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Phytochemical Constituents, Antibacterial and Antioxidant Activities of some Medicinal Plants

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Abstract

The present study provides a detailed description of the phytochemical analysis, physiological features and biological activities of four selected plant species, namely *Calligomum polygnoides* subsp. *comosum* and *Nicotiana glauca* collected from the coastal desert in the Delatic Mediterranean Sea, as well as *Hyoscyamus muticus* and pulicaria undulate subsp. Undulata collected from the inland desert in Wadi Hagul and Wadi El-Molaak in the North of Eastern Desert in Egypt. The obtained results showed that, the studied plant species are relatively rich in proximate constituents, elements and bioactive secondary metabolites. Hyscyamus muticus leaves appeared to accumulate ABA in highest magnitude if compared to Nicotiana glauca, Pulicaria undulata subsp. undulata and *Calligonum Polygonoides* subsp. *comosum*. Antioxidant enzymes as well as DPPH scavenging activities in leaves of *Nicotiana glauca*, found to be the highest activity in catalase, perioxidase and polyphenol oxidase as well as antioxidant activity and IC50 as compared to other selected plant species. On the other hand, Calligonum polygonoides subsp. comosum attained the highest activity of phenylalanine ammonia lyase and the weakest antioxidant activity with IC50 value (0.29 mg ml^{-1}).

The highest ratio of total saturated fatty acids to unsaturated fatty acids was recorded in *Nicotiana glauca*, while the lowest one was found in Calligonum polygonoides subsp. comosum. Concerning the biological activities, the methanolic extract of Nicotiana glauca showed the highest antioxidant activity, while the extract of *Calligomum polygnoides* subsp. comosum exhibited the weakest antioxidant activity. The methanolic extract of *Pulicaria undulata* subsp. undulata showed the highest antimicrobial activity, followed by the extracts of *Hyoscyamus muticus*, *Nicotiana glauca* and finally by *Calligomum polygnoides* subsp. *comosum.* On the other hand, the aqueous extract of *Nicotiana glauca* showed, the highest inhibition percentages of seed germination, radical growth and plumule growth of summer weed Portulaca oleracea, followed by the extracts of *Pulicaria undulata* subsp. *undulata, Hyoscyamus muticus* and finally by *Calligomum polygnoides* subsp. *comosum*. Therefore, it can be concluded that, the selected plant species in the present study are obviously considered as tolerant species against extreme drought and salinity. These species could be also used as natural renewable resources in numerous beneficial purposes such as medicine industry, natural antioxidants, antimicrobial agents and allopathic potential for biological control of weeds.

Keywords: Biological activity; Medicinal plants; Phytochemicals

Introduction

Medicinal plants are vital curative agents for curing human ailments. The over-exploitation of 35 natural plant resources encouraged various programs such as conservation, micro-propagation 36 and incremental plant architecture [1]. The interest of many researchers in understanding the biology of different medicinal plant has increased in the last few decades. Approximately 85% of the world's population relies on traditional medical treatments based on plant remedies, and around 25% of the world's pharmaceutical medicines demands are derived from plants [2]. The medicinal value of plants lies in some active chemical substances that produce a definite physiological effect on the human. The most important bioactive constituents of plants are phenolic compounds, flavonoids, alkaloids and tannins. Many of indigenous medicinal plants are used as food plants. They are also sometimes added to foods meant for pregnant and nursing mothers for medicinal purposes [3].

The contents of amino acids and alkaloids of the medicinal *H. muticus* in response to some salinization treatments were determined. The contents of *proline* increased progressively with the rise of salinization level [4]. Members of genus *Pulicaria* contain various bioactive compounds such as monoterpenes, flavonoids, acetylenes, isocomene, and sesquiterpene lactones [5]. The oil of Pulicariais rich in phenolic compounds, monoterpene hydrocarbons and has insecticidal and antibacterial activities [6]. Biological actions reported for Pulicaria species include the antibacterial and antispasmodic activities of *P. undulata*, *P. odora*, and *P. dysenterica.* In an experiment, *Calligonum* species germinated easily between 18 to 22°C [7]. Seed germination was not affected by salinity up to 5 and 6 dS/m for *Calligonum* species; however, the germination percentage decreased with an increase in salinity. Radicle growth decreased more than plumule growth with an increase in salinity in it [8].

Chemical analysis from previous studies showed that, anthraquinones and flanovonids are the common chemical constituents in *C. polygonoides.* Besides, dehydrodicatechin which had cytotoxic effect and antioxidant activity was also isolated from *C. polygonoides* when treated with organic solvents [9]. Interestingly, anthraquinones of *C. polygonoides* showed high antimicrobial potential [10]. The present study was undertaken to evaluate the phytochemical constituents, physiological features as well as biological activities of four wild medicinal plants namely, *Calligonum polygonoides, Nicotiana glauca*, *Hyoscyamus muticus* and *Pulicaria undulata.*

Materials and Methods

In the present study, shoots of four wild medicinal plants namely, *Calligonum polygonoides* m . subsp. *comosum* (L' Her.) Soskov, Nicotiana glauca R.C. Graham, *Hyoscyamus muticus L.* and *Pulicaria undulata* (L.) C.A. Mey. Sub sp. undulate were collected from the coastal desert of the Deltaic Mediterranean Sea coast, while *Hyoscyamus muticus* and *Pulicaria undulate* were collected from the inland desert (Wadi Hagul and Wadi El-Molaak) in the North Eastern Desert of Egypt. These species were homogenized (well mixed) and taken immediately to the laboratory after collection, the shoot system (aerial parts) of each species was cleaned, air dried at room temperature (25 °C) prior to grinding, then ground to fine powder and preserved in well stopped bottles [11].

Analysis of various chemical constituents

Total phenolics and tannins were determined according to Sadasivam & Manicka [12]. Flavonoids were estimated as the method adopted by Bohm & Kocipai- Abyazan [13]. Alkaloids and saponins were determined using the methods of Obadoni & Ochuko [14]. Anthocyanin were extracted according to Mirecki & Teramura [15] and estimated by using the methods of Mancinelli et al. [16]. Lycopene and β-carotene were determined according to the method of Nagata & Yamashita [17].

The method adopted for the estimation of proline was essentially that described by Snell & Snell [18].

The method used for extraction of Abscisic acid was that originally described by Shindy & Smith [19], while ABA was determine using two-dimensional HPLC. The methanolic extract of dried plant samples prepared as described by Kosem et al. [20]. 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical according to a procedure of Lim & Quah [21].

Antioxidant enzymes

The antioxidant enzymes assayed in the present study included peroxidase, catalase, polyphenol oxidase and phenylalanine ammonia lyase. For the extraction of antioxidant enzymes, the method of Agarwal & Shaheen [22] was adopted. Peroxidase activity was assayed as the increase in absorbance at 420 nm due to the formation of purpurogallin [23]. Polyphenol oxidase activity was assayed as the absorbance at 420 nm due to also the formation of purpurogallin [23]. Phenylalanine ammonia lyase activity was determined according to Havirand Hanson [24]. Catalase activity was assayed as described by [25].

Fatty Acids Analysis

The method of lipids extraction was adopted by Neumann [26]. The method of methylation of fatty acids for gas-liquid chromatography analysis was essentially that adopted by Sink et al. [27]. The identification of methyl esters of fatty acids was based on a comparison of the retention times with those of reference compounds. Log10, values of the retention time of authentic methyl esters of fatty acids were plotted against the number of carbon atoms. Unknown fatty acids were identified by consulting the plot. The peak areas in the chromatogram were measured with a planimeter and the relative percentage of individual fatty acids was calculated as recommended by Radwan [28].

Antimicrobial Activity

The antimicrobial activity of the plant extract was estimated by filter paper disc method [29]. Diameters of inhibition zone (mm) were measured after 6 days for fungi and 18-24 hours for bacteria [30].

Preparation of aqueous plant extract

For bioassay tests, stock extracts (10% w/v) were diluted with distilled water for aqueous extract to obtain concentrations of 10, 8, 6, 4 and 2% (v/v) test extracts [31]. The solutions were filtered and kept in refrigerator at 4 °C until further use [32].

Germination bioassays

Two layers of Whatman No. 1 filter paper were placed in 90 mm diameter glass Petri dishes. In each Petri dish, 20 seeds were placed and 10ml of each aqueous plant extract added in a concentration of 10, 8, 6, 4 and 2% (v/v). A check treatment was assigned with distilled water and let at room temperature (25 °C). Starting from the first day after experiment set on, germinated seeds were counted and removed daily. A seed with 0.5cm of radical was considered germinated. Rate of germination was calculated by dividing the number of germinated seeds each day by the number of days and summing the values. The inhibition percentage was calculating using the following equation:

$$\text{Inhibition Percentage}=[\frac{(CG\text{-}TG)}{CG}\times 100]$$

Where, CG: Germination Rate in Control; TG: Germination Rate in Extract Treatment.

The seeds of the *Portulaca oleracea* were germinated on filter paper in the dark at room temperature (25°C) for 4 days. Fifteen germinated seeds were transferred to Petri dishes which were filled with 25g quartz sand and 10ml of shoot powder extract added in concentrations of 10, 8, 6, 4 and 2% (v/v). In addition, a check added to experiment without any powder treatment. The plumule and radicle lengths of seedlings were measured on 14 day after treatment (DAT). Growth inhibition for plumule and radicle lengths was calculated using the following equation:

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$$\text{Growth inhibition}=[\frac{(LC-CT)}{LC}\times 100]$$

Where, growth inhibition in %; LT: Shoot or Root Length of Powder Treated Weed; LC: Shoot or Root Length of Untreated Check Weed.

Results and Discussion

In the last decade, secondary metabolites become a subject of dramatically- increasing interest relevant to their significant practical implication for medicinal, nutritive and cosmetic purposes as well as to their indisputable importance in plant stress physiology [33]. Over the centuries human have relied on plants for basic needs such as food, clothing, healthcare and shelter, all produced or manufactured for plants [34]. In the present study, the phytochemical analysis including the determination of secondary metabolites in the selective four medicinal plant species revealed that, *Nicotiana glauca* showed the highest values of total phenols, flavonoids, alkaloids and tannins (Table 1). These results may be promoting the usage of these studied plant species as a good natural renewable resource of bioactive secondary metabolites which could be used in folk medicine on the large scale. Nature has been a source of medicinal agents for thousands of years with an impressive number of modern drugs isolated from natural sources; many of these isolations were based on the uses of the agents in the traditional medicine [35]. According to World Health Organization (WHO), out of 252 drugs that are considered basic and essential more than 11% are of plant origin [36]. For safety, efficacy, lesser side effects and cultural acceptability, plants are invaluable, incredible and traditional sources for the curability of various diseases [37]. The potent use of a plant species for the health care may rely on its ability to synthesize primary or secondary metabolites that could be involved in the prevention and or the cure of various diseases and ailments. Rather than the primary metabolites, plant secondary metabolites have been implicated in most of the plant therapeutic activities [38].

Table 1: Changes in secondary metabolites (mg g-1 d wt.) in the shoot of some coastal and inland desert plants. Each value is the mean of triplicates sample ± S.E.

Parameter	Coastal Desert Species		Inland Desert Species	
	Calligonum Polygonoides	*Nicotiana Glauca*	*Hyoscyamus Muticus*	*Pulicaria Undulata*
Total phenolics	10.44±0.66	24.11±0.10	12.15±0.86	15.06±0.01
Flavonoids	4.78±0.15	7.28±0.11	5.59±0.30	6.39±0.13
Alkaloids	3.89±0.86	14.61±1.60	8.23±0.58	0
Tannins	1.20±0.08	10.81±0.23	8.32±1.10	5.30±0.14
Saponins	0.67±0.02	2.80±1.50	3.45±0.98	0

Table 2: Changes in physiological features in the shoot of some coastal and inland desert plants. Each value is the mean of triplicates sample ± S.E.

Parameter	Coastal Desert Species		Inland Desert Species	
	Calligonum Polygonoides	*Nicotiana Glauca*	*Hyoscyamus Muticus*	*Pulicaria Undulata*
Relative water content (%)	54.94±0.90	64.03±1.09	79.01±0.01	50.54±0.52
Saturation water deficit (%)	45.06±0.90	35.97±1.09	20.99±0.01	49.46±0.52
Total chlorophylls (mg g^{-1} d wt.)	1.54±0.14	3.94±0.39	6.53±0.14	1.58±0.19
Carotenoids (mg g^{-1} d wt.)	0.17±0.05	1.02±0.14	0.70±0.09	0.2±0.01
Carotenoids/Total chlorophylls	0.11±0.01	0.26± 0.01	0.11 ± 0.01	0.13±0.01
Total pigments (mg g^{-1} d wt.)	1.75± 0.09	5.38 ± 0.32	7.43 ±0.03	2.20±0.04
Lycopens (mg g^{-1}d wt.)	0.01±0.0	0.06±0.0	1.54±0.04	1.11±0.06
β-carotens (mg g^{-1} d wt.)	0.28±0.0	0.39±0.03	0.67±0.10	0.14±0.0
Anthocyanin (mg g^{-1}d wt.)	1.17±0.12	0.15±0.0	1.23±0.13	0.89±0.10
Proline (mg g^{-1} f wt.)	9.55±0.09	14.21±0.35	43.89±1.06	30.46±1.4
Abscisic acid (μg g^{-1} f wt.)	0.18±0.06	0.22±0.01	1.28±0.10	0.87±0.02

Environmental stress is well documented to severely restrict the distribution and productivity of plants [39]. These stressful conditions could affect the plant performance causing specific as well as unspecific reactions, damages and adaptation reactions [40]. Among the plant metabolites recognized with potent pharmaceutical activates, most of the plant non-photosynthetic pigment was documented to have health care attributes (Table 2). Therefore, they are gaining significance to enhance the health benefits with a great demand for supplements containing an efficacious dose of these natural colors [41]. Examples of this medically-active pigment are anthocyanin that possesses cationic aglycones with distinct chemical structures. Several studies exhibited that; anthocyanin could exhibit antioxidant and anti-inflammatory activities [42].

Thus, the results obtained from this study indicate that, the highest value of anthocyanin (1.23 mg g^{-1} dwt.) was determined in *Hyoscyamus muticus*, while the lowest value (0.15 mg g^{-1} dwt.) was estimated in *Nicotiana glauca. Calligonum polygonoides* subsp. *comosum* and *Pulicaria undulata* subsp. undulata attained

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the values of 1.17 and 0.89 mg g^{-1} dwt., respectively. Anthocyanin is the most conspicuous class of flavonoids that represent a large class of secondary plant metabolites [43]. Anthocyanin are involved in attraction of pollinators with a 38 pronounced effect under defenses against environmental stresses like ultraviolet radiation, 39 herbivores, cold temperature and drought [44,45]. With respect to drought, plant tissue containing anthocyanin is usually rather resistant to drought [46]. For example, a purple cultivar of pepper resists water stress better than the green cultivar [47]. In this connection, water deficit (Table 2) was found to enhance anthocyanin accumulation in grape berries [48]. The roles of anthocyanin as antioxidants, compatible solutes in osmotic regulation and photo-protectants under stress conditions were reported [49].

The highly drought-tolerant resurrection plant contains anthocyanin at a level that is several-fold more during dehydration that at a hydrated stage. The proposed mechanism behind anthocyanin-enhanced drought resistance is that anthocyanin can stabilize water potential [46]. In addition, the anthocyanin shows various function in counteracting the negative effects of reactive oxygen species (ROS) as well maintaining the redox homeostasis of biological fluids [50]. Some environmental stresses induce the accumulation of anthocyanin in plants by activating their biosynthesis as well as inhibiting their oxidation [51]. Phenyl alanine ammonia lyase (PAL) is a key enzyme in phenylpropanoid metabolism as it catalyzes the conversion of phenylalanine to trans-cinnamic acid, the first step in the biosynthesis of anthocyanin. It was also reported that, PAL is significantly induced by abiotic stresses such as salt, cold and drought [52].

Furthermore, the obtained results revealed that, the highest value of lycopene (1.54 mg g^{-1} dwt.) was determined in *Hyoscyamus muticus*, while the lowest value (0.01 mg g^{-1} dwt.) was estimated in *Calligonum polygonoides* sub *sp. comosum. Nicotiana glauca* and *Pulicaria undulata* sub sp. *undulata* attained the values of 0.06 and 1.11 mg g^{-1} dwt., respectively. In addition, these results reported that, the value of β-carotens varied from 0.14 g g^{-1} dwt. in *Pulicaria undulata* sub sp. *undulata* to 0.67 mg $g^{-}1$ dwt. in *Hyoscyamus muticus*. While Nicotiana glauca and *Calligonum polygonoides* sub sp. *comosum* attained the values of 0.39 and 0.28 mg $g^{-}1$ dwt., respectively. Under drought conditions (Table 2), increases in quantities of carotenoids and non- enzymatic antioxidants were reported in many studies [53,54]. For instance, the results obtained by Sedghi et al. [55] showed that, the concentration of carotenoids; namely lycopene and β-carotene, increased under water deficit in *Calendulla officinalis* compared with control plants. Similarity, carotenoids were accumulated in drought-stressed *Rosmarinus officinalis* [56]. Moreover, drought stress caused an increment in the amount of both lycopene and β-carotene in tomato plants [57].

Carotenoids are among the most abundant groups of lipid-soluble antioxidants in chloroplasts [58]. Increasing evidence suggests that, carotenoids are required for plant response to dehydration stress [59]. In plants, carotenoids play key role in photo protection and could enhance the ability of ROS scavenging [60]. In this regard lycopene and β-carotene contents were increased in drought-stressed rice plants when compared with their controls [61]. Furthermore, stress seemed to enhance the concentrations of lycopene and β-carotene in tomato plants [62]. Abcisic acid (ABA) is defined as a stress hormone because of its rapid accumulation in response to stresses and its mediation of many stress responses that helps plant to survive [63]. The involvement of ABA in mediating drought stress has been investigated by many researchers. ABA plays an important role in controlling plant water status through guard cells and controlling growth by inducing gene encoding enzymes and proteins involved in cellular dehydration tolerance [64].

The results presented in table 2 showed that *Hyoscyamus muticus* attained the highest concentration of abscisic acid (1.28 mg g^{-1} fwt.), while *Calligonum polygonoides* sub sp.*comosum* attained the lowest concentration (0.18 mg g^{-1} fwt). *Nicotiana glauca* and *Pulicaria undulata* sub *sp. undulata* attained the values of 0.22 and 0.8 mg g^{-1} fwt., respectively. The role as an inhibitor, when accumulated in a huge amount during stress, can help a plant to survive through regulating the opening and closing processes of stomata and increasing plant size. Its role to promote plant growth in low concentrations under normal conditions has an important role in the vegetative phase of some plant organs [65]. The increase of a plant's ABA content due to drought stress plays an important role in the opening and closing of stomata [63].

The high content of ABA promotes stomata closing. It is appeared that, drought generally increased diphenyl-picrylhydrazyl (DPPH)-scavenging activity but decreased the plant amount required to reduce the initial DPPH concentration by 50% (IC50). Furthermore, soil with 100% sand induced the highest DPPH-scavenging activity and ARP values with the lowest IC50 values in control and stressed plants. The extract of *Nicotiana glauca* showed the highest antioxidant activity with IC50 value of 0.12 mg ml^{-1}; while the weakest antioxidant activity obtained from the extract of *Calligonum polygonoides* with IC50 value of 0.26 mg ml^{-1}. The extract of *Pulicaria undulata* attained IC50 value of 0.14 mg ml^{-1}, while the extract of *Hyoscyamus muticus* attained IC50 value of 0.16 mg ml^{-1}. The extract of natural antioxidant catechol attained the IC50 value of 0.11 mg ml^{-1} (Table 3).

The stable radical DPPH has been widely used to determine primary antioxidant activity; that is the free radical-scavenging activity of certain antioxidant compounds. The assay is based on the reduction of DPPH radicals in methanol which causes an absorbance drop at 517 nm [66]. The active oxygen species, viz superoxide radical, hydrogen peroxide, hydroxyl radical and singlet oxygen, constitute the oxidative stress [67]. Plants scavenge ROS by detoxification mechanism via different stuffs of the antioxidant enzymes [68]. These overproduced ROS react

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directly with lipids, proteins and nucleic acids causing lipid peroxidation-mediated membrane injury, protein degradation, enzyme inactivation, pigment bleaching and disruption of DNA strands [67]. *Nicotiana glauca* attained the highest activities of catalase (54.78 enzyme unit mg^{-1}), peroxidase (67.74 enzyme unit mg^{-1}) and polyphenol oxidase (10.67enzyme unit mg^{-1}). *Calligonum polygonoides* attained the highest activity of phenylalanine ammonia lyase enzyme (5.34 enzyme unit mg-1) but the lowest activity of polyphenol oxidase (3.20 enzyme unit mg^{-1}). *Hyoscyamus muticus* attained the lowest activities of peroxidase enzyme (28.14 enzyme unit mg^{-1}) and phenylalanine ammonia lyase enzyme (0.88 enzyme unit mg^{-1}). *Pulicaria undulata* attained the lowest activity of catalase enzyme (19.10 enzyme unit mg^{-1}) (Table 3).

Table 3: Changes in antioxidant enzymes activity (unit mg^{-1}) and DPPH scavenging activity (IC50) in the shoot of some coastal and inland desert plants. Each value is the mean of triplicates sample ± S.E.

Parameter	Coastal Desert Species		Inland Desert Species		Catechol
	Calligonum Polygonoides	*Nicotiana Glauca*	*Hyoscyamus Muticus*	*Pulicaria Undulata*	
Catalase	30.15±1.32	54.78±1.60	44.89±1.11	19.10±1.2	
Peroxidase	34.54±0.90	67.74±1.07	28.14±0.8	45.30±1.34	
Polyphenol Oxidase	3.20±0.08	10.67±0.67	7.18±0.6	6.90±0.5	0.11
Phenylalanine Ammonia Lyase	5.34±0.7	1.20±0.02	0.88±0.0	1.43±0.08	
DPPH (IC50) mg ml^{-1}	0.29	0.12	0.16	0.14	

Similar responses to stress conditions were reported in soybean [69] and marigold [55]. Plants have developed the scavenging mechanism of ROS categorized as enzymatic and non-enzymatic defense system. When ROS increase, chain reactions start in which SOD 119 catalyses the disputation of superoxide radical to molecular O_2 and H_2O_2. The H_2O_2 is then 120 detoxified either by CAT/POX or in ascorbate glutathione cycle which involves oxidation/reduction of ascorbate and glutathione through APX and GR action. While CAT reduces H_2O_2 into H_2O and O_2, POX decomposes H_2O_2 by oxidation of co-substrate such as phenolic compounds. The equilibrium between the production and scavenging of ROS may be perturbed under adverse abiotic stresses resulting in the reduction of plant growth and development [67]. The main roles of fatty acids in plants are related not only to cell membrane functions but also to metabolic processes. Hyoscyamus muticus attained the highest concentrations of auric acid (0.32%), tridecanoic (0.54%), myristic (3.70%), pentadecanoic (5.12%), stearic (5.68%) and 129 linoleic (47.53%). *Nicotiana glauca* attained the highest content of palmitic (39.43%), 130 heptadecanoic (1.54%), arachidic (1.95%), lignoceric (0.21%), palmitoleic (4.71%) and arachidonic (0.43%). *Pulicaria undulata* attained the highest values of behenic acid (0.85%) and linolenic acid (48.28%). The highest percentages of total saturated fatty acids (50.62%) and total unsaturated (94.15%) were recorded in *Nicotiana glauca*, while *Calligonum polygonoides* attained the lowest values of both total saturated (15.83%) and unsaturated (33.35%). These results were found to be against with those obtained by Taarit et al. [70] in *Salvia officinalis* leaves under NaCl stress. Moreover, Xu & Beardall [71] stated that, in a *Dunaliella sp* with increasing salinity level, the proportion of total saturated fatty acids (TSFA) increased while (PUFA) decreased (Table 4).

Table 4: Changes in fatty acids content (%) in the shoot of some coastal and inland desert plants.

Plant Species		Inland Desert Species		Coastal Desert Species	
Fatty Acids		*Calligonum Polygonoides*	*Nicotiana Glauca*	*Hyoscyamus Muticus*	*Pulicaria Undulata*
Saturated acids fatty (%)	Lauric (C12)		0.26	0.32	
	Tridecanoic (C13)			0.54	
	Myristic (C14)	1.3	2.94	3.7	0.9
	Pentadecanoic (C15)	0.4	0.85	5.12	4.64
	Palmitic (C16)	9.8	39.43	24.61	13.68
	Heptadecanoic (C17)	1.1	1.54		
	Stearic (C18)	3.23	3.08	5.68	1.87
	Arachidic (C20)		1.95		0.74
	Behenic (C22)		0.36		0.85
	Lignoceric (C24)		0.21		
Unsaturated fatty acids (%)	Palmitoleic (C16:1)		4.71		0.46
	Oleic (C18:1)	26.25	26.25	26.25	26.25
	Linoleic (C18:2)	7.1	45.27	47.53	5.72
	Linolenic (C18:3)		17.49		48.28
	Arachidonic (C20:1)		0.43		

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Total saturated fatty acids (TSFA)	15.83	50.62	39.97	22.68
Total unsaturated fatty acids (TUFA)	33.35	94.15	73.78	80.71
TSFA/TUFA	0.47	0.54	0.54	0.28

Natural products from plants have provided the pharmaceutical industry with one of its most important sources of lead compounds and up to 40% of modern drugs derived from natural sources, using either the natural substance or a synthesized version [72]. The herbal preparations are becoming more popular and used increasingly in USA. Currently a large and ever-expanding global population base prefers the use of natural products in treating and preventing medical problems. This has influenced many of pharmaceutical companies to produce new antimicrobial formulations extracted from plants. Herbs are from natural plants and therefore often considered harmless compared with western medicines [73]. Plant species still serve as a rich source of 147 many novel biologically active compounds.

However, very few plant species have been 148 thoroughly investigated for their medicinal properties. Thus, there is renewing interest in phyto-medicine during last decade and nowadays many medicinal plant species are being screened for pharmacological activites [74]. In the present study the methanolic extract of *Pulicaria undulata* exhibited the highest diameter of inhibition zone against Bacillus subtilis (15mm), *Klebsiella pneumonia* (15mm), *Staphylococcus* aureus (15mm) and *Candida albicana* (13mm). Also, *Hyoscyamus muticus* showed the highest inhibition zone against *Erwinia carotovora* (16mm) and *Escherichia coli* (12mm). On the other hand, *Nicotiana glauca* attained the highest inhibition zone against *Streptococcus pyogenes* (11mm). *Calligonum polygonoides* exhibited a relative moderate inhibition effect on *Staphylococcus aureus* (11mm) and *Erwinia carotovora* (10 mm). Therefore, the antimicrobial activity of the selected plant species could be ranked as follows:

Pulicaria undulata>Hyoscyamus muticus>Nicotiana glauca>Calligonum polygonoides (Table 5).

Table 5: Changes in the antimicrobial activity of the shoot extracts as demonstrated by diameters of the inhibition zone (mm) (mean value ± standard error) of some coastal and inland desert plants.

Tested Organism	Coastal Desert Species		Inland Deserts Species	
	Calligonum Polygonoides	*Nicotiana Glauca*	*Hyoscyamus Muticus*	*Pulicaria Undulata*
Bacillus subtilis	9.00±0.11	14.50±0.50	13.00±0.18	15.00±0.43
Klebsiella pneumonia	9.50±0.16	13.00±0.66	7.50±0.67	15.00±1.20
Erwinia carotovora	10.00±0.78	8.50±0.50	16.00±0.15	12.00±1.09
Streptococcus pyogenes	8.00±0.10	11.00±0.12	Nil	Nil
Escherichia coli	Nil	7.50±0.36	12.00±0.56	9.50±0.89
Staphylococcus aureus	11.00±0.90	Nil	Nil	15.00±1.20
Candida albicans	9.00±0.12	11.00±0.35	10.00±0.18	13.00±1.50

These finding agree with many studies such as Abd El-Aal [75] & El-Shazly [76]. Allelopathy can be defined as any effect either inhibitory or stimulatory, mediated by chemicals released into the environment by a given plant [77]. These chemicals known as allele- chemicals, phyto-growth inhibitors on natural products are a prime factor in regulating the structure of plant communities [78]. Allelopathic compounds are secondary plant products. They came to the medium by many ways such as volatilization, leaching, root exudation and decomposition of plant residues in soil. Phenolic, flavonoids, alkaloids, terpenoids and cyanogenic glycosides have attracted the attention of scientists to investigate their structure and biological function [79]. The choice of measurement parameters for the demonstration of allelopathy must be considered. In several bioassays, seed germination and seedling growth is measured after exposure to alleged allelochemicals because seed and seedling growth are generally considered to be the most susceptible stages [80]. In the present study, the allopathic bioassay of Pulicaria undulata, Hyoscyamus muticus, Nicotiana glauca and Calligonum polygonoides were demonstrated by studying the phytotoxic effects of aqueous extracts on seed germination and seedling growth of the targeted nuisance of summer weed Portulaca oleracea.

Table 6: Changes in allelopathic effects of the shoot aqueous extracts of some coastal and inland desert plants on the seeds germination inhibition percentage (mean value ± SE) of *Portulaca oleracea.*

Plant Species	Concentration of the Extract (gl^{-1})				
	2	4	6	8	10
Calligonum polygonoides	28.33±0.33	37.0±0.00	43.33±0.33	50.0±0.00	70.33±0.33
Nicotiana glauca	18.0±0.56	38.33±0.67	68.33±0.33	89.50±0.00	100±0.00
Hyoscyamus muticus	33.33±0.67	41.67±0.33	58.33±0.67	71.67±0.33	75.0±0.58
Pulicaria undulata	53.33±0.88	61.67±0.33	65.0±0.58	75.0±0.58	83.33±0.67

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The highest seed germination, radicle and plumule growth inhibition percentages were recorded at concentration of 10 gl^{-1} in Nicotiana glauca, followed by Pulicaria undulata, then Hyoscyamus muticus and finally Calligonum polygonoides (Table 6). These results are in agreement with many studies such as Ramez [81] & Yahia [82].

Conclusion

In the above study, the obtained data confirmed that stress resulted in an inhibitory effect on two studied costal desert plants (*Nicatiana glauca* and *Calligonium polygonoides*) as well as two inland desert plants (*Hyoscyamus muticus* and *Pulicaria undulata*) the degree of inhibition differs according to the habitat and the plant species. Also, the results confirmed that either stress in costal land and inland desert habitats induces modifications in biochemical aspects of the studied plants as an adaptive strategy to stress. In summary, the results presented here confirm also that, costal desert plants are moderately salt sensitive species, while inland desert plants are extreme drought tolerant species. It can be concluded that, the four selected plant species in the present study are obviously tolerant species to both of extreme drought and salinity, as well as these species could be used in the different beneficial purposes such as medicine, antioxidant, antimicrobial and allelopathic potenitialities.

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Conflicts of Interest

The author declares no conflicts of interest.

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