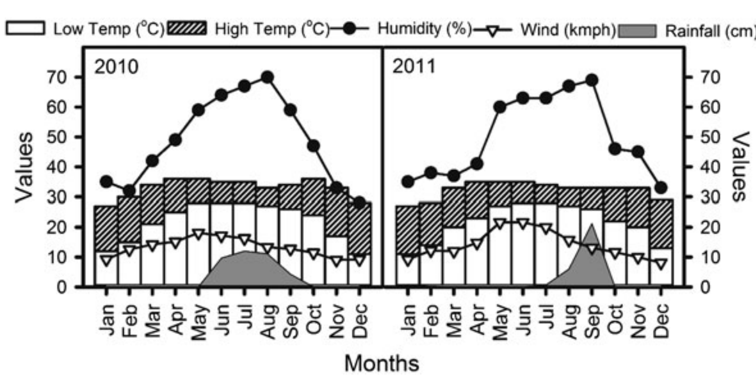

Figure 2. Environmental data of study area comprising mean annual temperatures (low and high), rainfall, humidity and wind velocity during period (2010 and 2011) of sample collection (Pakistan Meteorological Department).

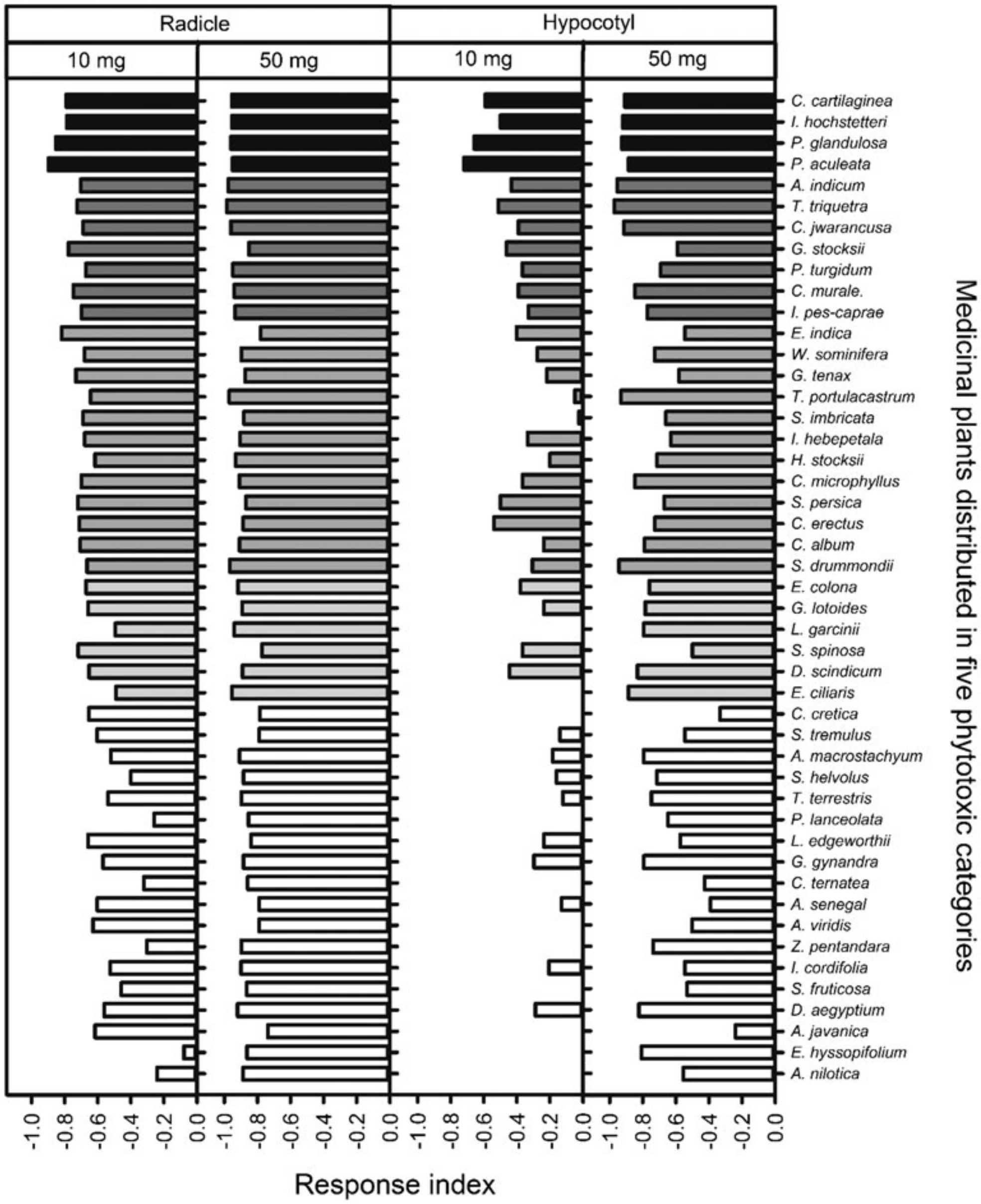

Figure 3. Response index of 47 medicinal plants distributed in five phytotoxic categories.

was followed with 10 μL injection volume and 1 mL min^{-1} flow rate at 35 °C. Phytotoxic compounds were identified at 280 nm wavelength by comparing retention time and UV-Vis spectra of individual peaks with authentic reference standards (purchased from Sigma Aldrich).

Statistical analyses

Length of radicle and hypocotyl is presented in terms of either percent inhibition (<100%) or promotion (>100%), as compare to control. The entire data was normalized before statistical analyses and normality test was conducted to ensure the homogeneity of variance. Mean, standard deviation (SD) and standard deviation variance (SDV) were calculated by SPSS (version 11 for windows). Data were classified into five different categories based on the inhibitory effect on radicle length using normal distribution, where values of SD (σ) were subtracted from the mean value (M) at three levels i.e. 0.5σ, 1.0σ and 1.5σ. Single (†), double (††) and triple daggers (†††) represent M-0.5σ, M-1.0σ and M-1.5σ, respectively (Fujii et al. 2003). Daggers from both 10 and 50 mg treatments were added for category four (††††) and five (†††††). Response index (RI) was also calculated for those species which lies under phytotoxic categories using equation (RI = T/C − 1; T – lettuce seedling growth treated with leaf leachates; C – lettuce seedling growth using distilled water), described by Williamson and Richardson (1988). Two-way ANOVA using SPSS ver. 11.0 (SPSS for windows version 11) was performed to compare significant differences among plant type and their distribution. Graphs were plotted using Sigmaplot (version 10).

Results and discussion

Phytotoxic potential of 105 medicinal plants collected from arid/semiarid areas along the Arabia Sea coast of Pakistan

Table 1. Distribution of medicinal plants in different groups and sub-groups in terms of their toxicity levels (Group of species containing <10% of total species are not included in this table; ns – non-significant).

Groups / Sub-groups	Species (%)	ns	†	††	†††	††††	†††††
		(% of particular species sub-group under phytotoxic categories)					
Plant Type							
Halophytes	65	51	15	5	9	11	9
Non-halophytes	35	71	19	3	7	0	0
Distribution							
Coastal	50	43	18	8	14	11	6
Near-coastal	50	67	15	4	6	4	4
Plant habit							
Tree	10	64	18	0	9	0	9
Grass	18	53	16	16	5	11	0
Shrub	30	52	16	0	19	6	6
Herb	30	47	25	6	13	6	3
Plant morphotype							
Dicotyledonous (D)	80	54	18	4	13	6	5
Monocotyledonous (M)	20	61	13	13	4	9	0
Life cycle							
Perennial (P)	80	58	17	4	11	7	4
Annual (A)	20	45	18	14	14	9	0

was investigated (supplementary material Table S1). Leaf leachates were used to evaluate the phytotoxic effects of medicinal plants on the growth of lettuce seedlings. The degree of inhibition varied across species, concentration of leachates, and part of lettuce seedling. Leachates (10 and 50 mg) of most of the medicinal plants significantly reduced lettuce seedling length, and this effect was more prominent in 50 mg treatment (supplementary material Table S1). Leaf leachates of all medicinal plants inhibited radicle length of lettuce seedlings. Hypocotyl length was also inhibited by most plants however, it was promoted by 50 mg leachates of *Heliotropium currassavicum*, *Prosopis cineraria* and *Sida ovate* (supplementary material Table S1). Similar results were also reported from the extract of other medicinal plants, where seedling growth was promoted at low concentration but inhibited at higher concentration (Anjum et al. 2010). For instance, seedling growth of rye grass and creeping red fescue was promoted by diluted leaf extracts of *Pinus sylvestris*, but similar extracts in high doses inhibited the seedling growth of both test species (Bulut and Demir 2007). Some phytochemicals in low concentrations may contribute to the synthesis of plant growth regulators (Li et al. 1993), caffeic and ferulic acids can be presented as an example (Li et al. 1993).

Our data indicate the higher degree of inhibition in radicle length than the hypocotyl length (Figure 3), which could be due to the direct exposure of roots to the inhibitory compounds (Shao et al. 2013). Furthermore, greater permeability of phytotoxins has been reported to (1) cease meristematic activity of roots, (2) restrict nutrient absorption by altering plasma membrane permeability and (3) modify phyto-hormone synthesis (Fujii et al. 1990). Bais et al (2003) supported the 'novel weapon hypothesis' and observed that phytotoxins may help in triggering excess production of reactive oxygen species (ROS), which may induce programed cell death in root tissues. By absorbing them through roots, these chemicals may also affect various other physiological processes (e.g. photosynthesis, mineral regulation, cell division or elongation), which in turn reduce growth of recipient plant (Fernandez et al. 2013).

In this study, medicinal plants represent 31 families (28 dicot and 3 monocot), in which most of the species were from Poaceae (18) and Leguminosae (16), followed by Amaranthaceae (12), while, rest of the families had less than 10 species (supplementary material Table S1). Most of the species (75%) showing severe phytotoxicity (†††††) were from Leguminosae. Among all tested plants, 47 species showed significant inhibition and based on the level of inhibition they were classified into five different categories, i.e. severe (†††††), high (††††), moderate (†††), low (††) and very low (†), represented by 4, 7, 12, 16 and 18 species, respectively (Figure 3). These species also showed negative RI (response index) values, which indicated the increasing level of inhibition among different phytotoxic categories (Figure 3). Among all medicinal plants, leaf leachates of *Capparis cartilaginea*, *Indigofera hochstetteri*, *Parkinsonia aculeata* and *Prosopis glandulosa* showed maximum inhibition (>90% for both hypocotyl and radicle) and lies under severe phytotoxic category (†††††) with most negative RI values (Figure 3). Inhibitory effect of these species was higher than other known phytotoxic plants, including *Peganum harmala* (Shao et al. 2013), *Inula falconeri*, *Inula koelzii*, *Lactuca dissecta*, *Anthemis nobilis* (Khan et al. 2009), *Foeniculum vulgare* and *Rhazya stricta* (Gilani et al. 2010). Except for *C. cartilaginea* rest of the species are leguminous, of which *P. glandulosa* was found to be toxic for wheat cultivars (Alam and Azmi 1989), while *P. aculeata* and *P. cineraria* also showed damaging effects on other crops (Noor et al. 1995). Fujii et al (2003) screened more than 200 medicinal plants and reported strong inhibitory effects of leguminous species. Leguminous plants are known for their symbiotic relationship with soil microbes, which could bring about changes in plant metabolic pathways thereby altering the profile of different secondary metabolites. This association facilitates leguminous plants to grow better by fixing nitrogen and could also help to reduce a competitive effect through the production of phytotoxic compounds (Fernandez et al. 2013). Such a relationship of leguminous plants in perspective of phytotoxicity would be an interesting idea for future studies.

Table 2. Comparison between halophytic and non-halophytic medicinal plants for phytotoxic potential.

	10 mg		50 mg	
	Radicle	Hypocotyl	Radicle	Hypocotyl
Non-halophytes	52.68 (2.36)	97.81 (3.58)	22.68 (1.78)	52.29 (3.52)
Halophytes	42.05 (1.68)	84.01 (3.30)	17.81 (1.29)	42.21 (3.24)
ANOVA	14.127***	7.559**	5.147**	4.162*

Note. Values represent mean (standard error) of lettuce seedling length. *F* values from ANOVA are given and asterisks in superscripts show significance level (***at $p < .001$, **at $p < .01$ and *at $p < .05$).

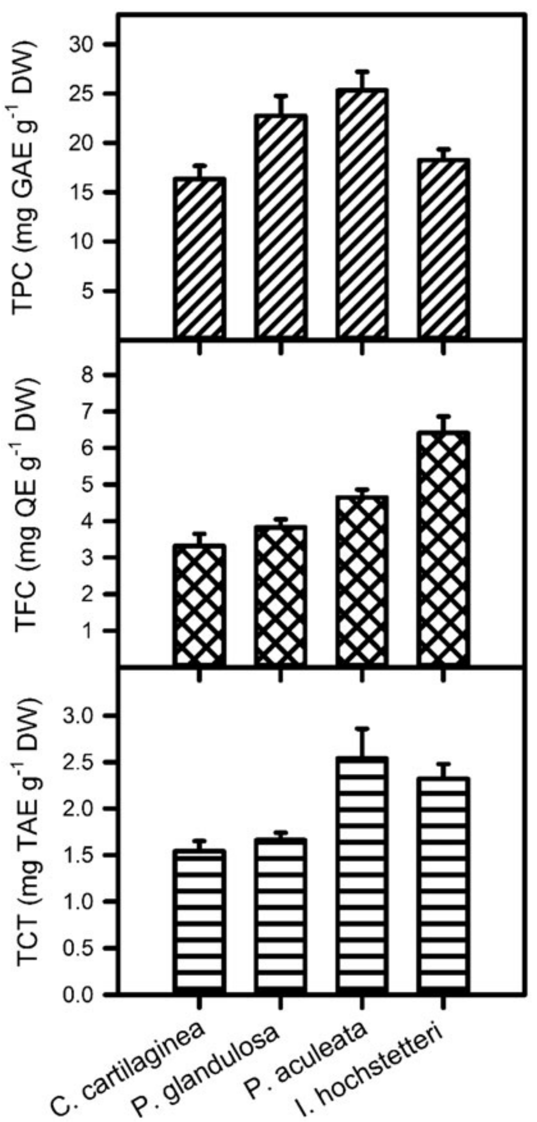

Figure 4. Total phenols (TPC), flavonoids (TFC) and condensed tannins (TCT) of four most phytotoxic species.

We found that, perennial trees, grasses and shrubs appear to have higher phytotoxic potential compared to other life forms (Table 1). Perennial plants appeared to invest more energy in chemical defense than on reproductive allocation. Shrubs have relatively longer life span and are subjected to greater environmental variations. Besides competing for resources, these plants produce a variety of chemicals to suppress the growth of competitors (Suresh and Vinaya Rai 1987). Our results are in accordance with previous studies, which shows high phytotoxic potential of perennial plants such as *Platanus occidentalis*, *Celtis laevigatus*, *Quercus rubra*, *Quercus alba*, *Eucalyptus camaldulensis* (Lodhi 1976), *Eucalyptus tereticornis*, *Casuarina equisetifolia* and *Leuceana leucocephala* (Suresh and Vinaya Rai 1987).

Synthesis of phytochemicals is often associated with plant genetic make-up as well as duration and quantum of environmental stresses. In this study, significantly higher phytotoxic potential was found in halophytes than non-halophytes (Tables 1 and 2), which may be associated to their survival in harsh environmental conditions such as salinity, water deficit, heat, nutrient imbalance, etc. Under such conditions, plant produce hormones, phytochemicals and bioactive secondary metabolites for their survival, which could also serve as phytotoxic agents at the community level (Mahmood et al. 1993). Some halophytes are reported to inhibit growth of neighboring plants by changing soil chemistry (Ungar 1998). *Prosopis juliflora* can be presented as a good example of a potentially toxic plant for co-occurring species, especially in arid/saline habitats (Noor and Khan 1994). Other coastal halophytes i.e. *Bruguiera gymnorrhiza*, *Kandelia candel* and *Salicornia herbacea* also possess considerable phytotoxicity (Mo and Hangqing 2001; Li et al. 2004). Mahmood et al. (1993) observed autoallelopathy as well as toxic effects of salt tolerant plants on seed germination and productivity of *Leptochloa fusca*. Furthermore, enhanced production and exclusion of phytotoxic root exudates was observed when these plants were exposed to abiotic stresses (Pramanik et al. 2000).

Phytotoxic effect of both simple (phenolic acids) and complex (flavonoids and tannins) phenolic compounds on germinating seeds and growing plants has been reported (Li et al. 2010; Einhellig 2004). In this study, four species (three belonging to family Leguminosae) with higher phytotoxic potential were found to contain high amount of TPC (16.35–25.33 mg GAE g^{-1}), TFC (3.32–6.41 mg QE g^{-1}) and TCT (1.54–2.54 mg TAE g^{-1}) (Figure 4). Previous reports suggested that species with high levels of such compounds showed high phytotoxic potential (Hierro and Callaway 2003). Phenolic compounds are known to interfere with major physiological processes of recipient plants (such as cell division, membrane permeability, synthesis of macro-molecules, enzyme activity, photosynthesis, respiration, mineral uptake and water balance) (Li et al. 2010). Basile et al. (2000) reported that flavonoids released from *Castanea sativa*, inhibited seed germination and seedling growth of *Raphanus sativus*. Similarly, tannins released from Engelmann spruce were toxic for other conifers (Taylor and Shaw 1983). Green and Corcoran (1975) tested five different tannins, each exhibited growth inhibition in the dwarf pea plants.

Although a number of active compounds may be present in test species, owing to their strong phytotoxic effects and higher contents, phenolic compounds were analyzed by HPLC against available standards. Ten major phenolic compounds were identified in extracts of selected species, including gallic acid, hydroxybenzoic acid, pyrocatechol, catechin, caffeic acid, ferulic acid, coumarin, cinnamic acid, quercetin and kaempferol (Figure 5). Of the total identified compounds, eight were found in *P. aculeata*, six in *P. glandulosa* and *I. hochstetteri*, while *C. cartilaginea* had five phenolic

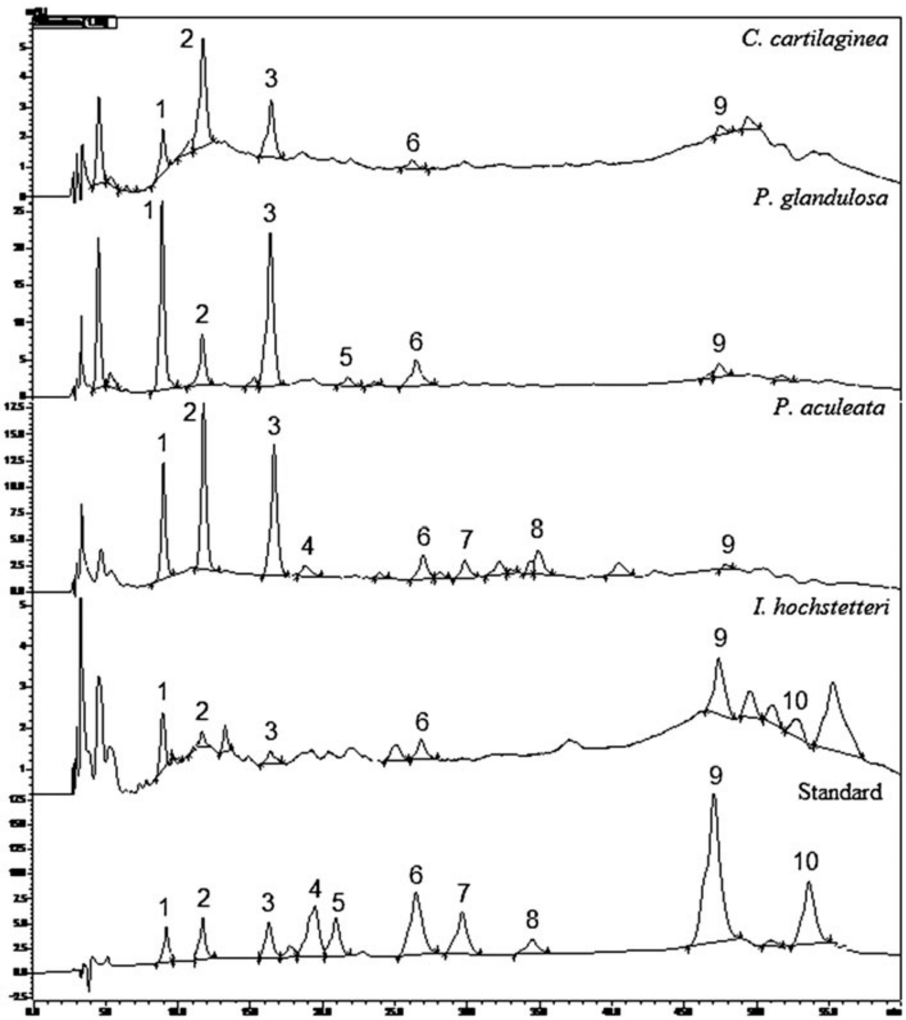

Figure 5. HPLC chromatogram showing phenolic profile (1: Gallic acid; 2: Hydroxybenzoic acid; 3: Pyrocaticol; 4: Catechin; 5: Caffic acid; 6: Ferulic acid; 7: Coumarin; 8: Cinamic acid; 9: Qurcetin; 10: Kaempferol) of standard compounds as well as four highly phytotoxic species.

Table 3. Quantification of individual phenolic compound (mg g^{-1}) in leaves of four highly phytotoxic plants using HPLC.

Phenolic compounds	*Capparis cartilaginea*	*Prosopis glandulosa*	*Parkinsonia aculeata*	*Indigofera hochstetteri*
Gallic acid	0.512 (0.026)	8.203 (0.013)	3.203 (0.012)	0.440 (0.003)
Hydroxybenzoic acid	0.987 (0.034)	1.797 (0.009)	3.617 (0.028)	0.131 (0.001)
Pyrocatechol	0.531 (0.012)	5.538 (0.011)	3.147 (0.016)	0.090 (0.001)
Catechin	nd	nd	0.180 (0.001)	nd
Caffeic acid	nd	0.295 (0.001)	nd	nd
Ferulic acid	0.019 (0.001)	0.466 (0.002)	0.183 (0.002)	0.054 (0.001)
Coumarin	nd	nd	0.298 (0.002)	nd
Cinnamic acid	nd	nd	1.492 (0.015)	nd
Quercetin	0.011 (0.001)	0.045 (0.001)	0.016 (0.001)	0.067 (0.001)
Kaempferol	nd	nd	nd	0.073 (0.001)

nd: not detected.

compounds (Table 3). Four phenols (gallic acid, hydroxybenzoic acid, pyrocatechol, ferulic acid) and one flavonoid (quercetin) were common in four test species, of which gallic acid, pyrocatechol and quercetin were in higher quantities (Table 3). Gallic and hydroxybenzoic acids were reported as strong phytotoxins in *E. tereticornis*, *E. camaldulensis*, *E. polycarpa*, *E. microtheca* (Sasikumar et al. 2004), *Vigna mungo* (Li et al. 2010), and *Rorippa sylvestris* (Yamane et al. 1992). Ferulic acid was previously identified as one of the major phytotoxins in aerial parts as well as rhizosphere of *Merostachys riedeliana* and *Ageratum conyzoides*. Ferulic acid is also reported to have negative impact on the growth of tomato, rice and maize seedlings (Devi and Prasad 1992; Batish et al. 2009; Jose et al. 2016). Hydroxybenzoic, ferulic and cinnamic acids inhibited seed germination and seedling growth of sesame and radish (Chon et al. 1988). Pyrocatechol isolated from *Populus tremuloides* bark inhibited growth of lettuce (Yamane et al. 1992) and *Hypoxylon mammatum* (Hubbes 1962, 1966). Quercetin was one of the main phytotoxins in leaves of *Salsola kali* (Lodhi 1979) and *C. sativa* (Basile et al. 2000) and its derivatives showed inhibitory effects on *Arabidopsis thaliana* and *Neurospora crassa* (Parvez et al. 2004).

It can be concluded from our study that a large number of studied medicinal plants (distributed in harsh arid/semi-arid environment) showed considerable phytotoxic effects. Among these, halophytes (considerably more stress tolerant plants) showed higher phytotoxcity than non-halophytes. Among selected medicinal plants, *P. aculeata*, *P. glandulosa*, *C. cartilaginea* and *I. hochstetteri*, showed higher content of total phenols, flavonoids and tannins. HPLC analyses revealed a distinctive composition of phytotoxic compounds including pyrocatechol, quercetin, gallic acid, hydroxybenzoic acid and ferulic acid. The type and quantity of naturally occurring phytochemicals makes these plants a better choice for extracting valuable agro-chemicals. In addition, these plants could be grown on arid/saline wastelands using brackish water to obtain natural alternatives of harmful synthetic herbicides/weedicides.

Acknowledgements

Authors would like to thank Higher Education Commission (HEC) of Pakistan for providing financial support to Prof. M.A. Khan under collaborative project between Pakistan and Japan entitled 'Conversion of traditional knowledge and resources into modern sciences, industries and environmental protection using Pakistan indigenous plant genetic resources'. M. Qasim is a recipient of indigenous PhD scholarship from HEC. This research was partly supported by Grant-in-Aid # 21248001 from Japan Society for Promotion of Science and Ministry of Agriculture of Japan. We are also thankful to Pakistan Meteorological Department for providing environmental data.

Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Muhammad Qasim http://orcid.org/0000-0002-3255-7378

References

Abideen Z, Qasim M, Rasheed A, Adnan MY, Gul B, Khan MA. 2015. Antioxidant activity and polyphenolic content of *Phragmites karka* under saline conditions. Pak J Bot. 47:813–818.

Ahmed M, Fatima H, Qasim M, Gul B, Haq IU. 2017. Polarity directed optimization of phytochemical and in vitro biological potential of an indigenous folklore: *Quercus dilatata* Lindl. ex Royle. BMC Complement Altern Med. 17:386–401.

Alam S, Azmi A. 1989. Influence of *Prosopis glandulosa* water extract on the seedling growth of wheat cultivars. Pak J Sci Ind Res. 32:708–709.

Alqarawi AA, Hashem A, Kumar A, Al-Arjani AB, Abd_Allah EF, Dar BA, Wirth S, Davranov K, Egamberdieva D. 2018. Allelopathic effects of the aqueous extract of *Rhazya stricta* on growth and metabolism of *Salsola villosa*. Plant Biosys 1:1263–1273.

Anjum A, Hussain U, Yousaf Z, Khan F, Umer A. 2010. Evaluation of allelopathic action of some selected medicinal plant on lettuce seeds by using sandwich method. J Med Plant Res. 4:536–541.

Bais HP, Vepachedu R, Gilroy S, Callaway RM, Vivanco JM. 2003. Allelopathy and exotic plant invasion: from molecules and genes to species interactions. Science. 301(5638):1377–1380.

Basile A, Sorbo S, Giordano S, Ricciardi L, Ferrara S, Montesano D, Cobianchi RC, Vuotto M, Ferrara L. 2000. Antibacterial and allelopathic activity of extract from *Castanea sativa* leaves. Fitoterapia. 71: S110–S116.

Batish DR, Kaur S, Singh HP, Kohli RK. 2009. Role of root-mediated interactions in phytotoxic interference of *Ageratum conyzoides* with rice (*Oryza sativa*). Flora. 204(5):388–395.

Bulut Y, Demir M. 2007. The allelopathic effects of Scots Pine (*Pinus sylvestris* L) leaf extracts on turf grass seed germination and seedling growth. Asia J Chem. 19:3169–3177.

Chang C-C, Yang M-H, Wen H-M, Chern J-C. 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal. 10:178–182.

Chon J, Han K, Jang B, Shin H. 1988. Determination of phenolic compounds responsible for allelopathy in upland weeds. Korean J Weed Sci. 8:258–264.

Chung I, Ahn J, Yun S. 2001. Identification of allelopathic compounds from rice (*Oryza sativa* L) straw and their biological activity. Can J Plant Sci. 81(4):815–819.

Devi SR, Prasad M. 1992. Effect of ferulic acid on growth and hydrolytic enzyme activities of germinating maize seeds. J Chem Ecol. 18(11): 1981–1990.

Einhellig F. 2004. Mode of allelochemical action of phenolic compounds. In: Einhellig F, Galindo J, Molinillo J, Cutler H, editors. Allelopathy: chemistry and mode of action of allelochemicals. Boca Raton: CRC Press; p.217–238.

Fernandez C, Santonja M, Gros R, Monnier Y, Chomel M, Baldy V, Bousquet-Mélou A. 2013. Allelochemicals of *Pinus halepensis* as drivers of biodiversity in Mediterranean open mosaic habitats during the colonization stage of secondary succession. J Chem Ecol. 39(2):298–311.

Fujii Y, Parvez SS, Parvez M, Ohmae Y, Iida O. 2003. Screening of 239 medicinal plant species for allelopathic activity using the sandwich method. Weed Biol Manage. 3(4):233–241.

Fujii Y, Shibuya T, Yasuda T. 1990. Survey of Japanese weeds and crops for the detection of water-extractable allelopathic chemicals using RICHARDS' function fitted to lettuce germination test. J Weed Sci Tech. 35:362–370.

Gasnier C, Dumont C, Benachour N, Clair E, Chagnon M-C, Séralini G-E. 2009. Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines. Toxicol. 262(3):184–191.

Gilani SA, Fujii Y, Shinwari ZK, Adnan M, Kikuchi A, Watanabe KN. 2010. Phytotoxic studies of medicinal plant species of Pakistan. Pak J Bot. 42:987–996.

Green FB, Corcoran MR. 1975. Inhibitory action of five tannins on growth induced by several gibberellins. Plant Physiol. 56(6):801–806.

Hierro JL, Callaway RM. 2003. Allelopathy and exotic plant invasion. Plant Soil. 256(1):29–39.

Hubbes M. 1962. Inhibition of *Hypoxylon pruinatum* by pyrocatechol isolated from bark of aspen. Science. 136(3511):156.

Hubbes M. 1966. Inhibition of *Hypoxylon pruinatum* (Klotzsche) Cke by aspen bark meal and the nature of active extractives. Can J Bot. 44(4): 365–386.

Islam MS, Iwasaki A, Suenaga K, Kato-Noguchi H. 2018. Evaluation of phytotoxic potential and identification of phytotoxic compounds in *Rumex maritimus*. Plant Biosys. 152(4):804–809.

Jose CM, Torres LMB, Torres MAMG, Shirasuna RT, Farias DA, dos Santos NA, Grombone-Guaratini MT. 2016. Phytotoxic effects of phenolic acids from *Merostachys riedeliana* a native and overabundant Brazilian bamboo. Chemoecology. 26(6):235–246.

Khan AL, Hamayun M, Hussain J, Khan H, Gilani SA, Kikuchi A, Watanabe KN, Jung EH, Lee I. 2009. Assessment of allelopathic potential of selected medicinal plants of Pakistan. Afr J Biotechnol. 8:1024–1029.

Khan MA, Qaiser M. 2006. Halophytes of Pakistan: characteristics distribution and potential economic usages. In: Khan MA, Böer B, Kust GS, Barth H-J, editors. Sabkha ecosystems. Vol.2. Netherlands: Springer; p.129–153.

Li H-H, Inoue M, Nishimura H, Mizutani J, Tsuzuki E. 1993. Interactions oftrans-cinnamic acid its related phenolic allelochemicals and abscisic acid in seedling growth and seed germination of lettuce. J Chem Ecol. 19(8):1775–1787.

Li M, Liao BW, Zheng SF, Chen YJ. 2004. Allelopathic effects of *Sonneratia apetala* aqueous extracts on growth performance of some indigenous mangroves. Forest Res. 17:641–645.

Li ZH, Wang Q, Ruan X, Pan CD, Jiang DA. 2010. Phenolics and plant allelopathy. Molecules. 15(12):8933–8952.

Lodhi M. 1976. Role of allelopathy as expressed by dominating trees in a lowland forest in controlling the productivity and pattern of herbaceous growth. Am J Bot. 63(1):1–8.

Lodhi M. 1979. Allelopathic potential of *Salsola kali* L and its possible role in rapid disappearance of weedy stage during revegetation. J Chem Ecol. 5(3):429–437.

Mahmood K, Malik KA, Lodhi M, Sheikh KH. 1993. Competitive interference by some invader species against Kallar grass (*Leptochloa fusca*) under different salinity and watering regimes. Pak J Bot. 25145–145.

Mo Z, Hangqing F. 2001. Allelopathy of *Bruguiera gymnorrhiza* and *Kandelia candel*. J Guangxi Sci. 8:61–62.

Nazir S, Qasim M, Gul B, Khan MA. 2018. Antioxidant properties and phenolic composition of coastal halophytes commonly used as medicine. Int J Biol Biotech. 15:473–482.

Noor M, Khan MA. 1994. Allelopathic potential of *Albizia samans* Merr. Pak J Bot. 26:139–139.

Noor M, Salam U, Khan MA. 1995. Allelopathic effects of *Prosopis juliflora* Swartz. J Arid Environ. 31(1):83–90.

Parvez MM, Tomita-Yokotani K, Fujii Y, Konishi T, Iwashina T. 2004. Effects of quercetin and its seven derivatives on the growth of *Arabidopsis thaliana* and *Neurospora crassa*. Biochem Syst Ecol. 32(7): 631–635.

Pramanik M, Nagai M, Asao T, Matsui Y. 2000. Effects of temperature and photoperiod on phytotoxic root exudates of cucumber (*Cucumis sativus*) in hydroponic culture. J Chem Ecol. 26(8):1953–1967.

Qasim M, Abideen Z, Adnan M, Gulzar S, Gul B, Rasheed M, Khan M. 2017. Antioxidant properties phenolic composition bioactive compounds and nutritive value of medicinal halophytes commonly used as herbal teas. S Afr J Bot. 110:240–250.

Qasim M, Aziz I, Rasheed M, Gul B, Khan MA. 2016. Effect of extraction solvents on polyphenols and antioxidant activity of medicinal halophytes. Pak J Bot. 48:621–627.

Qasim M, Abideen Z, Adnan MY, Ansari R, Gul B, Khan MA. 2014. Traditional ethno-botanical uses of medicinal plants from coastal areas of Pakistan. J Coast Life Med. 2:22–30.

Qasim M, Ahmed MZ, Fujii Y, Watanabe KN, Khan MA. 2012. Phytotoxic studies of medicinal plants from coastal areas of Pakistan. J Weed Sci Technol. 57(Suppl.):84.

Sasikumar K, Vijayalakshmi C, Parthiban K. 2004. Allelopathic effects of four Eucalyptus species on cowpea (*Vigna unguiculata*). J Tropic Forest Sci. 16:419–428.

Shao H, Huang X, Zhang Y, Zhang C. 2013. Main alkaloids of *Peganum harmala* L and their different effects on dicot and monocot crops. Molecules. 18(3):2623–2634.

Singleton V, Rossi JA. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Viticult. 16: 144–158.

Sun B, Ricardo-da-Silva JM, Spranger I. 1998. Critical factors of vanillin assay for catechins and proanthocyanidins. J Agric Food Chem. 46(10):4267–4274.

Suresh K, Vinaya Rai R. 1987. Studies on the allelopathic effects of some agroforestry tree crops. Int Tree Crop J. 4(2–3):109–115.

Taylor RJ, Shaw DC. 1983. Allelopathic effects of *Engelmann spruce* bark stilbenes and tannin-stilbene combinations on seed germination and seedling growth of selected conifers. Can J Bot. 61(1):279–289.

Toqeer S, Qasim M, Abideen Z, Gul B, Rasheed M, Khan MA. 2018. Chemical composition and antioxidant activity of seeds of various halophytic grasses. J Am Oil Chem Soc. 95(10):1285–1295.

Ungar IA. 1998. Are biotic factors significant in influencing the distribution of halophytes in saline habitats?. Bot Rev. 64(2):176–199.

Williamson GB, Richardson D. 1988. Bioassays for allelopathy: measuring treatment responses with independent controls. J Chem Ecol. 14: 181–187.

Yamane A, Nishimura H, Mizutani J. 1992. Allelopathy of yellow fieldcress (*Rorippa sylvestris*): identification and characterization of phytotoxic constituents. J Chem Ecol. 18(5):683–691.

Zahra SS, Ahmed M, Qasim M, Gul B, Zia M, Mirza B, Haq IU. 2017. Polarity based characterization of biologically active extracts of *Ajuga bracteosa* Wall. ex Benth. and RP-HPLC analysis. BMC Complement Altern Med. 17(1):443–458.

Zribi I, Ghezal N, Sbai H, Richard G, Fauconnier ML, Haouala R. 2018. Biochemical composition of Tunisian *Nigella sativa* L. at different growth stages and assessment of the phytotoxic potential of its organic fractions. Plant Biosys.1. doi:10.1080/11263504.2018.1454530.