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Original Research

Phytochemical Analysis and Evaluation of the Antioxidant and Antimicrobial Activities of Five Halophytes from Qassim Flora

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Abstract

Halophytes are salt tolerating plants that grow in variable habitats, such as deserts and steppes. *Chenopodiaceae* and *Amaranthaceae* are two families widely prevailing in Saudi Arabia. They include plenty of previously unexplored halophytes. In a research program aimed at exploring the phytochemical content and biological potential of Qassim region halophytes; the current study presents phytochemical analysis as well as assessment of the antioxidant and antimicrobial activity of the five halophytes: *Agathophora alopecuroides* var. papillosa, *Atriplex leucoclada, Halothamnus bottae, Salsola villosa,* and *Salicornia persia* ssp. Iranica. Results of qualitative phytochemical analysis indicated the presence of saponins, tannins, sterols, carbohydrates, and flavonoids in all tested species. The comparison of the phenolic content of the five species revealed the highest total phenolics content and total flavonoids content in *S. villosa* (135.2 mg GAE/g and 18.2 mg QE/g, respectively) and *A. leucoclada* (134.1 mg GAE/g and 21.2 mg QE/g, respectively). Investigation of the antioxidant potential demonstrated the highest activity of *H. bottae* followed by *S. villosa* (IC_{50} 263.7 and 290.7 µg/mL, respectively). Additionally, screening of the antimicrobial activity revealed potent activity of *Salicornia persia, H. bottae,* and *S. villosa* against *Staphylococcus aureus* while none of the tested extracts displayed antifungal activity against tested fungal species.

Keywords: halophytes, TPC, TFC, antioxidant, antimicrobial activity

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Introduction

Halophytes are described as plants that can withstand harsh conditions including high salinity. They are common in places where other conventional plants cannot grow. Throughout the world there are more than 2500 species included under halophytes; several of them belong to the plant families *Aizoaceae*, *Chenopodiaceae*, and *Zygophyllaceae*. In such tough environmental conditions, halophytes usually experience high levels of reactive oxygen species (ROS), which can lead to cell damage or even plant death. However, certain plants exhibit certain strategies to adapt, whether morphologically or physiologically, to such conditions. Among these strategies, the most important is the synthesis of biologically active metabolites with antioxidant potential. Such valuable metabolites could be effective chemicals used in the food industry to protect against food oxidation or as medicinal drugs used for the treatment of many diseases. Some of these secondary metabolites are restricted to halophytes, or at least present in higher concentrations than in glycophytes [1-2].

Due to their rich phytonutrient content, some halophytes species have been used traditionally as herbs and vegetables, feed, and fodder. Accordingly, they are considered one of the alternative solutions to resolve problems associated with food safety, freshwater shortage, and salinization [3]. Recently, halophytes have become an interesting subject for qualitative and quantitative investigation of their metabolic content. Polyphenols, such as phenolic acids, flavonoid glycosides, tannins, stilbenes, and saponins are among the most frequent secondary metabolites detected in halophytes [3].

Many diseases such as cancer, diabetes, and heart diseases are influenced by cellular oxidative damage. Several reports discussed the mechanism of action of antioxidants and whether they specifically interrupt or remove free radicals from cells in the human body [4-5].

The antimicrobial potential of several halophytes was reviewed by Giordano et al. [6]. They discuss the availability of certain phytoconstituents such as: phenols and fatty acids, with potential antimicrobial activity, in these salt-tolerant plants.

Several reports considering the work of the angiosperm phylogeny group, merged the two families: *Amaranthaceae* and *Chenopodiaceae* into one cosmopolitan *Amaranthaceae* family consisting of about 180 genera and 2250 species [7-8]. The two families are also reported to share some common morphological and anatomical features as well as phytochemical content, including betacyanins, flavonoids, and saponins [9-10]. On the other hand, some authors consider *Chenopodiaceae* as a distinct family from *Amaranthaceae* [11]. The present study aims to compare the phytochemical content, the antioxidant activity, and antimicrobial potential of five halophytes from five genera: *Agathophora, Atriplex, Halothamous, Salsola,* and *Salicornia* classified under the *Amaranthaceae* family.

Materials and Methods

All the reagents and chemicals used in the experiments were of analytical grade. 2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, Folin-Ciocalteu reagent, gallic acid, quercetin, aluminum chloride (anhydrous powder), sodium bicarbonate (anhydrous powder), sodium nitrite, sodium hydroxide reagent, nutrient agar, and dimethyl sulfoxide (DMSO) were obtained from Sigma-Aldrich (St. Louis, Missouri, USA).

Plant Materials

The five halophytes: *Agathophora alopecuroides* var. papillosa, *Atriplex leucoclada, Halothamnus bottae, Salsola villosa* and *Salicornia persia* ssp. Iranica, were collected in October 2020 from five different locations within the Qassim region, Kingdom of Saudi Arabia (KSA), and identified by Ibrahim Aldakhil, botanical expert, Qassim Area. Voucher samples were deposited at the College of Pharmacy, Qassim University. The plant samples were cleaned using deionized water then dried in the shade for two weeks. The dried samples were then ground to a fine powder and weighed, where 100g of each sample was macerated in 80% Methanol (5x200 mL), with frequent shaking. The mixtures were filtered separately, and dried at 40ºC using a rotatory evaporator. The percentage yield was determined for each species as g extract per 100g dry plant. The dried crude extracts were stored in amber-colored vials at 4ºC till analysis. All the spectrophotometric measurements of the following assays were performed by UV/Vis spectrophotometer (Jenway's Model 6800, UK).

Preliminary Phytochemical Screening

One-gram extract of each of the five species was dissolved in 100 mL methanol and used for qualitative testing of the secondary metabolites content. Qualitative testing for the presence of various metabolites is done according to [12-14]. The relative abundance of the respective compound is presented in (Table 1).

Determination of Minerals Concentration

For determination of Nitrogen (N), Potassium (K) and phosphorus (P) concentrations in the five halophytes, the powdered plant sample is mineralized through pressurized digestion with mineral acid. The concentrations of N, K and P elements in each of the predigested samples of each of the five halophytes were determined by AOAC method for N and ICP-OES method for K and P [15-16].

Table 1. Preliminary phytochemical screening of the five halophytes.

Plant species	Carb.	Sterols and/or triterpenes	Saponins	Tannin	Flavonoids	Alkaloids	Anthr. glycosides	Cardiac glycosides
A. alopecuroides	+	+	+	+	+	-	-	-
A. leucoclada	+	+	++	++	++	-	-	-
H. bottae	+	+	+	+	+	-	-	-
S. villosa	+	+	++	++	++	-	-	-
S. persia	+	++	++	+	+	-	-	-

+: positive result, -: negative result, ++ detected in higher amount. Carb.: carbohydrates, Anthr.: anthraquinone

Determination of total Phenolic Content (TPC)

Folin-Ciocalteu method was adopted according to the method described earlier [17]. The stock solution of dried extracts was prepared in 50 µg/mL concentration. Gallic acid (0-60 mg/mL) was used for the establishment of the calibration curve. The diluted extract or gallic acid (1.6 mL) was mixed with 0.2 mL Folin-Ciocalteu reagent (5-fold diluted with distilled water) thoroughly for 3 minutes. Sodium carbonate (0.2 mL, 10% w/v) was added to the mixture then kept aside for 30 minutes at room temperature. The absorbance of the mixture was measured at 760 nm. Results are expressed as mg GAE/g crude extract.

Determination of Total Flavonoids Content (TFC)

TFC was determined using the $AlCl_3$ method [17]. Extracts of the five halophytes were diluted with methanol to prepare 100 µg/mL concentration. The calibration curve was constructed using different concentrations of quercetin (0-100 mg/mL). Two milliliters of the diluted extracts or quercetin were mixed with 0.1 mL of 10% (w/v) aluminum chloride solution and 0.1 mL of 0.1 mM potassium acetate solution. The mixture was kept for 30 minutes at room temperature. The absorbance of the mixture was recorded at 415 nm. TFC was calculated using the regression equation derived from the quercetin standard curve and results are expressed as mg QE/g crude extract.

DPPH Radical Scavenging Activity

The DPPH free radical scavenging activity of methanol extract of the five plants was assessed according to the method described by Ahmed et al. [18]. The stock solution of DPPH was prepared by dissolving 24 mg in 100 mL methanol, then kept in a refrigerator till further use. DPPH working solution was prepared by diluting the stock solution with methanol to obtain an absorbance of about 0.98 (±0.02) at 517 nm. In a test tube, 300 µL plant extract (1 mg/mL) or Ascorbic acid solution is mixed with 3 mL DPPH working solution. The mixture was incubated for 30 min., then the absorbance was measured at 517 nm. The control was prepared by using 300 µL methanol in place of the plant sample. The percent antioxidant activity was calculated using the following formula:

$$\%\text{Antioxidant activity} = [(Ac - As)/Ac] \times 100,$$

where, Ac and As is the absorbance of control and sample, respectively. All experiments were done in triplicates. Statistical analyses of the triplicate data were performed with Graph Pad Prism 6 statistical software (Graph Pad Software, San Diego, CA, USA). Results of the replicates were expressed as mean±standard error (SEM).

Antimicrobial Activity

The Susceptibility tests were performed according to NCCLS recommendations (National Committee for clinical laboratory Standards, 1993) [19]. Screening tests regarding the inhibition zone were carried out by the well diffusion method. The inoculum suspension was prepared from colonies grown overnight on an agar plate and inoculated into Mueller-Hinton broth (fungi using malt broth). A sterile swab was immersed in the suspension and used to inoculate agar plates. Holes (0.6 cm diameter) were dug in the agar using sterile cork borer in the sterile malt agar plates for fungi and sterile Mueller-Hinton nutrient agar plates for bacteria. The holes were filled by plant extracts dissolved in DMSO (100 µL). Plates were left in a cooled incubator at 4ºC for one hour for diffusion and then incubated at 37ºC for tested bacteria and 28ºC for tested fungi. The extracts were dissolved in dimethyl sulfoxide (DMSO) with different concentrations (10, 5, 2.5 mg/mL). The inhibition zone developed due to active antimicrobial metabolites, in each extract, was measured around each well after 24 h of incubation for bacteria and 48 h of incubation for fungi. Controls using DMSO were adequately done.

MIC Determination

The crude extract displaying activity in the preliminary testing was then re-evaluated for the antimicrobial activity via determination of the minimal inhibitory concentrations (MICs) using the microdilution broth method, according to the procedures recommended by the National Committee for Clinical Laboratory Standards [19].

Results and Discussion

Harsh environmental conditions force halophytes to experience several ways to survive. They develop many strategies to overcome the increased oxidative stress such as the synthesis of secondary metabolites with free radicle scavenging potential. Accordingly, these plants have great agricultural benefits in arid areas. Moreover, such metabolites could be of valuable nutritional or medicinal value [2].

Extraction Yield

Extraction yield is defined as the amount of extract recovered by certain solvent from a specified amount of plant. It measures the solvent efficacy to extract phytoconstituents. Hydroalcoholic mixture (methanol 80%) represents one of the most effective extracting mixtures. Comparison of the percentage yield of the five tested halophytes revealed a wide variation, using the same extracting solvent. The percentage yields ranged from 8.8-40% amid the tested halophytes. *Salicornia persia* showed the highest percentage yield (40%), which could be attributed to the high salt content in this species [20]. Closely, *S. villosa* and *A. leucoclada* gave 23% and 20% extractives yield, respectively. While the recorded yields for *H. bottae* and *A. alopecuroides* were: 15.3% and 8.8%, respectively.

Phytochemical Profiling

Preliminary testing of variable metabolites, in hydroalcoholic extracts of each of the five halophytes, revealed several phytoconstituents in different abundances (Table 1). Carbohydrates, sterols, triterpenes, saponins, tannins, and flavonoids were detected in all extracts, while alkaloids, anthraquinones, and cardiac glycosides were absent in all tested species. The presence of such classes of secondary metabolites is usually influenced by several environmental factors such as drought, light intensity, temperature, and salinity [12]. Several studies reported the redox properties of phenolic compounds, hence their potential as antioxidant agents. Also, saponins and flavonoids were reported to have several activities as antioxidant, antibacterial, anti-inflammatory, anti-allergy, and anti-mutagenic [14, 21-23]. Reviewing the relevant literature, no reports were found discussing the biological activity of any of the tested species, however, the detection of these metabolites in the tested extracts suggests their possible biological potential.

Analyses to determine the concentrations of N, K and P were performed using AOAC and ICP-OES methods [15-16]. Results indicated that the concentrations of N in *A. alopecuroides*, *A. leucoclada, H. bottae, S. villosa,* and *S. persia* were: 1.74, 1.40, 1.65, 1.33 and 1.54 g/100 g, respectively, the contents of K were: 1.83, 3.88, 2.00, 2.25 and 3.40 g/100 g, respectively, and the contents of P were: 17.12, 47.39, 60.48, 22.50 and 52.9 mg/100 g, respectively.

Total Phenolics Content (TPC)

The content of the phenolic constituents was assessed in each of the five species using the Folin-Ciocalteu method. Gallic acid was used as standard and the contents were expressed as mg GAE/g crude extract. Results of determination of the TPC in the five halophytes (Fig. 1) indicated the presence of appreciable TPC in all the tested halophytes extracts. The highest content was recorded in *S. villosa* and *A. leucoclada* (135.2±0.6 and 134.1±1.3 mg GAE/g), respectively. The recorded TPC in the remaining three halophytes was in the following order: *A. alopecuroides* (90.6±3.6), *H. bottae* (85.6±2.7), and *S. persia* (67.3±1.6). These results come in accordance with the preliminary screening results displayed in table 1, that indicated the presence of polyphenolic compounds e.g. tannins and flavonoides in all tested extracts. Phenolic compounds are known for their antioxidant potential that is believed to have a significant role in the treatment of several diseases such as: diabetes, cancer, liver, and neurodegenerative diseases [23]. Accordingly, determination of the TPC is a preliminary step for predicting the antioxidant potential of plant extract. It is noteworthy that this is the first report for the evaluation of TPC content in all tested species. However, the TPC content of other species e.g. *Salsola imbricate* and *S. cyclophylla*, were previously reported. Our recorded results for *S. villosa* greatly exceeded previous data for *S. imbricate* that ranges from (1.5-2 mg GAE/ml) [24]. Another research study reported higher content of phenolics in *S. imbricate* and close value for TPC in *S. cyclophylla* (360 and 126 mg GAE/g, respectively) [25].

Total Flavonoids Content (TFC)

The aluminum chloride method is used for the determination of flavonoid content in the tested plant extracts using quercetin as standard. The flavonoids content was calculated using the regression equation obtained from the quercetin standard calibration curve. The recorded flavonoids contents (Fig. 1) ranged from (12.4-21.2 mg QE/g), they were in the order: *A. leucoclada* (21.2±3.6)>*H. bottae* (20.5±1.6)>*S. villosa* (18.2±0.2)>*A. alopecuroides* (13.7±0.6)>*S. persia* (12.4±0.04). Reviewing the relevant literature, no

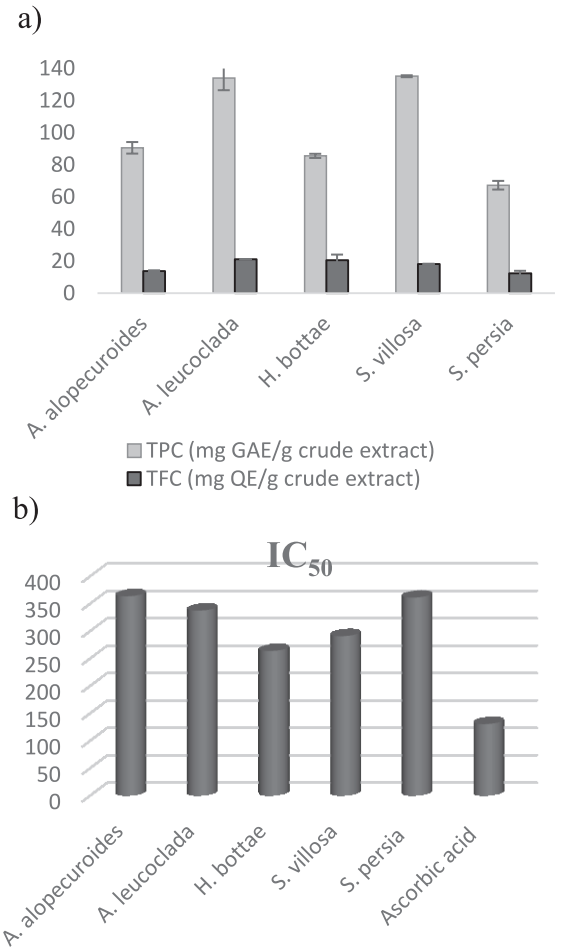

Fig. 1. a) Total phenolic content (TPC) and total flavonoid content (TFC) in different extracts of the five halophytes. b) DPPH scavenging activity of the extracts from the five halophytes and Ascorbic acid expressed as IC_{50} (µg/mL) values. Ascorbic acid IC_{50} (130.4 µg/mL).

previous reports for the TFC of the tested halophytes, were found. However, the TFC of another *Salicornia* species, *S. europaea*, was reported as (8.7±0.2 mg QE/g) [26]. This result is less than our results for the TPC of *S. persia* (12.4±0.04 mg QE/g).

The results of preliminary phytochemical screening, TPC, and TFC content indicated the existence of different classes of valuable metabolites, which in turn, suggested valuable pharmacological activities for these halophytes. Our results indicated a similar metabolic pattern in the five halophytes, however, variable phenolics and flavonoids contents were recorded in each species. This variation suggested different biological potentials between the five tested halophytes. This was stated by the observed results of the antioxidant and antimicrobial testing.

Antioxidant Activity Using DPPH Assay

Plants are well known to produce a diverse array of secondary metabolites to engage with its environment. These secondary metabolites are of variable chemical structures, e.g. alkaloids, terpenes, and phenols. Phenolic compounds comprise a wide variety of natural products that can be divided into several classes, such as phenolic acids and tannins. Due to their potent antioxidant capacity, some polyphenols are proposed as therapeutic agents for a variety of diseases or to promote general health [27-28]. Several assay methods are usually employed to evaluate the antioxidant capacity of plant extracts, but DPPH radical scavenging assay is frequently used. DPPH works in both electron transfer and hydrogen transfer systems and allows the determination of a substance or a complex mixture that donates either hydrogen atoms or electrons in a homogeneous system. The radical solution is discolored to pale yellow hydrazine according to the number of electrons captured by antioxidant [29]. Herein, the DPPH assay method was employed for comparing the antioxidant potential of the five halophyte extracts, using Ascorbic acid as the positive control. The IC_{50} value was calculated using Graph pad prism software. Results, presented in Fig. 1, indicated a difference in IC_{50} values of the five extracts. The lowest IC_{50} value, hence the highest antioxidant activity, was observed for *H. bottae* (263.7± 0.025 µg/mL). Comparison of the IC_{50} values for the five extracts revealed that the antioxidant potential was in the following order: *H. bottae* (263.7±0.025)> *S. villosa* (290.7±0.035)>*A. leucoclada* (337.3±0.041)> *S. persia* (361.1±0.048)>*A. alopecuroides* (363.5±0.055). Variation in the antioxidant potency could also

Table 2. Antimicrobial activities of crude extract of the five halophytes against some pathogenic bacteria and fungi.

Plant Species	*Staphylococcus aureus*		*Escherichia coli*		*Aspergillus fumigatus*	*Candida albicans*
	Inhibition zone (mm)	MIC (mg/mL)	Inhibition zone (mm)	MIC (mg/mL)	Inhibition zone (mm)	
A. alopecuroides	11.93±0.4	12.5	11.17 ±0.76	12.5	NA	NA
A. leucoclada	NA		NA		NA	NA
H. bottae	14.93±0.7	3.13	NA		NA	NA
S. villosa	12.9±0.46	6.25	NA		NA	NA
S. persia	14.0±1	1.56	NA		NA	NA

Ciprofloxacin and fluconazole acted as the positive control against the tested bacteria and fungi, respectively

be due to differences in abundance and/or type of the phenolic compounds in each species. Reviewing the relevant literature, no reports were found discussing the antioxidant activity of any of the tested halophytes. However, a recent research on another *Halothamous* species, *H. auriculus,* reported percentage inhibition ranging from 14.8-49.5% for different extracts of [30]. Also, a research discussing the radical scavenging effect of *Salsola stenoptera,* reported that the ethanol extract of the plant stem exhibited an IC_{50} value of 29.8 µg/mL. This result greatly exceeds our results for *S. villosa* (290.7 µg/mL), however, this could be due to different abundance and/or types of metabolites responsible for the antioxidant effect [31].

Antimicrobial Activity

Nowadays, antibiotic resistance is considered one of the most urging threats to mankind [32]. Many research programs are directed towards the exploration of different sources for new antimicrobials. In this concern, the authors are interested in the exploration of Qassim area plants, especially those with minor or no previous reports. Accordingly, the alcohol extracts of the five halophytes were screened for activity against two fungal species: *Aspergillus fumigatus, Candida albicans,* and two bacterial species: *Staphylococcus aureus* (Gram-positive species) and *Escherichia coli* (Gram-negative species). All tested extracts, except *A. leucoclada,* displayed inhibitory activity against *S. aureus,* with inhibition zones ranging from 11.94-14.93 mm. Comparison of the MIC of active extracts, indicated the highest activity for *S. persia* (1.56 mg/mL), *H. bottae* (3.13 mg/mL), *S. villosa* (6.25 mg/mL) and finally *A. alopecuroides* (12.5 mg/mL) (Table 2). Although, the presented results excluded *A. leucoclada* from observed activity against the four microbial species, other *Atriplex* species were previously reported to exhibit significant antimicrobial activity e.g. *A. halimus* [33-34], *A. tatarica* [35] and *A. semibacata* [36-37]. Regarding the antimicrobial activity of the five tested extracts against *E. coli*, only *A. alopecuroides* extract exhibited inhibitory effect with inhibition zone 11.17 mm and MIC 12.5 mg/mL. On the other hand, none of the tested extracts displayed antifungal activity against *A. fumigatus* or *C. albicans* (Table 2).

From the previous investigations, only one report was found for the antifungal potential of the butanol extract of *Salsola villosa* against *C. albicans* and *Fusarium oxysporum* [38]. Other *Salsola* species were investigated for antimicrobial potential e.g. *Salsola vermiculata* exhibit potent antimicrobial activity against pan-drug resistant (PDR) pathogens [39] and *S. kalli* against a list of microbes [40]. Concerning genus *Halothamous*, only one report was found stating good antimicrobial potential of *H. auriculus* [30]. *Salicornia* is a halophyte belonging to the *Amaranthaceae* family, that has been used for edible and non-edible purposes. Its name comes from the Latin Word meaning “salt” [41]. Several *Salicornia* species were reported for potent antimicrobial activity, e.g. *S. herbacea* and *S. brachiate* [42-44]. Herein, our results reported significant antibacterial activity of the alcoholic extract of *S. persia* against *S. aureus*. These results represent the first report for the antibacterial potential of the edible halophyte *S. persia*.

Conclusion

This study presents the first report for the phytochemical profiling, antioxidant activity evaluation, and antimicrobial screening of five halophytes prevailing in the Qassim region, Saudi Arabia. The results indicated richness of the secondary metabolite pattern of the five plants with several classes of metabolites, sounded for its biological efficacy. Tannins, flavonoids, and saponins were among the most characteristic metabolites. All the species showed high content of TPC and TFC as well as appreciable antioxidant activity. Four out of the five tested halophytes displayed good activity against *S. aureus*, where the edible halophyte *S. persia* exhibited the most significant activity. These results recommend these halophytes for more extensive phytochemical and biological exploration. This could lead to the discovery of new chemical entities and/or bioactive natural products.

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Conflict of Interest

The authors declare no conflict of interest.

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